PALACKY UNIVERSITY OLOMOUC

Faculty of science

Department of Biophysics

Voice Research Lab

Vibration and morphology of the vocal folds studied by excised larynx experiments, electroglottography, high-speed imaging, and computed tomography

Doctoral dissertation

Author: Mgr. Vít Hampala

Study program: P1703 / Physics

Supervisor: RNDr. Jan G. Švec, Ph.D. et Ph.D.

Olomouc 2015

Vít Hampala

Vibration and morphology of the vocal folds studied by excised larynx experiments, electroglottography, high-speed imaging, and computed tomography.
[Studium kmitů a morfologie hlasivek pomocí preparátů hrtanů, elektroglotografie, vysokofrekvenčních zobrazovacích metod a počítačové tomografie.]

Doctoral Thesis, Palacky University in Olomouc, the Czech Republic – with an abstract in Czech.

STATEMENT OF ORIGINALITY

I declare that I am the only author of this thesis and all sources are properly cited.

In Olomouc: ……. Signature: ……

Table of contents

Acknowledgements

Personal thanks

I would gladly like to thank to dr. Jan G. Švec for supervising me during my PhD. studies, for the opportunities to present my results at foreign conferences and for his support, helpfulness and time invested into a development of my research skills. I would also like to thank to dr. Christian T. Herbst and Maxime Garcia with whom I spent very productive amount of time in the Bioacoustic Laboratory in Vienna (Dept. of Cognitive biology), for dr. Herbst`s supervision during writing the publication Hampala *et al.* (2015a) of this thesis and to Prof. Tecumseh Fitch for allowing me to spend my 3-month stay in Vienna.

My last thanks belong to my family and to my fiancée Veronika for support and love which helped me to finish the studies successfully.

Support:

The research in original work of the author was supported by the following projects, grants and institutions:

- Palacky University students' project IGA_PrF_2013_017 (1.3.2013-28.2.2014)
- Palacky University students' project IGA_PrF_2014_029 (1.3.2014-28.2.2015)
- Palacky University students' project IGA_PrF_2015_025 (since 1.3. 2015)
- European Social Fund Project OP VK CZ.1.07/2.3.00/20.0057 "Progress and internationalization of biophysical research at Faculty of Science, Palacký University in Olomouc" (1.1.2012-31.12.2014)
- European Social Fund Project OP VK CZ.1.07/2.4.00/17.0009 "New methods and technologies for education, diagnosis, therapy and rehabilitation of human voice - connecting basic research in voice physiology with university education and stays at a specialized health institute" (1.6.2011-31.5.2014)
- Technology Agency of the Czech Republic project no. TA04010877 "Automatic evaluation of videokymographic recordings for early diagnosis and prevention of vocal fold tumors" (since 1.7. 2014)
- University of Vienna

Abstract

Vibration and morphology of the vocal folds studied by excised larynx experiments, electroglottography, high-speed imaging and computed tomography

Voice production is a very complex process. Voice research laboratories all over the world have brought important knowledge on voice production principles, particularly by observing vocal fold vibrations *in vivo* or *in vitro* using excised larynx experiments. Although general knowledge about the voice production principles has expanded during last decades many questions still remain to be answered. In order to find answers for some of them, methods for assessing the properties of the vocal folds and their vibration characteristics need to be improved. This dissertation is devoted to studying the morphologic and oscillatory characteristics of the vocal folds. In particular, three topics were addressed:

Firstly, an excised larynx setup was improved by designing and constructing **an adjustable subglottal resonance tract** in order to better control the influence of the subglottal tract on the oscillatory behavior of the vocal folds. Although the subglottal acoustics may considerably influence vocal fold vibrations the subglottal resonance properties have mostly not been properly specified in studies utilizing excised larynx experiments. The developed subglottal tract provides a possibility to set the desired subglottal resonance frequency or to change it continuously. This is desirable for obtaining comparable results from different laboratory setups.

Secondly, the **relationship between electroglottographic (EGG) signal and vocal fold contact area (VFCA)** was investigated. Electroglottography (EGG) is a widely used non-invasive method for assessment of vocal fold vibrations that purports to measure changes in relative vocal fold contact area (VFCA) during phonation. Despite its broad application, only a single study has so far attempted to verify the direct relationship between the EGG signal and vocal fold contact area [Scherer, R. C., Druker, D. G., and Titze, I. R. (1988). "Electroglottography and direct measurement of vocal fold contact area," in Vocal Physiology: Voice Production, edited by O. Fujimura (Raven Press, Ltd., New York), pp. 279-291]. That study found approximately linear relationship; nevertheless, the relationship was investigated under non-physiologic conditions and flow-induced vocal fold vibrations were not studied. A rigorous empirical evaluation of EGG as a measure of VFCA under proper physiological conditions was therefore performed in this thesis using three excised red dear larynges. A strong correlation between the EGG signal and VFCA was found, but some discrepancies were observed in the contacting and decontacting phases of the glottal oscillatory cycle. In the contacting phase the EGG signal systematically preceded the visually measured VFCA in all larynges. In the decontacting phase two of the larynges showed good agreement between the EGG signal and VFCA, but considerable disagreement was found in one of the larynges. This larynx differed from the other two by showing a slight anomaly of the medial vocal fold surface, known as *sulcus vergeture*. The role of pathology and other factors on the potential discrepancies between the EGG signal and VFCA should be explored in future studies.

Thirdly, computed tomography (CT) was used in order to **study changes in vocal fold configuration induced by a special therapeutic method, i.e. phonation into a tube**. This method uses a narrow constriction at lips formed by a tube in which the subject phonates. Previous studies showed that phonation into a tube caused clear changes in vocal tract cavities which endured also after the exercise was finished. The purpose of the present study

was to show whether the adjustment of the vocal folds and their morphology were also affected by the exercise or not. Two subjects underwent the examination using CT device. The collected data were used to measure vocal fold vertical thicknesses, cross-sectional areas and lengths. Interestingly, the results of this double-case study did not show any clear systematic common trends in vocal fold adjustments between the two subjects suggesting that the adjustments of the vocal tract shape play a more important role for the voice improvement after the therapy than the vocal fold adjustments. Nevertheless, more studies with larger number of subjects are needed to better understand the influence of the vocal fold and vocal tract adjustments caused by voice therapy on the resulting voice quality and verify these results.

Souhrn

Studium kmitů a morfologie hlasivek pomocí experimentů s preparáty hrtanů, elektroglotografie, vysokofrekvenčních zobrazovacích metod a výpočetní tomografie

Tvorba hlasu je velmi složitý proces. Výzkumné hlasové laboratoře z celého světa již přinesly celou řadu důležitých poznatků o principech tvorby hlasu, a to zejména díky pozorováním kmitů hlasivek *in vivo* či *in vitro* za použití preparátů hrtanů. Ačkoliv znalost principů tvorby hlasu za poslední desetiletí vzrostla, mnoho otázek je stále nezodpovězeno. Předpokladem nalezení odpovědí na některé z nich je zlepšení metod pro studium vlastností hlasivek a jejich kmitání. Tato dizertace se věnuje studiu morfologických a oscilačních vlastností hlasivek v rozsahu následujících 3 témat:

Za prvé, sestava pro experimenty s preparáty hrtanů byla vylepšena **navržením a zkonstruováním nastavitelného subglotického rezonančního traktu**, který slouží k lepší kontrole vlivu subglotického traktu na oscilační vlastnosti hlasivek. Navzdory tomu, že subglotické akustické jevy mohou značně ovlivnit kmitání hlasivek, subglotické rezonance většinou nebývají ve studiích s preparáty hrtanů dostatečně specifikovány. Vyvinutý subglotický trakt poskytuje možnost nastavit požadované frekvence rezonancí nebo je plynule měnit. Takové nastavení je důležité pro opakovatelnost experimentů i pro získání vzájemně porovnatelných výsledků mezi různými laboratorními sestavami.

Za druhé byl studován **vztah mezi elektroglotografickým (EGG) signálem a kontaktní plochou hlasivek (VFCA, z angl. "vocal fold contact area“)**. Elektroglotografie je neinvazivní metoda pro sledování kmitů hlasivek, jejímž cílem je měřit relativní změny kontaktní plochy hlasivek. I přes její časté použití v odborných pracích, však existuje jen jediná studie, jež se pokusila ověřit přímý vztah mezi EGG signálem a VFCA [Scherer, R. C., Druker, D. G., and Titze, I. R. (1988). "Electroglottography and direct measurement of vocal fold contact area," in Vocal Physiology: Voice Production, edited by O. Fujimura (Raven Press, Ltd., New York), pp. 279-291]. Zmíněná studie ukázala přibližně lineární vztah, nicméně neproběhla za podmínek podobných fyziologickým, tj. kmitání hlasivek nebylo vyvoláno prouděním vzduchu. Z tohoto důvodu byl vztah mezi EGG signálem a VFCA podrobně studován v této disertaci, a to za podmínek, které lépe odpovídají fyziologickým. Experimenty byly provedeny za využití 3 jeleních hrtanů. I přes to, že výsledky ukázaly silnou korelaci mezi EGG signálem a VFCA, byly nalezeny i významné rozdíly v rámci kontaktní a dekontaktní fáze hlasivkového kmitu. Během kontaktní fáze EGG signál systematicky předbíhal vizuálně měřenou VFCA. Během dekontaktní fáze dva ze tří hrtanů vykazovaly velmi dobrou shodu mezi EGG a VFCA, ale u jednoho hrtanu se průběh křivek značně lišil. U tohoto problematického hrtanu byla na mediální straně hlasivkové tkáně nalezena lehká anomálie odpovídající klinickému nálezu tzv. *sulcus vergeture*. Vliv patologií hlasivek a dalších faktorů na případné rozdíly mezi EGG signálem a VFCA je třeba podrobněji objasnit v budoucích studiích.

Za třetí, **změny v nastavení hlasivek vyvolané speciální terapeutickou metodou zvanou “fonace do trubice” byly studovány za pomocí počítačové tomografie (CT, z ang. “computed tomography”).** Hlasová cvičení pomocí fonace do trubice využívají zúžení v oblasti rtů způsobené rezonanční trubicí. Předchozí studie ukázaly, že tato terapeutická metoda vyvolává prokazatelné změny ve tvaru rezonančních dutin, jež setrvávají i po ukončení cvičení. Cílem této studie bylo zjistit, zda má fonace do trubice vliv také na nastavení hlasivek a jejich morfologii. Dvě osoby podstoupily CT vyšetření. Pořízená data byla použita pro měření vertikální tloušťky, plochy průřezu a délky hlasivek. Překvapivě, výsledky

této dvojité kazuistiky neprokázaly zjevné systematické změny v nastavení hlasivek podobné pro oba subjekty. To naznačuje, že pro zlepšení hlasu pomocí fonace do trubice jsou pravděpodobně podstatnější změny rezonančních dutin vokálního traktu než změny v nastavení hlasivek. K lepšímu pochopení vlivu změn v nastavení vokálního traktu a hlasivek způsobených fonací do trubice a k ověření obdržených výsledků je nicméně vhodné provést další studie s více subjekty.

Goals of the thesis

The aims of this thesis were to:

1) *design a new subglottal tract* with adjustable length and resonance characteristics to be used for excised larynx experiments;

2) *validate the method of electroglottography* on excised hemilarynges by directly comparing the electroglottographic signal and vocal fold contact area observed by means of high speed video recordings;

3) *investigate whether the therapeutic method called „phonation into a tube" causes distinct changes in the vocal fold adjustments,* in order to clarify the physiologic mechanisms of this method

List of publications

Paper I – utility model:

V. Hampala, J. Švec, P. Schovánek, D. Mandát (2013). Užitný vzor č. 25585: Model subglotického traktu. [Utility model no. 25585: Model of subglottal tract.] Soukup, P. 2013-27834(CZ 25505 U1), 1-7. 24-6-2013. Praha, Úřad průmyslového vlastnictví. [Prague, Czech Republic, Industrial property office].

Paper II –scientific article:

V. Hampala, M. Garcia, J.G. Švec, R.C. Scherer, C.T. Herbst (2015, in press). "Relationship Between the Electroglottographic Signal and Vocal Fold Contact Area." Journal of Voice.

Paper III – scientific article:

V. Hampala, A.-M. Laukkanen, M.A. Guzman, J. Horáček, J.G. Švec (2015, early online). "Vocal Fold Adjustment Caused by Phonation Into a Tube: A Double-Case Study Using Computed Tomography." Journal of Voice, doi: 10.1016/j.jvoice.2014.10.022.

Introduction

Voice research is a highly inter-disciplinary field. There are various aspects of voice production which are of interest for different kinds of professions. Physicists, physicians, biologists, singers, engineers, mathematicians and other specialists study voice from different perspectives. Large effort is devoted to improving examination methods ordinarily used in clinical medical practice or in voice science in order to understand principles of voice production. Despite of the fact that voice has been studied since the invention of laryngoscopic mirror in 19th century many aspects of its creation remain poorly understood.

This thesis consists of two main parts. The first part provides a theoretical overview of the topics related to the work of the author. The second part consists of the original work by the author.

1. Theoretical section and basic principles

1.1. Basics of voice anatomy

Voice is normally created in laryngeal part of the breathing airways by a pair of oscillating tissues, i.e. vocal folds (VFs). This process is called *phonation*. Phonation is, however, only the latest acquired function of the larynx (Asher *et al.*, 1996).The primary function of the larynx is to prevent foreign substances (i.e. liquids, food or any other solid particles which could potentially be inhaled) from entering the trachea and lungs.

A gap between the VFs is called *glottis*. The cavities above the glottis (superiorly, i.e. epilarynx, pharynx, oral and nasal cavities) are called supraglottal, whereas the cavities below (inferiorly) are called subglottal. The supraglottal cavities involved in the vocalization process are frequently denoted as the *vocal tract*.

Oscillation of the vocal folds is initiated by air flowing from the lungs using breathing muscles (diaphragm, intercostal muscles, etc.). Thus, the compressed air in lungs acts as a power source. The scheme of these anatomical structures is depicted in **Figure 1**. As soon as the VFs are approximated (see **Figure 2**, right) by laryngeal muscles and the airflow is established, the vocal fold oscillation begins.

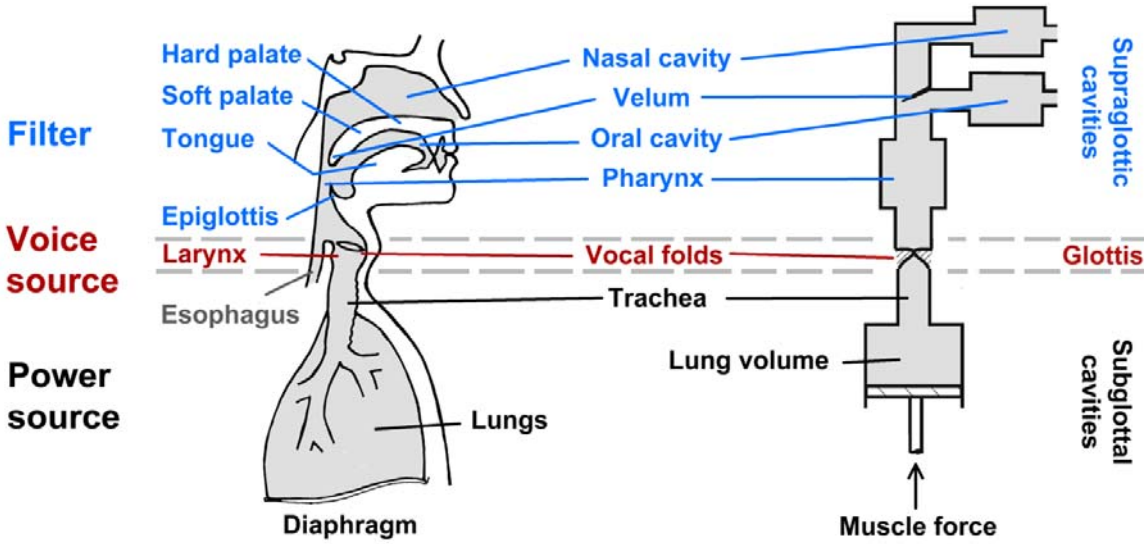


Figure 1. Scheme of the structures involved in the voice production (blue – supraglottal tract; red – voice source; black – subglottal tract): left – basic anatomical structures, right – scheme modified after Fant (1960).

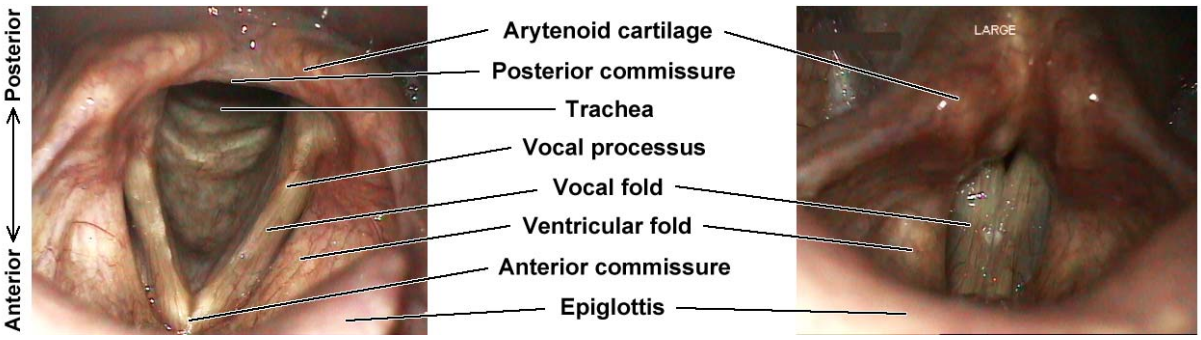


Figure 2. Laryngoscopic view of the vocal folds and surrounding structures. Left – open glottis during breathing; right –vocal folds closed during phonation.

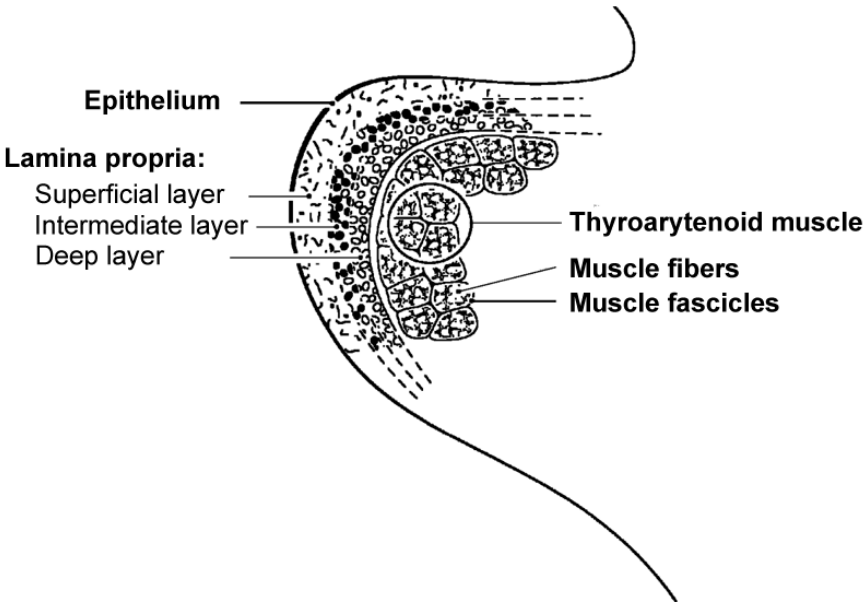


Figure 3. Vocal fold tissue structure (Titze, 2000).

The anatomical scheme of a vocal fold (VF) is depicted in **Figure 3**. The VF is formed by a layered structure (Hirano, 1975; Hirano *et al.*, 1981; Titze, 2000), which consists of epithelium, lamina propria (superficial, intermediate and deep layers) and thyroarytenoid muscle.

The VFs are anteriorly attached to the thyroid cartilage and posteriorly to the arytenoid cartilage. The length and tension of the vocal folds are mainly controlled by cricothyroid and thyroarytenoid muscle (Titze *et al.*, 1988; Titze, 2000).

Activation of the thyroarytenoid muscle shortens and bulges the VF body (Hirano, 1975). In contrast, when the cricothyroid muscle is activated, the thyroid cartilage is pulled inferior-anteriorly and rotated around the cricothyroid joint (**Figure 5**, arrow) resulting in elongation of the VFs (Van den Berg *et al.*, 1960; Titze, 2000).

Glottal contact is reached by approximating the VFs caused by activation of intrinsic laryngeal muscles [for more details on the anatomy see related literature; e.g. Čihák (1988), Diano (2006), Hampala (2011), Olias (2004), Svec (1996), Titze (2000) or any anatomical atlas]. The muscles taking part in the approximation are called adductors (i.e. lateral cricoarytenoid and interarytenoid muscles) whereas those pushing them apart laterally are abductors (i.e. posterior cricoarytenoid muscle) (**Figure 6**).

The terms adduction and abduction denote the process of approximating and spreading apart the VFs and are also commonly used to describe the level of vocal fold medialization. Thus, whenever the VFs are approximated and touching along their length it means they are adducted and vice versa. Since the activation of cricothyroid and thyroarytenoid muscles affects the VF shape (**Figure 4**) it influences also the overall adduction.

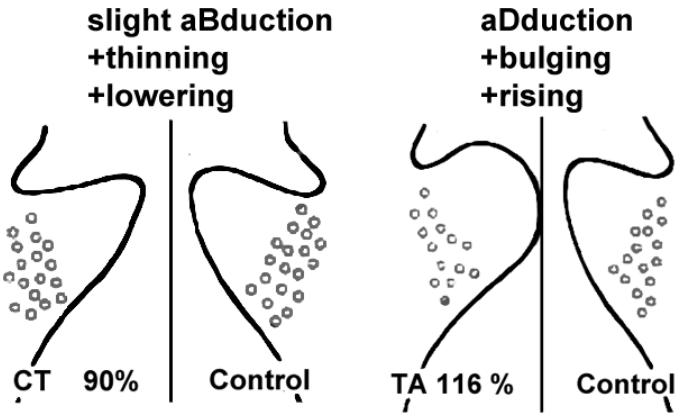


Figure 4. Effect of cricothyroid and arytenoid muscle activity on cross-sectional vocal fold shape according to Hirano (1975).

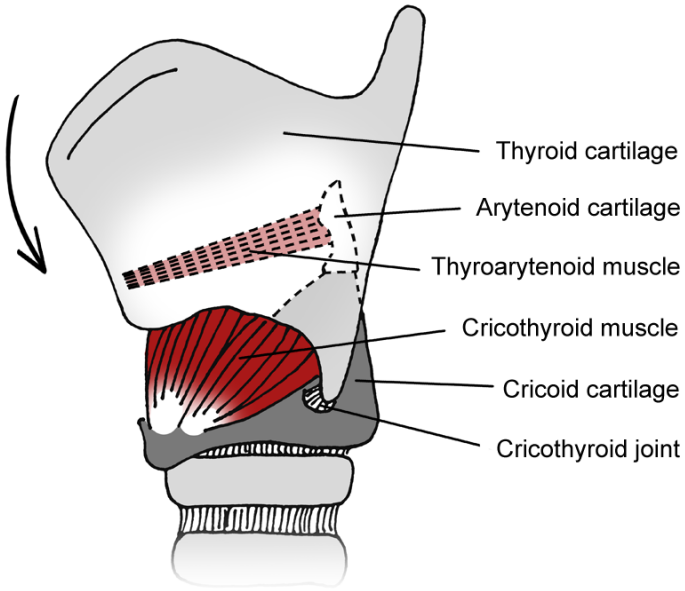


Figure 5. Principle of vocal fold elongation caused by activation of cricothyroid muscle. The contraction of cricothyroid muscle causes rotation of the thyroid cartilage around the cricothyroid joint while elongating the vocal folds.

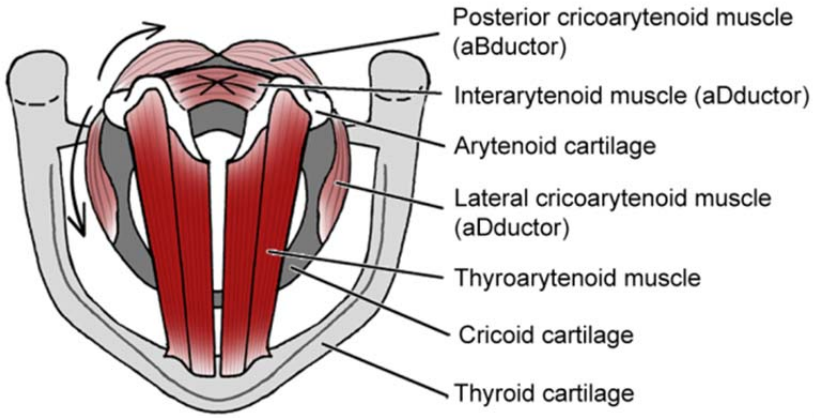


Figure 6. Intrinsic laryngeal muscles responsible for adduction and abduction of the vocal folds.

1.2. Principles of sound production

Although the presence of vibrating VFs is necessary for producing the primary acoustical signal, the resulting vocal output is influenced by resonance cavities. Therefore, the knowledge of basic voice production principles is crucial for voice research purposes. It is related also to **Paper II** of this thesis (Hampala *et al.*, 2015b) where the interaction between the vocal folds and the vocal tract is partially discussed.

1.2.1. Source-filter theory of voice production

The oscillating VFs turn a steady airflow into a sequence of flow pulses created by opening and closing of the glottis. This is a primary sound signal which acoustically excites the resonance cavities of the vocal tract and is modified by them (**Figure 7**). The resonances are damped (**Figure 7**) due to radiation loses and energy dissipation in soft tissues covering and surrounding the airways.

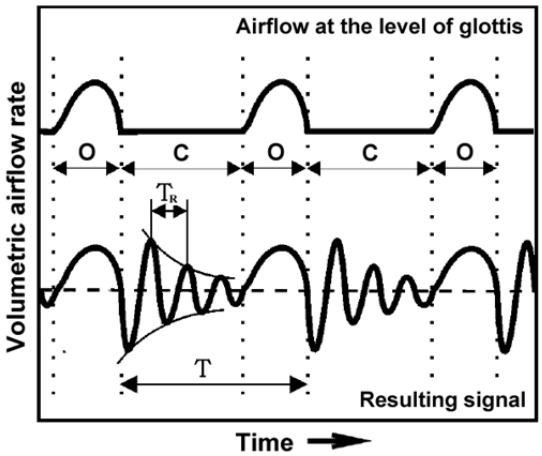


Figure 7. Airflow pulses at the level of glottis and the simplified view of the influence of resonance cavities on the resulting signal according to Rothenberg (1973) and Svec (1996) modified by the author. Legend: O – open phase, C – closed phase, T – period of vocal fold oscillation, T_R – period of damped supraglottal resonance.

The most important resonance cavities are particularly the supraglottal ones. However, since the sound is spread to all directions, the system of subglottal cavities (i.e. trachea, bronchi, lungs) also acts as a resonator (Hála and Sovák, 1941; 1962; Kitzing and Löquist, 1975; Koike, 1981; Cranen and Boves, 1985; 1987; Sundberg *et al.*, 2013).

Fant (1960) introduced the *source-filter theory* of voice production assuming that the resulting acoustical spectrum is created by a linear superposition of harmonic spectrum of the voice source, vocal tract transfer function and mouth radiation (**Figure 8**). In other words, the acoustical properties of vocal tract have an influence on the resulting acoustical output.

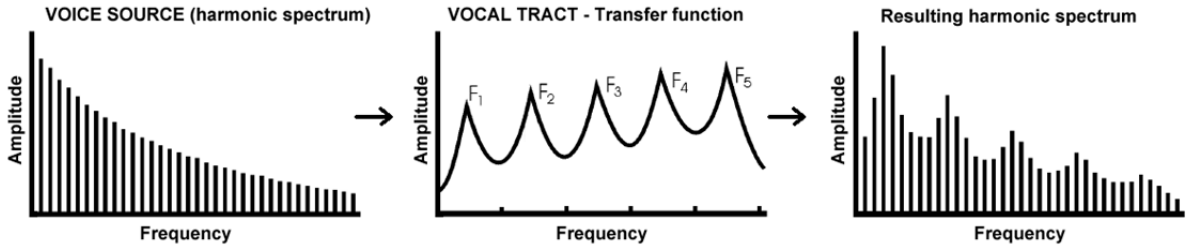


Figure 8. Simplified spectra illustrating the origin of acoustic output according to Fant (1960); Sundberg (1977); Svec (1996); Titze (2000); Howard and Murphy (2008) modified by the author. Legend: $F_{1\text{-}5}$ – formants.

1.2.2. Myo-elastic aerodynamic theory of vocal fold vibration

To explain the principles of vocal fold (VF) vibration, the voice science community generally accepts the so called myo-elastic aerodynamic theory. Its fundamentals were formulated by Van den Berg (1958) and later developed by many other scientists, including the notable work by Flanagan (1965), Titze (1980) and others. The most detailed information about this theory can be found in Titze (2006a).

The myo-elastic aerodynamic theory assumes that the vocal fold vibration is a result of the interaction between airflow which drives VFs by aerodynamic forces and elastic properties of VFs influenced by activity of laryngeal muscles. During phonation the properties of the VF tissues can be consciously and voluntarily controlled by laryngeal muscles (particularly cricothyroid and thyroarytenoid muscle). This is desired since the VF is not uniformly structured (Hirano *et al.*, 1981) and, when the tension is applied, different tissue layers are not tensed equally. In order to study the role of cricothyroid and thyroarytenoid muscle activity in control of VF vibration, a two-layer cover-body theory was established (Hirano, 1974; Fujimura, 1981; Hirano and Kakita, 1985; Titze *et al.*, 1988). The cover consists of the epithelium and the superficial and intermediate layers of lamina propria (recall **Figure 3**) whereas the body incorporates the deep layer of lamina propria and the thyroarytenoid muscle (Hirano and Kakita, 1985). The thyroarytenoid muscle forms a major part of VF; therefore, its active tension can significantly influence the overall VF tissue properties (e.g. thickness and tension) and thus partially compensate for the tension inequalities in different tissue layers when the VF is passively tensed and stretched by the cricothyroid muscle. This is demonstrated in **Figure 9** which shows possible ratios of thyroarytenoid vs. cricothyroid muscle activity for different lung pressures while keeping the same fundamental frequency.

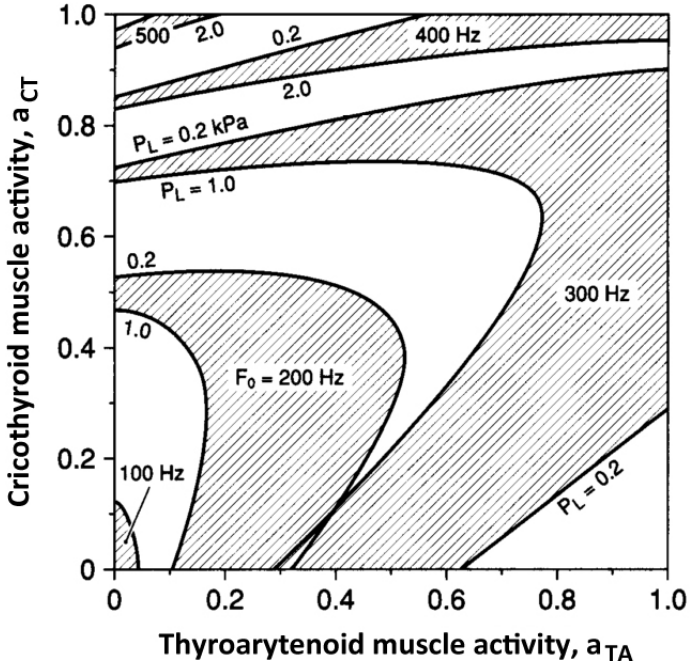


Figure 9. Relative cricothyroid and thyroarytenoid muscle activity (a_{CT} and a_{TA}) for different lung pressures (PL) after Titze (2000).

The airflow blown through the VFs is necessary to set them into a vibration and produce a time-varying glottal flow. The VF oscillation is self-sustained due to the flexibility of the VF tissues. During this process VFs vibrate with a vertical phase difference (Titze, 1988), i.e. they change their shape according to **Figure 10**. The vertical phase difference results in a wave-like motion referred to as *mucosal wave* traveling on the VF surface from its inferior to superior edge (Titze, 1988; Berke and Gerratt, 1993; Titze *et al.*, 1993) (**Figure 10**, arrows). The mucosal wave has been studied by a number of authors [e.g. Moore and von Leden (1958); Hollien *et al.* (1968); Sovák *et al.* (1971); Hirano (1974); Hirano *et al.* (1980); Titze (1988); (Berke and Gerratt, 1993); Yumoto *et al.* (1993a)] and its presence during phonation is considered to be very important for healthy VF vibrations. The mucosal wave propagation is dependent on the viscosity, mass, length or tension of vocal fold and any changes of these properties may cause abnormalities in the resulting phenomena (Qiu *et al.*, 2003; Svec *et al.*, 2007).

When opening, the shape of glottis is convergent, whereas while closing it is divergent. The airflow streaming through the open glottis is accelerated resulting in a local drop of transglottal pressure which "sucks" the VFs medially (Flanagan and Ishizaka, 1978; Broad, 1979). This phenomenon is known as the "Bernoulli effect" and in combination with VF elasticity and change of VF shape it closes the glottis (Titze, 1976; 1988). Also, an inertia of the air column above the closing glottis leads to a drop in the air pressure which aids VF closure (Titze, 1988).

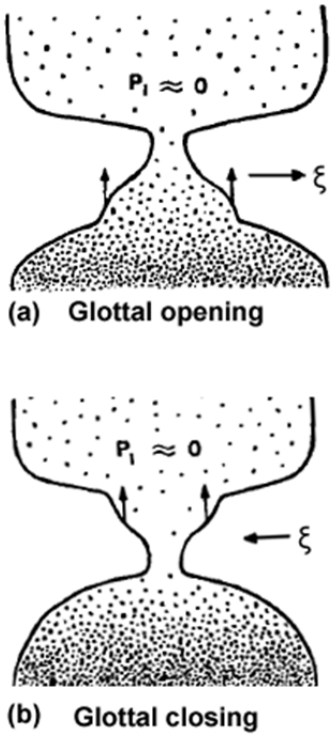


Figure 10. Change of the vocal fold (VF) shape during phonation according to Titze (1988): a – glottis is opening, glottal shape is convergent; b – glottis is closing, glottal shape is divergent. Legend: P_i – supraglottal pressure; ξ – displacement of the VF tissue; arrows – propagation of mucosal wave on the VF surface.

1.2.3. Supraglottal cavities

Supraglottal cavities are the most important part of the vocal resonance system. Since the cavities between VFs and mouth opening are most involved in voice production they are also called the *vocal tract*. For sake of simplicity, the nasopharyngeal port is often considered to be closed by velum. In such case the average male vocal tract is approximately 17,5 cm long (Titze, 2000). Its acoustical properties are described by the so called *formants,* i.e. maxima in a transfer function of the vocal tract (**Figure 8**, middle). Any change of vocal tract shape has an effect on its transfer function, and thus, on the position of formants in the spectrum. This can be nicely illustrated by an example of the shape changes induced by pronunciation of different vowels (**Figure 11**). Vowels are characterized by the frequencies of their first two formants (i.e. F_1, F_2) and together with higher formants they influence voice timbre. **Figure 11** compares the difference among vowels [a], [i] and [u] which are mainly based on a position of tongue (Singh and Singh, 1976; Mackay, 1978; Wolfram and Johnson, 1982; Ladefoged, 1990)(**Figure 11**, left). Corresponding vowel spectra and the example of positions of formants F_1, F_2 are shown in **Figure 11**, right.

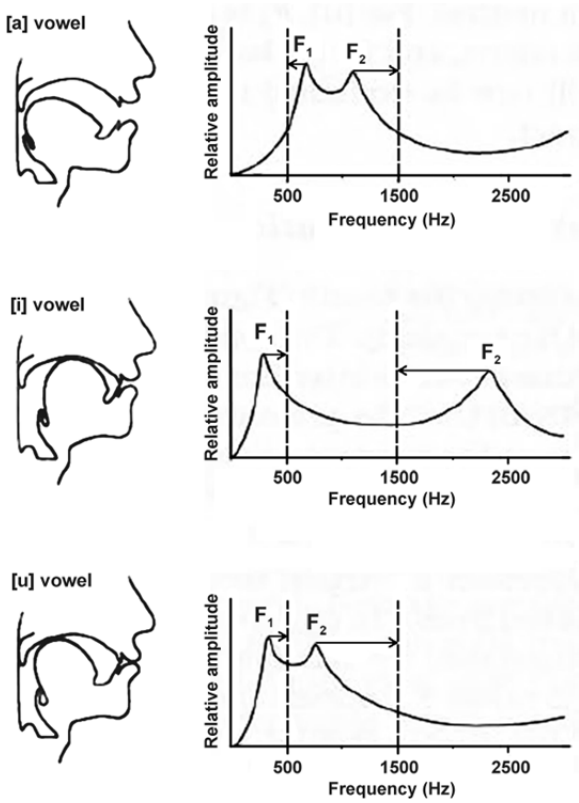


Figure 11. Shapes of the vocal tract (left) and corresponding spectra according to Titze (2000).

1.3. Instrumentation in voice research

Voice research often needs an inter-disciplinary approach. It uses techniques and instruments adopted from various fields including medicine, acoustics, explores technical solutions concerning electrical and physical measurements as well as imaging techniques. The following text describes the methods or techniques which are related to the subjects of this thesis.

1.3.1. Imaging techniques

1.3.1.1. Classical radiography

Classical radiography is based on the attenuation of X-rays while passing through examined tissue. The first imaging detectors of transmitted X-ray radiation consisted of photographic plates or films. Nowadays they are mostly replaced by digital flat detectors (Spahn, 2013).

The major disadvantage of the classical radiography is the superimposition of all the structures on the film. This makes it sometimes difficult to recognize detailed structures, especially when the tissue is of similar density (i.e. tumors and its surrounding tissue). One way how to deal with the superimposition is to move the film and X-ray bulb simultaneously which results in blurred tissue below and above the focal plane (Seeram, 2009). This method is called *laminagraphy*.

In voice research the radiography was particularly used in 1960`s when Hollien and his colleagues used the so called laminagrams to investigate the changes of VF thickness and tilting related to changes in fundamental frequency (Hollien, 1960; Hollien and Curtis, 1960; Hollien and Moore, 1960; Hollien and Colton, 1969; Hollien, 2014). For this purpose original systems of reference lines and points were designed.

Figure 12 illustrates the system for measurement of cross-sectional areas used by Hollien and Curtis (1960). First, the shapes of laryngeal cavity, i.e. supraglottal laryngeal walls (lines A and A`), VFs, and subglottal laryngeal walls (lines B and B), were carefully outlined on laminagrams. In order to measure the VF cross-sectional areas lateral boundaries had to be established. Since there were no sharp limits a system of parallel lines was used. First, the midline was identified using points Z and Z` which bisected the distance between most medial surface of VFs and most lateral parts of the subglottal laryngeal wall. Then, two sets of lines (CD, C`D` and EF, E`F`) parallel to the midline were drawn. Lines CD and C`D` were positioned to intersect points X and X`, i.e. the base of VFs where the laryngeal wall sharply inflected towards the midline. Since the laryngeal ventricles did not always reach the lines EF and E`F` the outlined superior VF surface was extended resulting in lines G and G`. This allowed the measurements of cross-sectional areas denoted by grey color in **Figure 12**.

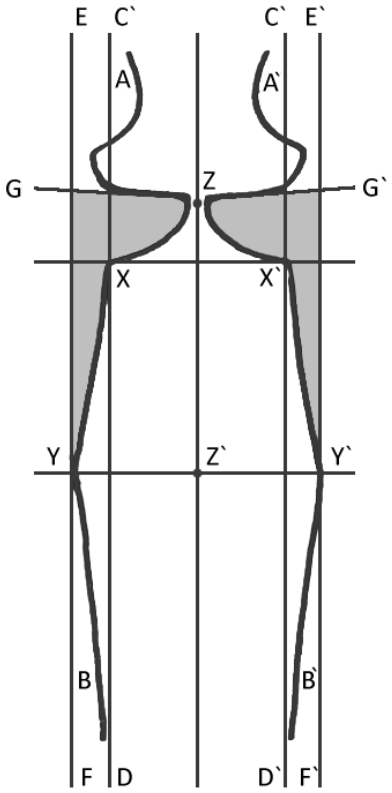


Figure 12. System of reference lines and points used for measurei areas (grey color) on laminagrams after Hollien and Curtis (1960)

Apparently, this system had limitations. In **Figure 12** the lines G and G` representing the VF surface were drawn manually. In such approach there was a room for potential error originating from wrong sloping of these lines.

Nevertheless, Hollien`s approach inspired the work of the author of this thesis for designing an alternative system of measurements (see Chapter 2.4.1.) used in Hampala *et al.* (2015b) which is the **Paper III** in this thesis.

1.3.1.2. Computed tomography (CT)

The computed tomography is based on transmission of X-rays through an examined object/subject similarly as the classical radiography. The limitation of classical radiography is the inability to image small differences in tissue density. This makes the tissue identification complicated. In contrast, the CT method provides a minimal superimposition, improved image contrast and it enables imaging of small differences in tissue contrast (Hounsfield, 1973). In CT X-rays are highly collimated into a very narrow beam which is transmitted through a specific cross-section. After the beam passes through the subject`s body it is captured by special electronic detectors. In order to reconstruct the image of the desired cross-section the analogue signal from the detectors is converted to digital data and then processed in computer using mathematical algorithms.

Basically, there are two ways of collecting the transmission measurements dependent on the acquisition geometries as illustrated in **Figure 13**. **Figure 13**a shows the CT device geometry where only the X-ray tube is rotating 360 degrees around the subject. The X-ray tube is placed inside the ring of the stationary detector array. **Figure13**b shows the second possibility. In this case, both the X-ray tube and the detector array are moving simultaneously around the patient. The second geometry is more commonly used in the modern CT devices (Zuna and Poušek, 2002).

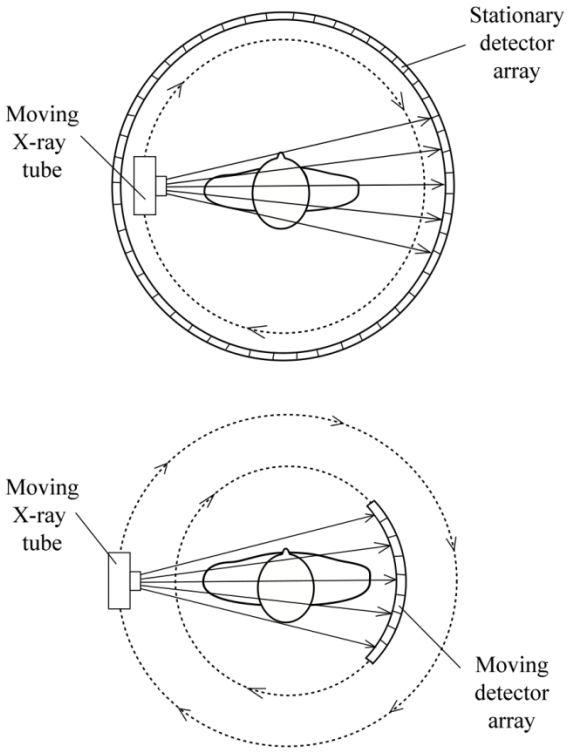


Figure13. Possibilities of CT device construction geometries.

According to the way how the x-ray tube rotates around the examined subject we distinguish between conventional slice-by-slice data acquisition and volumetric data acquisition (**Figure 14**). In the conventional method X-ray tube rotates to collect data from one slice. As soon as the tube finishes the revolution a table with the patient is moved to collect the data for the next slice (**Figure 14**a). This is repeated until whole the required segment is scanned. In the volumetric method the tube rotates in a helical way to scan certain volume of the tissue (**Figure 14**b). One revolution refers to a single slice, i.e. single slice computed tomography (SSCT). Nowadays, multislice helical CT (MSCT) has become available which rapidly increases the scanning procedure. The standard CT devices are able to scan 4, 8, 16, 32, 40, or 64 slices per revolution and since 2007 there has been commercially available MSCT scanner able to produce 320 slices (Seeram, 2009).

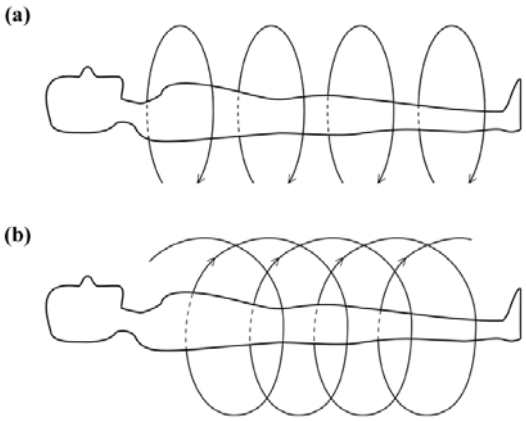


Figure 14. Conventional slice-by-slice (a) and volumetric method (b) for the CT data acquisition.

During the scanning procedure a large number of measurements from many different views are performed. Since the intensity of the x-ray source is known, the detectors measure the transmitted radiation. Thus, the relative transmission (T_r) can be calculated from these two values as follows:

$$\boldsymbol{T_r = log\ \frac{I_0}{I}}\text{, [Equation 1]}$$

where I_0 is the intensity of x-rays at the source and I is the intensity measured at the detector.

The energy of the transmitted x-ray beam is always lower because some photons are absorbed or scattered. The basic goal of CT is to determine the attenuation. In the first experiments homogenous radiation from a gamma source was used since it fulfills Labert-Beer law according to Seeram (2009):

$$\boldsymbol{I = I_0 e^{-\mu x}}\text{ , [Equation 2]}$$

$$\boldsymbol{\mu = (I/x) \cdot (\ln I_0/I)}\text{, [Equation 3]}$$

where I is the intensity transmitted through the tissue, I_0 is the initial intensity, e is Euler`s constant, μ is the linear attenuation coefficient and x is the thickness of the object. The homogenous beam is linearly attenuated as it passes through the homogenous substance. However, the disadvantage of this method was too long time period necessary to scan and produce image. The conventional X-ray tube produces a heterogeneous beam which results in a non-exponential attenuation due to photoelectric and Compton effects (Kallender, 2011). The photoelectric attenuation occurs mainly in a high density tissue (i.e. bone) whereas the Compton interactions are characteristic for the soft tissue. Therefore, it is

necessary to approximate the equation for the heterogeneous beam to the homogenous one as follows:

$$\boldsymbol{I = I_0 e^{-(\mu\rho+\mu c)x}}\text{ , [Equation 4]}$$

where $\mu\rho$ is the attenuation originating from photoelectric effect and μc is the attenuation due to the Compton effect (Kallender, 2011). Since the beam is heterogeneous it is better to use a number of photons rather than to calculate the intensity according to:

$$\boldsymbol{N = N_0 e^{-(\mu\rho+\mu c)x}}\text{, [Equation 5]}$$

where N is a number of photons passed through the tissue and N_0 is the initial number of the photons. These equations hold for a homogenous block of substance. However the patient body is a heterogeneous structure composed from different tissue types. This is solved by dividing the slice into a large number of regions where each of them is represented by its own attenuation coefficient. The readings are then preprocessed, some corrections are applied, and the image is reconstructed using special algorithms.

There is also another important parameter, i.e. the beam energy controlled by X-ray tube voltage [denoted as peak kilovoltage (kVp)]. Typical values of X-ray tube voltage range from 80 to 140 kVp (Shaw, 2014). The operator of CT device can change the tube voltage in order to reduce the artifacts and to keep an optimum detector response for particular scanned body region. The scans of higher attenuation regions (such as head or pelvis) are normally acquired with the highest kVp whereas pediatric patients are examined using the lowest values (Shaw, 2014).

Hounsfield units

Each pixel in the reconstructed image is represented by so called "CT number" which is related to the measured attenuation. This number is calculated as:

$$\boldsymbol{CT\ number = \frac{\mu_T - \mu_R}{\mu_R} \cdot K}\text{ , [Equation 6]}$$

where μ_T is the attenuation coefficient of the scanned tissue, μ_R is the reference attenuation coefficient of water, and K is a contrast factor. The scale which is given if the contrast factor equals to 1000, is called Hounsfield (H) scale. There are also other scales, e.g. E.M.I. scale which was used in the first E.M.I. x-ray devices (Hounsfield, 1977). The reference CT number for the Hounsfield scale is 0 (CT number of water). Then, the H numbers for bone and air are +1000 and -1000, for blood between 40 – 60 (Seeram, 2009; Molteni, 2013) and they are

around 50 (Seeram, 2009; Molteni, 2013) for muscle tissue which constitutes a major part of VF tissue. Based on this, the values for VF tissue may be expected somewhere in a range from slightly below +40 to slightly above +60 H units as illustrated in **Figure 15**.

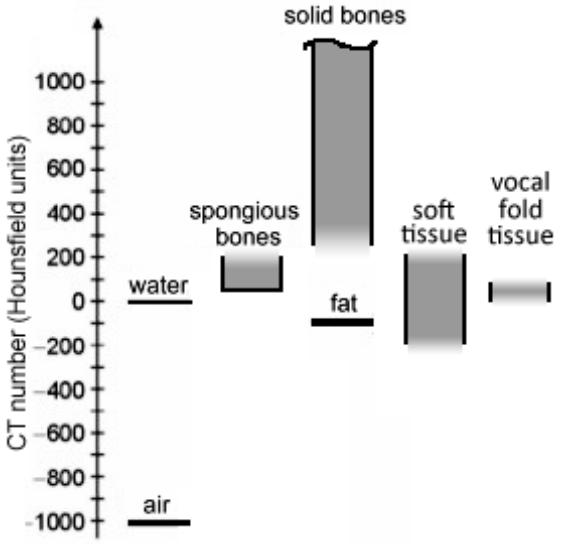


Figure 15. Hounsfield scale for several body tissues Kallender (2011) and expected H units corresponding to vocal fold tissue.

To visualize the image the H units are converted into shades of grey where air is black and bone is white. This is demonstrated in **Figure 16**. The collected CT data also enables 3D reconstruction of the scanned object. The basic volumetric unit is then called voxel (similarly as pixel in 2D image).

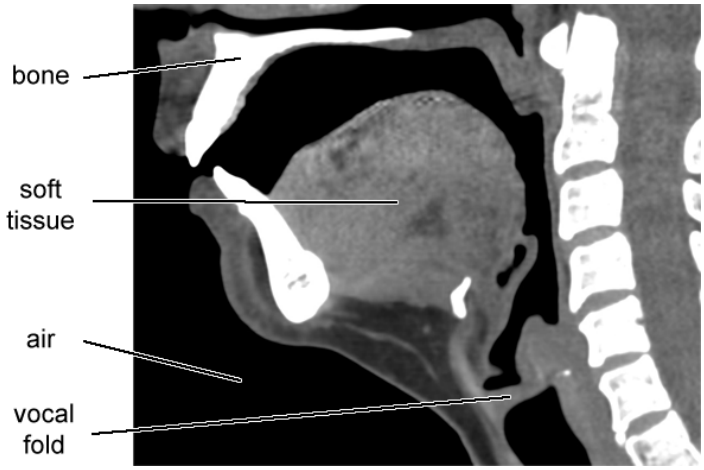


Figure 16. Sagittal CT image of a human vocal tract and vocal fold.

Voice research and CT method

The CT method has been successfully used in many studies concerning anatomical structures and their relationship with voice, e.g. in measuring the dimensions of vocal tract (Guzman *et al.*, 2013) or laryngeal structures (Shin *et al.*, 2009; Hiramatsu *et al.*, 2012) and their three-dimensional reconstruction including VFs (Bakhshaee *et al.*, 2013), investigation of pathologies concerning voice and vocal apparatus (Yumoto *et al.*, 2004; Rubin *et al.*, 2005; Berjawi *et al.*, 2010; Raj *et al.*, 2011; Chaudhry *et al.*, 2014), surgical interventions (Benninger *et al.*, 2015), and others. The CT data was also used for acoustical simulations which clarified the relationship between vocal tract shape and acoustical output (Vampola *et al.*, 2011; Vampola and Svec, 2013). Nevertheless, nobody has so far applied the approach of Hollien (see Chapter 1.3.1.1.) for measuring the vocal fold shape based on CT data.

The use of CT method is always related to potential radiation hazards. For this reason there is a general tendency to use less invasive imaging methods whenever possible. A magnetic resonance imaging [MRI, for extensive information see Bushong and Clarke (2015)] is a possible alternative to CT method since it does not employ any ionizing radiation. The MRI has been used in voice research in order to study e.g. the shape of the vocal tract [Baer *et al.* (1987); Demolin *et al.* (2003); Vasconcelos *et al.* (2011); Ventura *et al.* (2013); Miller *et al.* (2014) and others] or visualize laryngeal pathologies [e.g. Malcolm *et al.* (1997)]. Unfortunately, the resolution of the routinely available MRI method has been lower than that of the CT for displaying the tissues of vocal organs (Story *et al.*, 1996). When investigating MRI images from a study on the vocal tract adjustments caused by a therapeutic method *phonation into a tube* (Laukkanen *et al.*, 2012), the data did not allow visualizing the vocal fold shape clearly, whereas the CT method allowed displaying the vocal fold outlines in addition to the vocal tract shape [see the **Paper III**, which uses the same CT data as used for studying the vocal tract shape in the studies of Guzman *et al.* (2013) and Vampola *et al.* (2011)]. Therefore the CT method was used for the **Paper III** of this thesis, and the MRI method is not described here in detail.

1.3.2. Electroglottography (EGG)

Generally, there is an interest to obtain detailed information on VF behavior under various conditions of phonation. The most direct way is viewing the VFs using rigid or flexible laryngoscope. Methods as high-speed laryngeal videoendoscopy (Rubin and LeCover, 1960; Moore *et al.*, 1962; Hertegard, 2005; Deliyski *et al.*, 2008), videostroboscopy (Bless *et al.*, 1987), or videokymography (Svec and Schutte, 1996; Svec *et al.*, 2007; Svec and Sram, 2011) can be utilized. However, the laryngeal endoscopy has an impact on examined person`s habitual comfort and it provides only a superior view of the VFs *in vivo*. Therefore, other methods were developed (Fabre, 1957; Holmer *et al.*, 1973; Rothenberg, 1973; Sondhi, 1975; Rothenberg, 1977) to record a signal representing an oscillation cycle of the VFs.

A non-invasive method electroglottography (EGG) was introduced by Fabre (1957) who was the first to design an electroglottographic device. This device has also been called by some scientists “laryngograph”. EGG devices have been manufactured in various countries (e.g., USA, Sweden, Denmark) and the term “electroglottograph” (**Figure 17**) has been generally used.

Figure 17. Front panel of EGG device (a) and electrodes attached to the neck (b).

The EGG is based on variable electrical impedance of the neck tissues during the VF oscillation. As soon as VFs are separated the impedance increases due to the decreased contact between them. Depending on the particular instrument, the electrical signal generated by the oscillator may have a frequency ranging from hundreds of kHz to a few MHz and the current is limited to 10 mA or less (Baken, 1992). This typically corresponds to a voltage across the neck of about 0.5 V (Baken, 1992) which is not perceived by the examined subject.

In order to pass the electrical current through the neck tissues, two electrodes are placed laterally on sides of thyroid cartilage. Unfortunately, there are many paths for the current to flow through the neck and only a small portion goes through the VFs while the glottis is being closed. This results in quite low amplitude high frequency changes of the current. In addition, there are low frequency changes originating from other events such as movements of the larynx, muscle contractions, and changes of blood flow through arteries and veins (Orlikoff, 1998). Therefore, the EGG device contains circuits for high-pass filtering of the original signal and amplification of the dynamic changes caused by glottal opening and closing.

1.3.2.1. Interpretation of the EGG signal

Since the EGG signal waveform is quite simple in comparison to the acoustic signal, it has been successfully used for a quick extraction of fundamental frequency (Chevrie-Muller *et al.*, 1983; Baken and Orlikoff, 1987; 1988) but its true physiological correlate has been unknown and investigated for decades. First hypotheses assumed that the EGG signal correlated to glottal width or area [Chevrie-Muller and Grémy (1962); Grémy and Guérin (1963) cited in Baken (1992)] or that the variations of EGG signal were caused by compression of perilaryngeal tissue due to resonances in the vocal tract. However, this was disproved by Gilbert *et al.* (1984) who performed an experiment with insertion of a high-

resistance slit between the VFs. The EGG signal amplitude considerably increased after the slit was removed indicating that the EGG signal could rather be related to VF contact area (VFCA).

In order to validate the EGG waveform features and its relation to vibratory characteristics of VFs a number of comparative studies has been conducted using, stroboscopic photography (Fourcin, 1974; Lecluse *et al.*, 1975; Pedersen, 1977), photoglottography (Kitzing, 1977; Dejonckere, 1981; Kitzing, 1982; Baer *et al.*, 1983a; Baer *et al.*, 1983b; Kitzing, 1983; Titze *et al.*, 1984; Berke *et al.*, 1987), inverse filtering (Fourcin, 1981; Rothenberg, 1981; Childers *et al.*, 1983b; Rothenberg and Mahshie, 1988), videostroboscopy (Anastaplo and Karnell, 1988; Karnell, 1989), high-speed cinematography (Baer *et al.*, 1983a; Childers *et al.*, 1983b; Childers *et al.*, 1984; Childers and Krishnamurthy, 1985; Childers *et al.*, 1990), and measurements of subglottal pressure (Kitzing, 1982; Gilbert *et al.*, 1984). For an extensive review of these studies, see Childers and Krishnamurthy (1985). These studies indirectly supported the assumption that the EGG signal was strongly related to VFCA. The expected relationship between VF vibrational pattern and the EGG signal as a measure of VFCA is depicted in **Figure 18** as proposed by Orlikoff (1998).

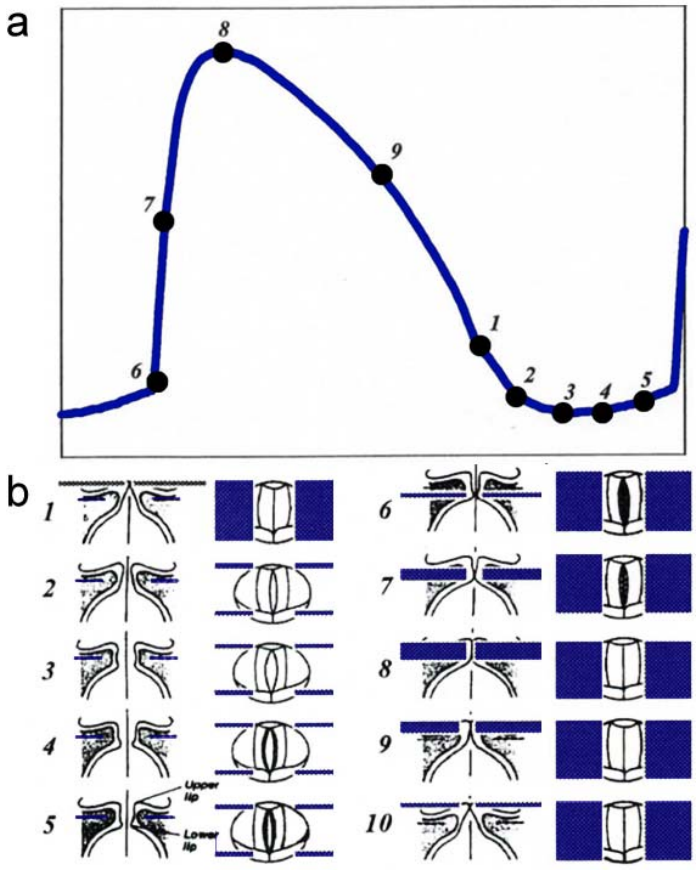


Figure 18. Schematic representation of the relationship between typical EGG signal waveform (a) and the corresponding phases of (VFs) during vibration (b) viewed coronally (left) and superiorly (right) as proposed by (Orlikoff, 1998). The blue panels represent vertical and horizontal conductive pathway dimensions throughout the VFs. The numbers of the pictograms in (a) correspond to the numbers denoted in the EGG curve (b): 1 and 10 – VFs just before the opening, 2 to 3 – glottis is open, 4 – 5 approximation of the lower VF lips, 6 – initial contact of lower VF lips, 7 – increasing contact between VFs, 8 – maximum VF contact, 9 - lower VF lips start to separate.

It is important to note that EGG curve is only a relative signal and that it does not reveal whether there is a complete closure or full opening of the glottis at its maximal or minimal levels. Also, it converts overall 3D vibrational pattern into 2D information, making the exact interpretation of the EGG signal rather complex as shown in **Figure 18**.

Interestingly, there has been only a single formal study directly investigating the relationship between EGG and VFCA (Scherer *et al.*, 1988). The authors conducted an experiment using two canine larynges. The scheme of the setup used in Scherer *et al.* (1988) is depicted in **Figure 19.**

The VF was driven against a glass plate covered with conductive layer with a mechanical arm covered with plastic. The VF movement was not flow-induced but mechanically pressed against the glass plate, which resulted in a sinusoidal movement at frequencies around 10 – 30 Hz. During the experiment the larynx specimens were placed in horizontal position and the VFCA was assessed using videostroboscopy which provided illusory slowed-down vibrations. Since the vibrations were not self-sustained and the VFCA was not visualized at high-speed frame rates, there has been a need to verify the EGG signal as a measure of relative VFCA using more advanced imaging techniques under more physiologic conditions. Such a study is described in (Hampala *et al.*, 2015a) which is **Paper II** of this thesis.

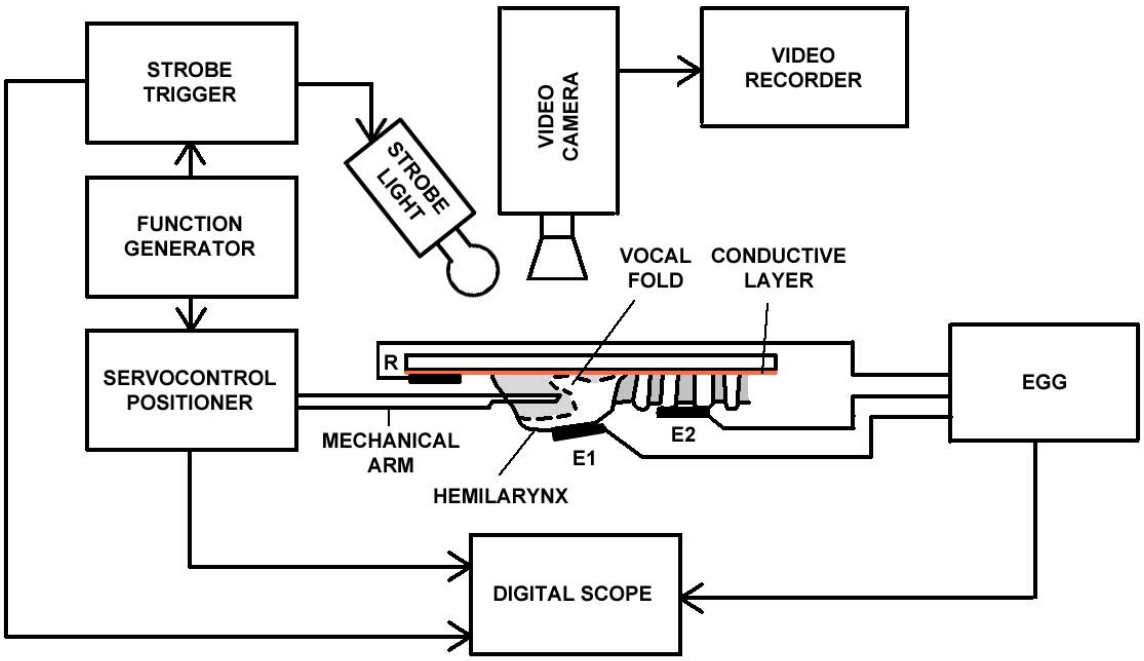


Figure 19. Scheme of the setup used in Scherer et al. (1988). The function generator provided the basic sinusoidal signal for a custom controller (servocontrol positioner) of the mechanical arm position and for the triggering of stroboscopic light. The EGG device used three electrodes (SynchroVoice) – two (E1 and E2) positioned on the hemilarynx and one on the conductive layer (R) of the glass electrode. The output signals from the egg, arm position controller and signal used for triggering the stroboscopic light were collected with a digital analyzer and stored to disk. The VFCA was recorded using a standard video camera and a cassette video recorder.

1.3.2.2. The EGG contact quotient

The EGG signal is frequently analyzed using the so called *contact quotient (CQ_{EGG})* which represents glottal contact phase relative to whole VF oscillation cycle period, i.e.:

$$\mathbf{CQ} = \frac{\mathbf{VF\ contact\ duration}}{\mathbf{glottal\ cycle\ period}} \quad \textit{[Equation 7]}$$

The concept of using CQ_{EGG} has its limitation. The problematic issue is an identification of the glottal contact within the glottal cycle. In most cases the EGG waveform does not provide a clear break point (particularly when the glottis is opening). Therefore, several methods for estimation of the glottal contact phase using CQ_{EGG} were developed.

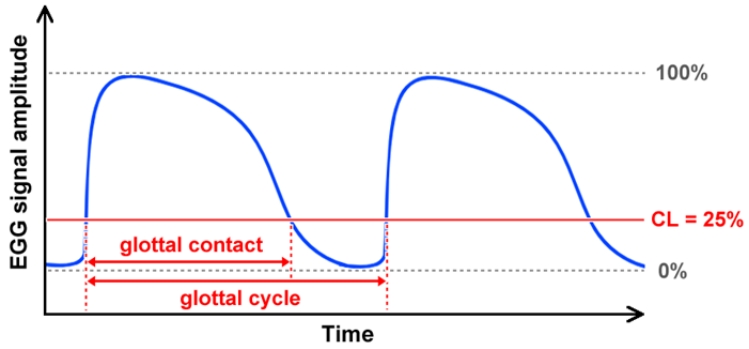


Figure 20. EGG waveform and glottal contact determined by criterion level (CL).

Rothenberg and Mahshie (1988) proposed a method using a *criterion level (CL,* **Figure 20***)* to estimate the CQ_{EGG}. The peak-to-peak amplitude is calculated for each cycle. The criterion level is usually determined as a percentage of the peak-to-peak amplitude. As soon as the signal exceeds certain threshold (i.e. CL) it is considered to be a point of the contact. The criterion levels between 20% and 50% have been used in various studies (Herbst, 2011). Alternatively, the first derivative of EGG signal (dEGG) was used by Childers *et al.* (1983a); Childers and Krishnamurthy (1985); (Childers *et al.*, 1986); Childers *et al.* (1990) and others in order to investigate VF physiological correlates of the EGG signal and to estimate the CQ_{EGG}. Henrich *et al.* (2004) described a sophisticated method called DECOM for estimating the CQ_{EGG} based on dEGG. The dEGG signal has recently also been explored for visualizing dynamic changes within the EGG signal by creating the so called *wavegrams* (Herbst *et al.*, 2010a). In addition, a hybrid method for calculation of CQ_{EGG} was proposed (Howard *et al.*, 1990; Howard, 1995). In this method the contacting event is determined by the strongest positive DEGG peak whilst 42% CL is used for the decontacting analysis. Despite of the fact that CQ_{EGG} has been used broadly in recent voice research (Herbst *et al.*, 2010b; Andrade, 2012; Awan and Awan, 2013; Enflo, 2013; Guzman *et al.*, 2013; Kankare *et al.*, 2013; Li *et al.*, 2013; Master *et al.*, 2013; Andrade *et al.*, 2014; Bier *et al.*, 2014; Granqvist *et al.*, 2014; Herbst *et al.*, 2014) it was shown that CQ_{EGG} is highly dependent on choice of algorithm (Kania *et al.*, 2004; Herbst and Ternström, 2006; Echternach *et al.*, 2010; Mecke *et al.*, 2012; Lã and Sundberg, 2015) and therefore it should be used cautiously.

1.3.3. Phonation into a tube

Phonations into resonance tubes and other semi-occluded vocal tract exercises are therapeutic methods used by speech-language pathologists for vocal training or rehabilitation purposes (Laukkanen, 1995). The name "resonance tube" comes from feeling vibrational sensation (i.e. "resonance") in the face and lips while the subject is phonating (Laukkanen and Titze, 2007). Phonations into resonance tubes or straws can positively influence VF vibrations and the subject can practice e.g. high frequency voice with high lung pressures without risking injury of VFs (Titze, 2002a; b). The method is simple and user friendly. One end of the resonance tube is inserted between the patient`s lips while the opposite one can be inserted into water or left pointing into free airspace.

The phonation into resonance tubes started to be used in early 20th century [Spiess (1904) cited in Bele (2005)] and was originally found to be useful in treatment of hypernasality (Habermann, 1980; Bele, 2005). The phonation into tubes became popular in voice therapy and training especially in Finland (Simberg and Laine, 2007; Laukkanen *et al.*, 2008). It has also been used for vocal care in heavy voice users because the voice is perceived to be louder and easier to produce after such an exercise (Laukkanen *et al.*, 2008). The resonance tube is considered to be an extension of the vocal tract. It was found that the interaction between the voice source and the vocal tract can change the vibration properties of the VFs due to the changes in the supraglottal tract (Titze, 2008; Titze *et al.*, 2008). Since the vocal tract is artificially elongated during the phonation into a tube, the first formant F_1 drops (Story *et al.*, 2000) which increases the inertance of the vocal tract resulting in increased source-filter interaction (Titze, 2006b).

The resonance tube creates a narrow constriction at the lips. This reduced diameter increases a supraglottal pressure during the phonation resulting in decreased transglottal pressure, i.e. difference between the subglottal pressure and supraglottal pressure (Laukkanen, 1995; Bele, 2005). The sufficiently low transglottal pressure is thought to preserve the VFs from powerful collisions and allows the phonation to be more economical in terms of achieving the maximal outcome with minimal effort (Bele, 2005; Titze, 2006b). A recent study of Enflo *et al.* (2013) reported an increase of the collision threshold pressure (i.e. the minimum subglottal pressure for which the vocal folds collide during phonation) after the phonation into a tube.

Recent study of Vampola *et al.* (2011) used computed tomography to show the changes in vocal tract shape after the exercise and reported transversally enlarged cross-sectional areas of the oropharyngeal and oral cavities, and tightened velo-pharyngeal closure during and after the resonance tube exercise. Finite element modelling in the same study confirmed increased input reactance of the vocal tract and acoustic output. These findings are in line with Laukkanen *et al.* (1995) and Guzman *et al.* (2013) who also measured acoustic output and vocal tract dimensions.

While the above mentioned studies showed changes in vocal tract shape caused by the resonance tube exercise, so far it has not been clear whether this exercise stimulates also changes in the configuration of the VFs. There are some indications that an increased effort (needed to overcome the resistance introduced by the narrow tube) might result in an increased activity of thyroarytenoid (TA) muscle (Laukkanen *et al.*, 2008). According to Hirano (1975) and Yumoto *et al.* (1995) the increased activity of TA muscle should be

projected in VF bulging and thickening. In addition, Chhetri *et al.* (2012) found that increased TA muscle activity also helped to close mid-membranous glottis. However, indirect studies using electroglottography (Laukkanen, 1992; Guzman *et al.*, 2013) report inconsistent results not allowing making any conclusive statement.

The changes in vocal fold configuration as a result of the phonation into a tube are investigated in **Paper III** of this thesis (Hampala *et al.*, 2015b).

1.3.4. Excised larynx experiments

1.3.4.1. History and overview

Excised larynges have been helpful in a very broad spectrum of fields within voice research. One of the first documented attempts to investigate general principles of voice production using excised larynges was performed in 18th century by Antoine Ferrein [Műller (1837) cited in Cooper (1986)]. First he tried to blow into a trachea of a cadaver with no apparent acoustical output. Then, he used a canine (dog) larynx with approximated VFs which resulted in a sound production. His further research showed that the fundamental frequency of phonation was not primarily influenced by changing the airflow rate but by changing the VF tension. Subsequent experiments were performed by Johannes Műller, a German physiologist. He reported his findings about the relationship of subglottal pressure and VF tension [Műller (1839) cited in Cooper (1986)]. In his experiments he used the so called "compressorium" which was a set of pulleys used for medialization of VFs and rotating the thyroid cartilage around cricothyroid joint to simulate the activity of CT muscle.

. The formulation of the classic *Myo-elastic aerodynamic theory of phonation* (Van den Berg, 1958) was also supported by studies of excised larynges (Van den Berg and Tan, 1959; Van den Berg, 1962). The theory combined fluid mechanics of the airflow interacting with the mass of the VFs whose tension was controlled by muscular activity.

The role of mucosa was found to be important for VF oscillation. Fournié (1866) [cited in Cooper (1986)] removed superficial VF layer and found that it was no longer possible to establish the phonation.

In order to find out how VFs vibrate various techniques have been used in combination with excised larynges. There were attempts to observe VF vibrations with a light beam projected through the glottis and recording it on a sensitive moving film tape [(Weiss, 1914) cited in Cooper (1986)] or a method using capacitive displacement transducer for recording of vertical VF movement [(Trendelenburg and Wullstein, 1935) cited in Cooper (1986)]. A stroboscopic imaging method was introduced to excised larynx experiments by Harless (1853) and then applied by Réthi (1896) and later by Baer (1975) to discover vibrational patterns of VF surface, i.e. downward and upward movement of the VF edges. Fukuda *et al.* (1983) adopted the idea of using lead particles from Baer (1975) and injected them into the VF mucosa (*in vivo*) with and without the muscle stimulation using X-ray stroboscopy. The trajectories of the particles enlarged under the stimulation of recurrent nerve. Both Baer (1975) and Fukuda *et al.* (1983) suggested that in high-pitched voices (i.e. falsetto) only the

superficial VF layers were involved. Also studies of Yumoto *et al.* (1993b); Kusuyama *et al.* (2001); Jiang *et al.* (2008); Zhang *et al.* (2009) investigated VF vibration dynamics. Under certain conditions nonlinear phenomena (irregular vibrations, amplitude and frequency modulation, subharmonics, chaos etc.) can occur. Berry *et al.* (1996); Švec *et al.* (1999); Jiang *et al.* (2003); Zhang *et al.* (2007); Tokuda *et al.* (2008) studied phonation instabilities thoroughly, particularly in phonation onset, offset, or during transitions between different vibrational modes. The excised larynges have also been used for validating the signals obtained from electroglottography [Lecluse *et al.* (1975); Lecluse (1977); see Chapter 1.3.2] which is based on dynamic changes of laryngeal impedance.

Nowadays, technologies such as high speed video (HSV) cameras and particle image velocimetry (PIV) cameras allow more detailed investigations of the voice production principles such as advanced analysis of vortex creation during each oscillation cycle (Murugappan *et al.*, 2009). The collected data from excised larynx experiments are helpful in computational models which provide a simplified version of VFs and allow simulating changes of parameters which are difficult or impossible to control in laboratory approach.

1.3.4.2. Experimental specimens

Animal larynges have been used as physiological models for over hundred years. Each animal species has its specific features, i.e. dimensions and shapes of cartilages, VFs (true VFs), ventricular folds (false VFs) and their tissue composition and structure. Canine larynx has been the most commonly used specimen due to its size, gross structure, ease of manipulation and similarity with VF vibrations observed in humans (Garrett *et al.*, 2000; Regner *et al.*, 2010). Alipour *et al.* (2011) compared VF elasticity properties of pig, sheep and cow larynges and found that pig VFs had highly non-linear stress-strain relationship similarly as the human ones. In later study Alipour *et al.* (2013) concluded that the pig VFs oscillation behavior and wide frequency range were similar to human larynges. However, canine larynges are being used more frequently [e.g. Herbst *et al.* (2014)] since practical experience shows that pig larynx contains two pairs of VFs (true and false) where both of them seem to participate in phonation. In humans, the structures corresponding to false VFs in pigs are called ventricular folds and do not usually participate in phonation; therefore, suitability of pig larynges for experiments related to human phonation is questionable. The pig larynges are more accessible than canine larynges, however, since they can be easily collected from slaughter houses. There, however, the pig cadavers are commonly put into a bath with water heated to approximately 70°C for cleaning purposes. This may constitute a problem since VF mucosa is likely to be damaged during the cleaning procedure. In this sense a wild boar larynx may be considered as a proper replacement for domestic pig larynx, because no potentially damaging procedures are needed before the collection of larynx specimens. Not only dog, pig, sheep and cow larynges have been investigated. Hertegård *et al.* (2009) studied phonation in models of rabbits with phonotrauma and Maytag *et al.* (2013) designed a setup for rabbit excised larynges. Despite its physiological differences (Hertegård *et al.*, 2003) the rabbit larynx appeared to be useful in *in vivo*/*ex vivo* and histologic studies of VFs, mainly for studies investigating biological processes during treatment and healing (Flint *et al.*, 1997; Dufresne and Lafreniere, 2000; Thibeault *et al.*, 2002). Lately, the deer larynx was

found to be suitable for certain kinds of experiments (Herbst, 2014) due to a very stable phonation and clearly defined VF shape. This makes the experiment easier if one is interested e.g. in tracing the VF edges or when long-term stable phonations are needed. For these reasons the red deer larynges were chosen for the experiment in Chapter 2.3.

The voice research is generally not only focused on mammals. In the last decade, the sound production in birds has also been successfully studied using excised syringes, which fulfill similar function as the larynx in mammals (Elemans *et al.*, 2004; Elemans *et al.*, 2006; Elemans *et al.*, 2008).

1.3.4.3. Adduction of vocal folds (VFs) in excised larynges

A practical way how to reach the VF adduction in excised larynges is using prongs as described in Titze (2006a) (**Figure 21**). The prongs are equipped with needles (**Figure 21**). The insertion of the needles allows for direct positioning of arytenoid cartilages as illustrated in Titze (2006a) (**Figure 21**). Each needle is attached to a holder with a screw enabling the needle to be moved in or out according to desired position.

The same or similar prongs to those described by (Titze, 2006a) have been often utilized in studies with excised larynges [e.g Alipour *et al.* (1997); Alipour and Jaiswal (2009); Finnegan and Alipour (2009); Herbst *et al.* (2014) and others] and are also used in the **Paper II** of this thesis (Hampala *et al.*, 2015a).

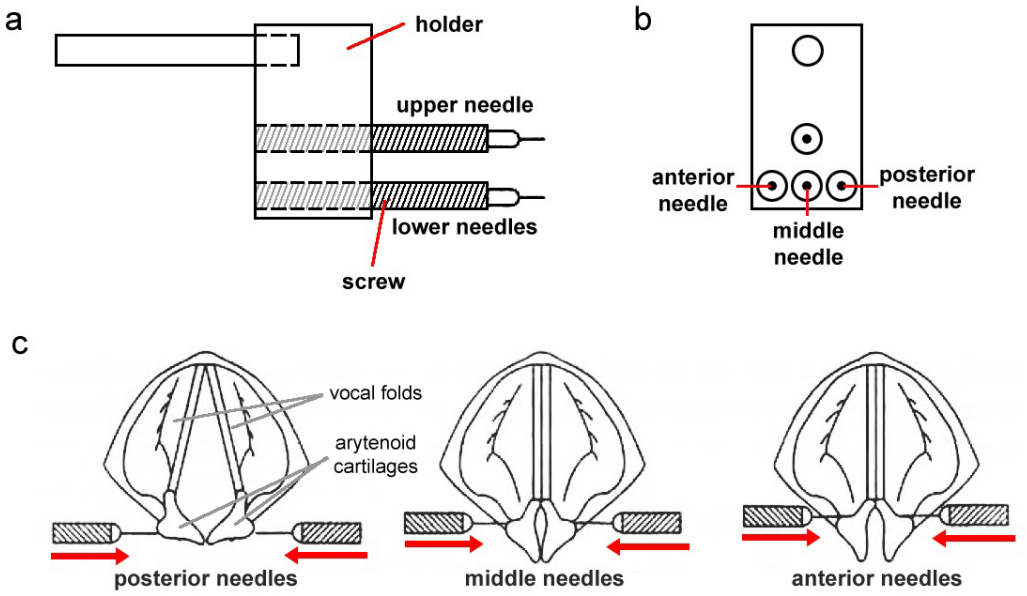


Figure 21. Schema of prongs as described in Titze (2006a) used for VF adduction: a – side view; b – front view; c – positioning of arytenoid cartilages.

1.3.4.4. Hemilaryngeal setup

The excised larynx experiments provide a good insight into voice production mechanisms. However, the use of full larynx has its limitations. The superior and inferior views do not provide much information about what is happening on the medial surface because it is not visible especially when VFs are closed. Thus, it is difficult to quantify, e.g., the medial mucosal wave propagation which is directly related to glottal airflow dynamics or the collision forces between the VFs (in case the contact occurs). To gain knowledge about these phenomena a hemilarynx setup can be used as it allows an experimenter to observe the VF from superior, inferior and medial view during the oscillation.

The early version of hemilaryngeal setup was developed by Hiroto (1968). Later, Jiang and Titze (1993) validated the use of hemilaryngeal setup and f found that vibrations of single VF in hemilarynx had similar amplitude and frequency of VF vibration (as a function of subglottal pressure) to those of the full larynx. Also, the subglottal pressure at which the phonation started, i.e. the phonation threshold pressure (PTP), and the range of subglottal pressures over which the larynges phonated, were similar to full larynx experiments. The average airflow in hemilaryngeal setup was approximately half that of the full larynx and the sound pressure level (SPL) was about 6 dB less, under similar conditions. The difference in airflow and SPL appears to be logical since the second VF was not present. These results validated the use of the hemilarynx setup as an analogue for full larynx preparation. Later, Berry *et al.* (2001) conducted hemilaryngeal experiments in order to reveal spatio-temporal eigenmodes of self-sustained oscillation with micro-sutures placed on the medial VF surface using high-speed video camera. Eigenmodes of VF vibrations in human hemilarynges were further analyzed by Döllinger *et al.* (2005) and Döllinger and Berry (2006b). Together with the computational study of Döllinger and Berry (2006a) they analyzed two essential eigenmodes of which the largest one covered the alternating convergent/divergent shape of the medial surface, while the second largest represented the lateral vibrations of the VF.

2. Original work by the author

2.1. Motivation for the work of the author

Despite the fact the voice production has been studied for centuries, some aspects of the voice production mechanisms have remained under-researched. Studies utilizing excised larynx experiments have strived to explain, e.g. the influence of various factors on the characteristics of the vocal fold vibrations. Often, however, these studies did not provide any information on subglottal acoustic properties. Nevertheless, the subglottal resonances potentially could affect the gathered results since it has been found that supra- and subglottal acoustic pressures interact with VF vibrations (Titze and Story, 1997; Titze, 2008). In addition, another aspect has mostly been neglected in excised larynx experiments - the subglottal systems and the corresponding resonances in various species differ. Therefore, there has been a need for a setup which would allow setting the subglottal resonances so that they correspond to the acoustic properties of examined species *in vivo*. This problem has been addressed in

Paper I: V. Hampala, J. Švec, P. Schovánek, D. Mandát (2013). Užitný vzor č. 25585: Model subglotického traktu. Soukup, P. 2013-27834(CZ 25505 U1), 1-7. 24-6-2013. Praha, Úřad průmyslového vlastnictví. / Utility model no. 25585: Model of subglottal tract. Soukup, P. 2013-27834(CZ 25505 U1), 1-7. 24-6-2013. Prague, Industrial property office.

The paper presents a technical design of a new subglottal tract which allows smooth adjustment of the length of the subglottal resonator and thus provides the means to smoothly change the resonance frequencies of the subglottal tract. Such model can also be used to investigate behavior of excised larynges from different species and adjust the subglottal resonance frequencies accordingly.

In order to understand the voice production in living subjects, there has been a need to investigate the VF vibrations *in vivo*. The most direct imaging methods for humans observe the VF using laryngoscopy. These imaging methods, however, are limited to observing mainly the superior surface of the VFs. The phenomena happening in the inferior and medial part of the VFs remain hidden in the laryngoscopic view, especially during the phase when the VFs are closed. Furthermore, the laryngoscopic methods are practiced under medical supervision as they require inserting endoscopes into the vocal tract potentially causing discomfort to the examined subject. In contrast to these, the method of electroglottography (EGG) is noninvasive and can be used to monitor the vocal fold vibrations without any discomfort also in non-medical setup. Therefore, electroglottography (see Chapter 1.3.2.) has frequently been used in research studies, although the exact interpretation of the EGG signal has been rather complex. Previous research has strongly indicated that the EGG signal during phonation is directly proportional to the vocal fold contact area, VFCA, which is varying as the vocal folds vibrate during phonation. Nevertheless, such a relationship between the EGG signal and VFCA has not yet been directly

investigated under proper physiological conditions Therefore, **Paper II** of this thesis is devoted to investigating the relationship between the EGG signal and VFCA on excised hemilarynges using a sophisticated setup with two high-speed cameras:

Paper II: V. Hampala, M. Garcia, J.G. Švec, R.C. Scherer, C.T. Herbst (2015). "Relationship Between the Electroglottographic Signal and Vocal Fold Contact Area." Journal of Voice (in press).

The experiments for this study were conducted during the author`s stay at Bioacoustics Laboratory, Dept. of cognitive biology (University of Vienna, Austria) and the setup contained the subglottal tract described in **Paper I**.

Vocal fold vibrations play a crucial role for voice production and changes in their shape and adduction cause changes in voice quality [i.e.Hirano (1974); Herbst (2011)]. These changes can be assumed to play important role also for voice exercises used during voice therapy. One of the currently used therapeutic methods is the *phonation into a tube*. Previous studies discovered that this exercise causes changes in the shape and resonance properties of the vocal tract (Vampola *et al.*, 2011; Guzman *et al.*, 2013). However, it has not been clear whether this exercise stimulates also changes in the adjustment of the vocal folds. **Paper III** therefore devotes attention to studying the effect of the phonation into a tube on the changes in vocal fold configuration:

Paper III: V. Hampala, A.-M. Laukkanen, M.A. Guzman, J. Horáček, J.G. Švec (2015). "Vocal Fold Adjustment Caused by Phonation Into a Tube: A Double-Case Study Using Computed Tomography." Journal of Voice, doi: 10.1016/j.jvoice.2014.10.022.

The paper explores CT data originally obtained during previous studies aiming at monitoring the changes in the vocal tract configuration caused by the phonation into a tube (Vampola *et al.*, 2011; Guzman *et al.*, 2013). The CT images included also the region of the vocal folds and therefore this study took advantage of these unique CT data to study also the changes in the focal fold configuration caused by the phonation into a tube.

2.2. Paper I - Subglottal tract with adjustable length - utility model (Hampala *et al.*, 2013)

Previous research has shown that there is an interaction between the voice source and resonance cavities, (Titze and Story, 1997; Titze, 2008), i.e. the voice source exhibits acoustic coupling with supra- and subglottal tract. Many previous experiments with excised larynges investigated non-linear phenomena of phonation including phonation onset and offset, register changes, frequency jumps, sub-harmonics, chaos etc. (Berry *et al.*, 1996; Švec *et al.*, 1999); Jiang *et al.* (2003); (Zhang *et al.*, 2007; Tokuda *et al.*, 2008). These phenomena could be influenced and sometimes even caused by the acoustic coupling between the voice source and resonators.

Some authors proposed a relationship between subglottal resonances and vocal registers (Van den Berg, 1968a; Large, 1972; Austin and Titze, 1997) and it has been found that the subglottal resonances can considerably influence the VF vibrations (Zhang *et al.*, 2006). Most of the previous studies [e.g. Van den Berg and Tan (1959); Van den Berg (1968b); Berry *et al.* (1996); Švec *et al.* (1999); Jiang *et al.* (2003)], however, did not specify acoustic properties of the subglottal system (i.e. measured resonances or the length of a subglottal tube for their estimation). In order to minimize the effect of subglottal resonance upon VF vibration Alipour and Scherer (2001) and Thomson *et al.* (2005) used a tube long enough to lower the first subglottal resonance below the expected fundamental frequency. Nevertheless, the higher resonances could have potentially influenced these experiments.

Zhang *et al.* (2006) used segments of tubes in order to change the acoustic properties of the subglottal space. However, the segments are impractical for everyday laboratory practice. Therefore, there has been a need for a device allowing easy setting of the subglottal resonances according to particular demands.

The author of this thesis designed a new subglottal tract inspired by an unpublished prototype of an excised larynx setup used by Tjouwke van Kalkeren in cooperation with prof. H. Mahieu a prof. H.K.Schutte in the Netherlands during the years 2004 - 2006. The subglottal tract utilizes a sliding piston. **Figure 22** and **Figure 23** show the components of the subglottal tract **Figure 24** and illustrate its basic concept. The subglottal tract`s body (**Figure 22***,* 6) is formed by a plexi-glass tube going through a working desk. Two tightening nuts (**Figure 22**, 3) fix the tract and stabilize it. The piston consists of a body (7), head (4), and handle (**Figure 22**; 7, 4, 12). The head contains a groove for rubber sealing (**Figure 22***,* 5). The piston slides inside of the tube and can be locked in a desired position by a locking screw (**Figure 22**, 11) placed at the bottom (**Figure 22**, 10) of the subglottal tube. Air is supplied through a side pipe (**Figure 22**, 8) above the top position of the piston. On the top of the subglottal tube there is a metal adapter (**Figure 22**, 1) which can be inserted into a trachea in order to attach the artificial subglottal tract to an excised larynx. In order to attach larynges of different sizes, various diameters of the metal adapters can be used. In order to keep the resonance properties as clear and simple as possible, the adapter and the subglottal tube are connected by an insert smoothly reducing its inner diameter (**Figure 22**, 2). The insert is specific for each adapter in order to reduce creation of additional resonances caused by a sudden change of the inner diameter. Finally, condensed water can be drained out through a hole at the bottom of the subglottal tube (**Figure 22**, 9).

It is important to note that the air supply tubing also influences the overall acoustic properties of the system. Because each setup is different it is appropriate to measure the acoustic resonances before an experiment and, in case of undesired resonances of the system, use an acoustic terminator placed inside the piping (**Figure 22**, 8)

The described utility model improves the use of excised larynx setup by another known adjustable parameter, i.e., the length of the subglottal tract and consequently by the adjustable subglottal resonance frequencies.

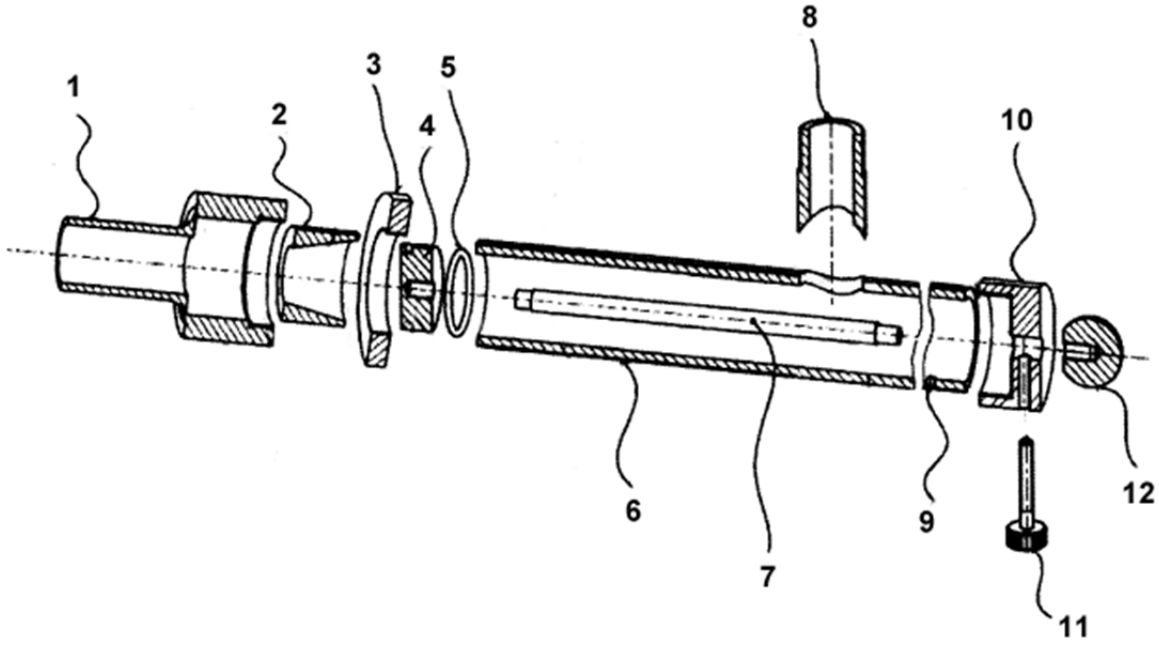


Figure 22. Subglottal tract model components: 1 – adapter mounting tube; 2 – adapter body; 3 – diameter reducing insert; 4 – tightening screw; 5 – piston head; 6 – sealing; 7 – tract body, 8 – piston body; 9 – air supply tube; 10 – bottom cover; 11 – draining hole; 12 – locking screw; 13 – piston handle.

Figure 23. Photo of the dismounted subglottal tract.

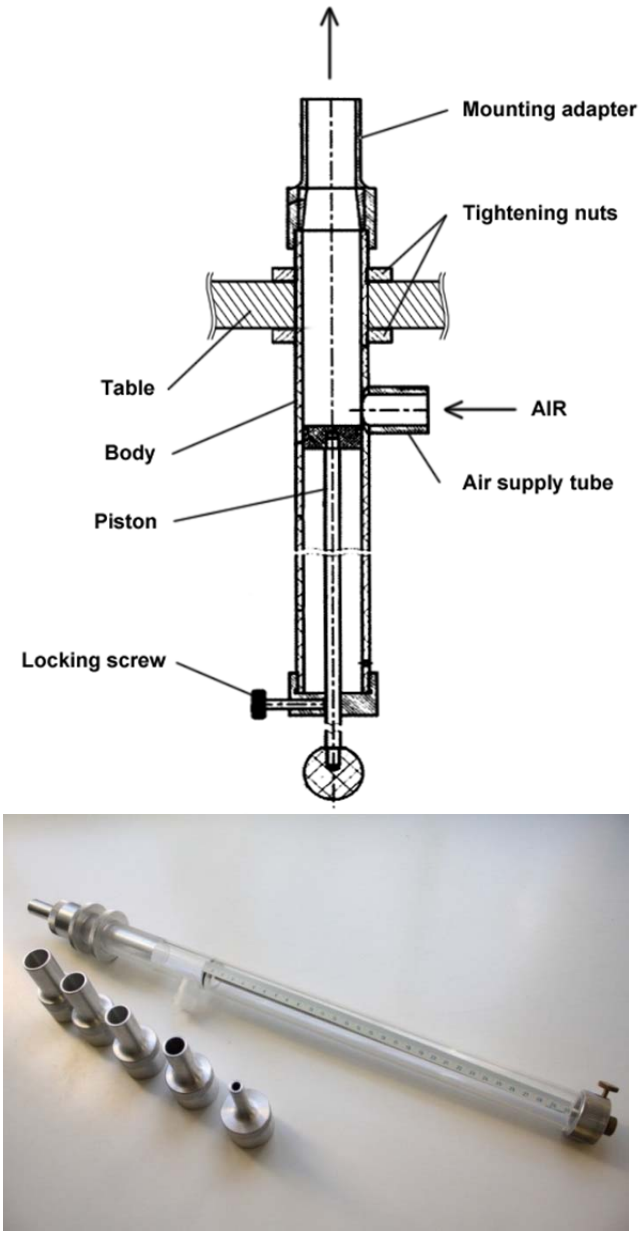


Figure 24. Model of the subglottal tract ready to use (top – mounting of the tract and its position in the table; bottom – the assembled tract and the adaptors for different tracheal diameters).

2.3. Paper II – Relationship between EGG signal and vocal fold contact area (Hampala *et al.*, 2015a)

The relationship between the landmarks in the EGG curve (see Chapter 1.3.2.) and phenomena occurring during voice production has been investigated since the electroglottographic device was developed by Fabre (1957). Although the electroglottography has become a frequently used method in voice science, one important issue has remained to be solved – there are indications that EGG signal is proportional to VFCA but the direct evidence has still been lacking. The **Paper II** has therefore been devoted to investigating the mutual relationship between the EGG signal and VFCA. A special hemilaryngeal setup was designed for this purpose.

2.3.1. Methods

Preparation of hemilaryngeal specimens

This chapter extends the information provided in the Paper II of this thesis. The preparation of a hemilarynx (**Figure 25** to **Figure 28**) encompasses removing one VF (**Figure 25** to **Figure 27**) in a way that one half of the thyroid cartilage, the arytenoid cartilage and a part of the cricoid cartilage are cut off. This cut makes an incision forming an L-shaped mortice (**Figure 28**). Although the preparation does not involve any special techniques, sometimes it may be difficult due to an advanced ossification of the thyroid or cricoid cartilage. Moreover, one has to be careful with cutting the thyroid cartilage particularly in its anterior bottom part - if the ligament connecting the cartilage and the thyroarytenoid muscle of the examined VF is damaged the larynx cannot be used anymore for studying the vocal fold vibrations. After the successful preparation is finished, the removed part forms an L-shaped mortice.

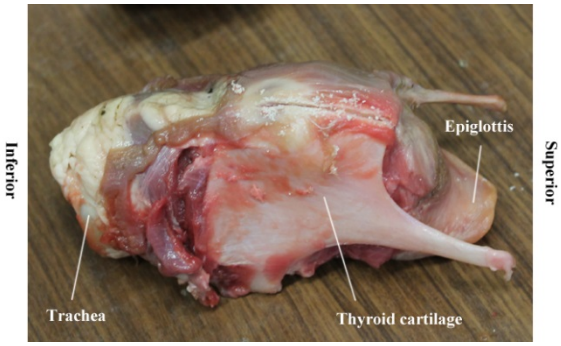


Figure 25. Red deer larynx with an initial cut in the anterior part of the thyroid cartilage.

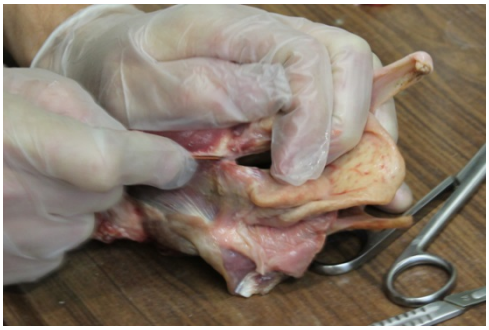


Figure 26. The cricoid cartilage of the red deer larynx being cut into halves in its posterior part

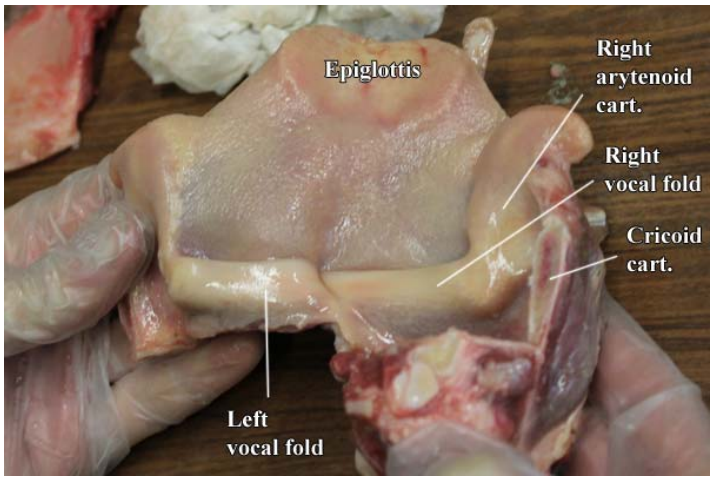


Figure 27. The red deer larynx before the removal of the left part.

Figure 28. The L-shaped mortice covered with Dental cement (red) for sticking the hemilarynx onto the glass plates.

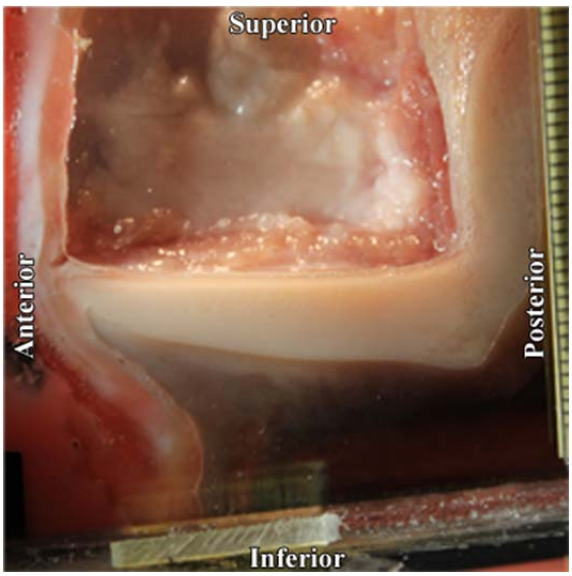


Figure 29. The resulting hemilaryngeal view with an adducted vocal fold.

Experimental setup

The experiments were conducted using three female red deer (*Cervus elaphus*) larynges. Each larynx was cleaned and the hyoid bone was removed together with the external laryngeal muscles. About 5 cm of trachea was left for mounting the larynx on the air supply tube with a circular clamp. The hemilarynges were prepared according to previous chapter. The L-shaped mortice formed a housing for an EGG glass electrode. This special EGG glass electrode consisted of two glass plates – one of them was covered with a conductive layer (**Figure 30**, denoted as cGP) in order to convey the VF contact, the other one was supportive (**Figure 30**, denoted as sGP). The larynx was glued onto the glass plates using dental cement (**Figure 30**).

The attachment of the larynx to the conductive glass electrode is depicted in **Figure 30**a-d. The larynx was mounted on the adapter at the top of the subglottal tract [described in Paper I of this thesis, Hampala *et al.* (2013)]. The schematic of the setup for obtaining the EGG signal is illustrated in **Figure 30**e. In addition, two high-speed video cameras were used to record the oscillatory movements of the VF from the top and from the side (**Figure 30**e). The adduction of VFs was performed via prongs as described in Chapter 1.3.4.3.

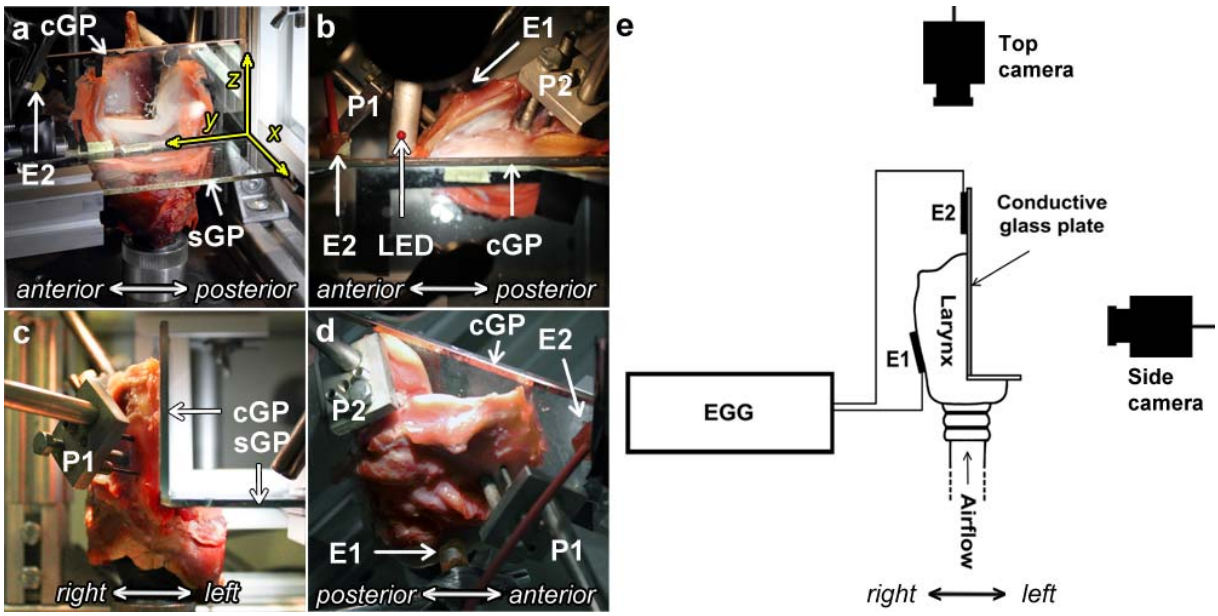


Figure 30. Hemilarynx setup with a conductive glass-plate and a system for collecting the EGG signal: a –view through the glass plate with x, y, z – axis orientation, b – top view, c – anterior view, d – anterolateral view (right side of the hemilarynx attached to the glass plate), e - scheme of the experimental hemilaryngeal setup. Legend: cGP – conductive glass plate; sGP – supportive glass plate; E1 – EGG electrode attached to muscles around the thyroid cartilage; E2 – EGG electrode attached to the glass plate; LED – led diode used for synchronization of cameras; P1 – prong stabilizing the thyroid cartilage; P2 – prong adducting VF.

The rest of the setup was identical to that described in the study of Herbst *et al.* (2012): the pressurized air tank was used as a source of the air which was humidified and warmed in a custom-made heating system and led to the larynx. The setup also included a flow sensor (flow head with a differential pressure transducer) and a pressure sensor (measuring average DC pressure). Subglottal pressure during flow induced oscillation was varied via computer-controlled pressure sweeps. ***Table 1*** contains a list of equipment used for the data acquisition.

The synchronization of all the involved devices was achieved using a TTL signal (rectangular pulses) generated by Labjack U6. The TTL signal was routed via IC555 electrical circuit to obtain rapid rise time for each TTL pulse. This TTL signal was then recorded by all the required devices (cameras and Dewe-43 data acquisition card) for post-synchronization purposes. The camera for side view had the TTL signal encoded in one pixel in the corner of the frame. Similar principle was used for the top view camera, except that a blinking LED (**Figure 30**b, labeled as LED) was placed into a camera view.

Table 1: Data acquisition – list of equipment used for recording the data for the analysis

Purpose	Device	Details
EGG signal collection	Electroglottograph EG2 -1000 (Glottal Enterprises; Syracuse, NY)	- high-pass filter set to 2 Hz - 11 mm electrodes with 100 Ohm resistor in series to avoid clipping (Glottal Enterprises, Syracuse, NY) - Electrode gel (Spectra 360; Parker Laboratories, Inc.; Fairfield, NJ)
Side view video	MotionBLITZ LTR 1 camera (Mikrotron, Unterschleissheim, Germany)	- 6000 FPS - resolution: 400x200 px
Top view video	MotionBLITZ Cube 7 camera (Mikrotron, Unterschleissheim, Germany)	- 6000 FPS - resolution: 400x200 px
Lighting	Cymo 7300.03 300W xenon continuous light source (Cymo, Groningen, The Netherlands)	
	standard flexible 1-bulb LED table lamp	
	5 W MR16 LED bulbs (SLV Elektronik GmbH, Übach-Palenberg, Germany)	- array of 12 bulbs
Subglottal pressure	Keller PR-41X pressure sensor (Winterthur, Switzerland)	- sensor positioned 32 cm upstream from the VFs
A/D conversion of EGG signal	Dewe-43 (Dewetron, Germany) data acquisition card	- 44100 Hz sampling frequency

Data analysis

The goal of the analysis was to trace the borderlines of the VF contact area (VFCA) on each consecutive frame within video recordings captured by the side camera. Since lighting conditions played an important role here (especially anterior-posterior difference in lighting quality) the outlines had to be traced manually instead of using automated image analysis. For each larynx (denoted as L1, L2, and L3), one sequence where the VF performed periodic vibration and its outlines were best visible was used for further analysis. The resulting sequences were chosen without prior knowledge on corresponding EGG signal.

The annotation of the VFCA contours on the side view was not always easy, since the focal fold edges were sometimes not clearly defined. Therefore, the corresponding recordings from the top and side camera views were extracted and merged to one video sequence. This allowed assessing the VF movement from both the views simultaneously. The

top camera view was extremely helpful for recognizing and clarifying the anterior-posterior (A-P) VF contact and its extension. These composed videos were then manually annotated by three experimenters using custom built scripts within an image processing software FIJI (Schindelin *et al.*, 2012). All the experimenters annotated the same three recordings containing one VF oscillation cycle.

The annotation process was performed in two iterations. After the first iteration all three experimenters discussed the obtained annotated VFCA contours in order to reach a common consensus for the approach in the second iteration. After the second iteration the disagreement between the experimenters was resolved by considering a pixel to be contacting the glass plate when at least two out of three experimenters included this particular pixel in their annotated area. The number obtained by counting all the pixels in which the VF was judged to be contacting the glass plate resulted in the overall VFCA measure ($VFCA_{OVR}$) expressed in pixels. The resulting $VFCA_{OVR}$ and its temporal relation to EGG signal is depicted in **Figure 31**.

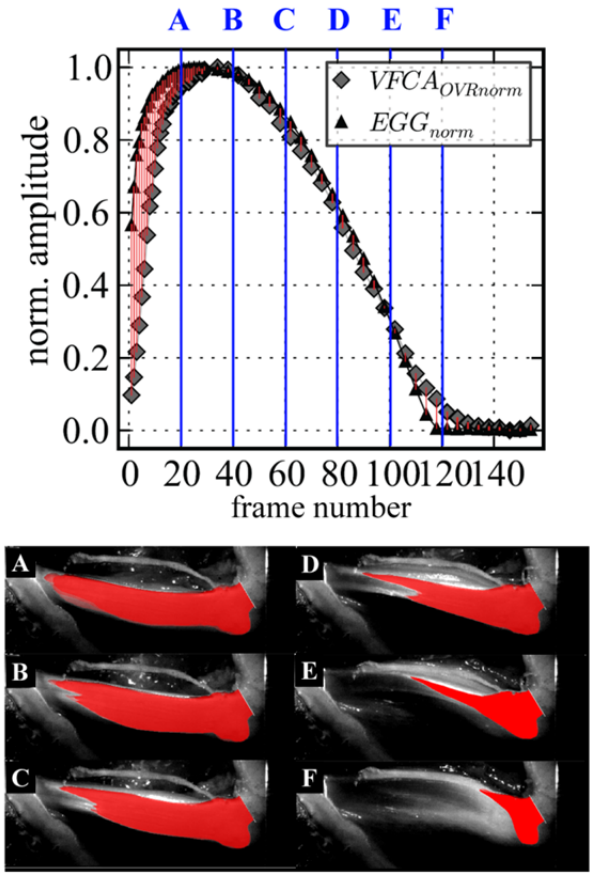


Figure 31. Development of the VFCA shape in larynx L3 during the analyzed oscillation cycle. Top: Normalized EGG signal (black triangles) and normalized $VFCA_{OVR}$ (gray diamonds); Bottom (A-F): high-speed video images (side-view camera) with superimposed $VFCA_{OVR}$ (red), corresponding to time instances marked by A-F lines in the top part of the figure.

2.3.2. Results

Figure 32A-C shows the comparison and relationship between the normalized EGG_{norm} signal and the normalized overall VFCA ($VFCA_{OVRnorm}$) variations during a single VF oscillatory cycle for the three larynges (L1, L2, and L3). The figure reveals that the EGG_{norm} waveform constantly precedes the measured $VFCA_{OVRnorm}$ during the contacting phase of VF oscillation cycle in all three larynges (**Figure 32**). The maximum difference in relative amplitude between EGG_{norm} and $VFCA_{OVRnorm}$ was 0.42, 0.40, and 0.54 in the contacting phase in the three larynges (L1, L2, and L3). The values corresponded to about half of the possible range [0, 1].

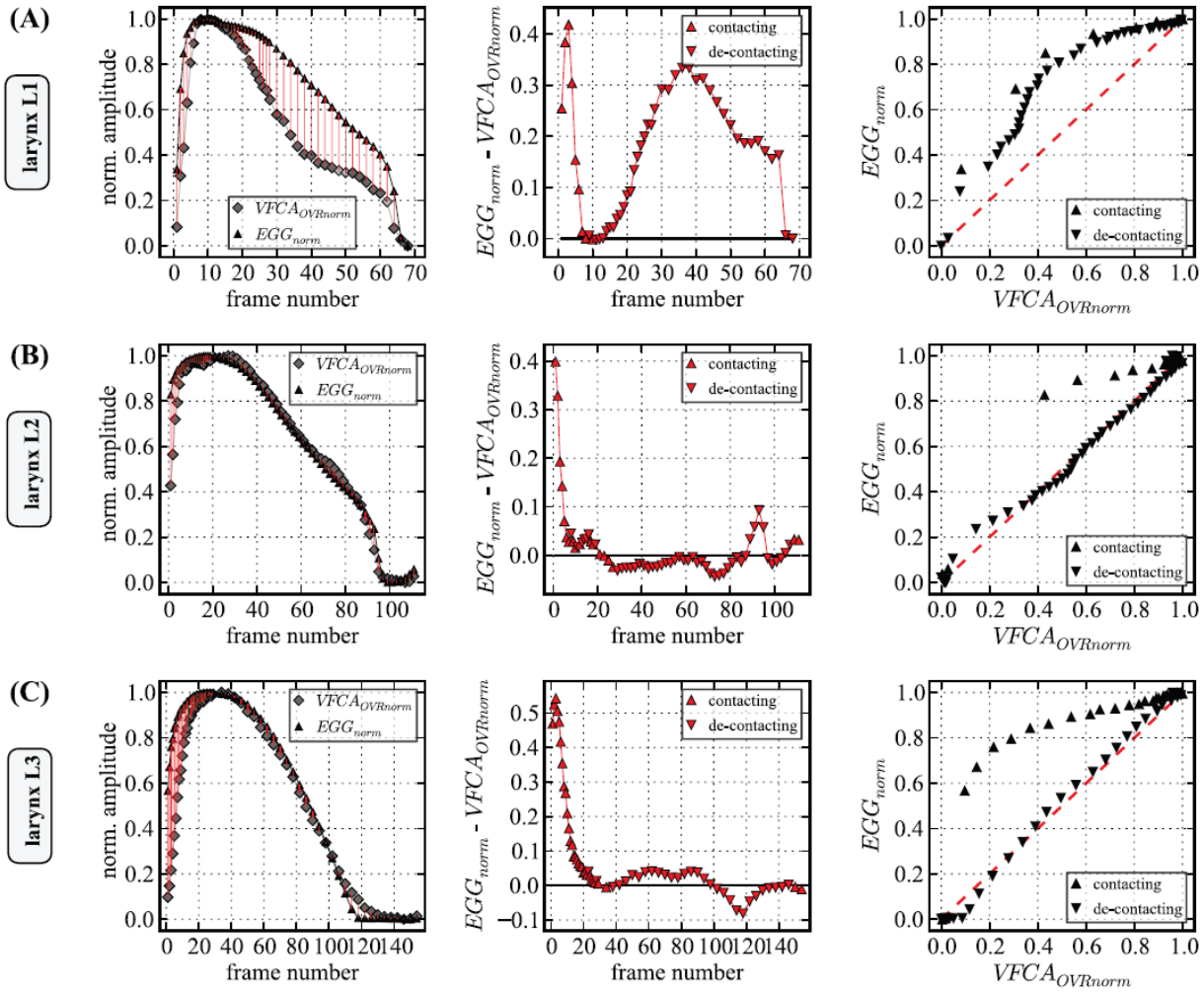


Figure 32. Comparison between the normalized EGG (EGG_{norm}) and VFCA ($VFCA_{OVRnorm}$) waveforms for the three larynges L1, L2, and L3 (A, B, and C). Left panels: EGG_{norm} (black triangles) and $VFCA_{OVRnorm}$ (gray diamonds); middle panels: difference between EGG_{norm} and $VFCA_{OVRnorm}$; right panels: relation between $VFCA_{OVRnorm}$ and EGG_{norm} waveform.

The decontacting phase in L2 and L3 showed very good agreement (differences mostly below 5% of the maximum normalized values; **Figure 32**, middle panel) between the $VFCA_{OVRnorm}$ and EGG_{norm} waveforms, whereas the data for L1 showed a noticeable disagreement (differences up to 33% of the maximum normalized amplitude) between $VFCA_{OVRnorm}$ and EGG_{norm}. This is projected in the relationsips between $VFCA_{OVRnorm}$ and EGG_{norm} (**Figure 32**, right panel). The decontacting phase showed approximately linear relationship (values around the dashed line) in larynges L2 and L3 but not in L1. The contacting phase in all the three larynges showed a deviation from the linear relationship between the $VFCA_{OVRnorm}$ and EGG_{norm} waveforms.

2.3.3. Discussion

The collected data suggested a linear relationship between the VFCA and EGG signals for the major portion of the oscillation cycle (i.e. decontacting phase) in two out of three examined larynges. This is in line with study of Scherer *et al.* (1988) who also found relatively linear relationship. On the other hand, there were some noteworthy disagreements between EGG and VFCA as discussed below:

1) Contacting phase – systematic disagreement in all three larynges

There was a consistent systematic offset between EGG and VFCA waveforms in all the three larynges (**Figure 32**). One could search for a potential source of error in the synchronization assessment of collected data which corresponded to ±1 frame at 6000 FPS. However, the time shift between EGG and VFCA was found to be approximately 5 frames for larynx L1 and L2 and about 10 frames for larynx L3. Such an error could therefore not be caused by the synchronization issues only. A potential explanation of these discrepancies could stem from signal processing procedures performed by the EGG device hardware circuitry.

2) Larynx L1 – overall disagreement

The origins of disagreement between the EGG signal and the VFCA in Larynx 1 were searched in various factors. One potential source of discrepancies could theoretically be electrode placement. One of the electrodes was positioned above the VF and more anteriorly in order not to obstruct the view of the side camera. This could likely influence the resulting EGG signal in a way that the superior part of the VF would contribute more to the overall signal since the current would be expected to flow mainly through the direct paths between the electrodes (Titze, 1990). Previous research confirmed that the change of electrode placement influences the EGG waveform (Colton and Conture, 1990; Baken, 1992; Tang *et al.*, 2015). However, the electrode placement was almost identical for all three larynges. This suggests that the systematic difference between VFCA and EGG found also in L2 and L3 could potentially be introduced by the electrode placement

whereas the difference in the decontacting phase in the larynx L1 had probably a different cause.

Another possible explanation of the disagreement could be searched in potential non-uniform tissue conductance properties of the VF. The tissue properties vary according to its type, i.e. muscle, bone etc., as showed by (Gabriel *et al.*, 1996) depending on their composition (Grimnes and Martinsen, 2015). Therefore, it might be possible that in case of a pathology (e.g. a lesion) where the tissue contains less water its admittance locally differs. The VFCA of the larynx L1 contained a visible groove indicating a sulcus-like structure aberration which might have been the cause of the found discrepancy. Moreover, according to Grimnes and Martinsen (2015) the tissue conductance properties are for the frequency modulated currents below 10 MHz governed by body electrolytes. Tissue conductance also vary under compression because of the exodus of the conductive fluids from the compressed regions (Belmont *et al.*, 2013). Such effect may occur as the VF collides with the glass plate or *in vivo*. Therefore, it is possible that the overall tissue conductance properties might have been influenced and exhibited spatio-temporal changes during vibrational cycle. The combination of electrode placement and potential VF pathology could theoretically cause such non-linear variations of its conductance properties projected in obtained EGG signal.

2.3.4. Conclusion

The purpose of the study was to find out whether the EGG signal is directly related to VFCA. Generally, similar trends in the shapes of the EGG and VFCA curve were observed. However, in all the larynges the EGG signal showed the contacting phase to occur slightly earlier than that indicated by the visual VFCA analysis. Furthermore, in one of three larynges the EGG signal deviated from the normalized VFCA curve also during the decontacting phase. This larynx differed from the other two by exhibiting a slight aberrance of the medial shape (furrow, resembling sulcus vergeture). How such a tissue aberrance can cause the EGG signal to deviate from the VFCA remains to be explored in future studies.

2.4. Paper III – Vocal fold adjustments caused by phonation into a tube (Hampala *et al.*, 2015b)

It has been found that the phonation into a resonance tube (see Chapter 1.3.3.) introduces changes in the shape of the vocal tract (Vampola *et al.*, 2011; Laukkanen *et al.*, 2012; Guzman *et al.*, 2013). However, it has not been clear whether the phonation into a resonance tube influences also the adjustment of VFs. Such a change has been theoretically predicted by Titze (2006b)), but has not been verified experimentally nor clinically.

The Paper III of this thesis (Hampala *et al.*, 2015b) therefore addresses this issue and investigates the changes in VF configuration caused by resonance tube exercise in two experimental subjects. For this purpose three following hypotheses were formulated based on the study of Laukkanen *et al.* (2008) who reported increased activity of TA muscle after the tube phonation:

1) *the VFs are going to be more bulged and thicker* –this hypothesis is based on previous reports indicating that increased TA muscle activity bulges and thickens the VF (Hirano, 1975),

2) *the glottal width is going to decrease and its margin is going to shift medially* – this hypothesis stems from the studies of Hirano (1975) and (Yumoto *et al.*, 1995) who reported that the medial VF margin shifts towards the center of the glottis when TA is activated,

3) *the length of VFs will remain unchanged* – this hypothesis is based on the fact that no change of fundamental frequency was required and VF length has been shown to change mainly with the fundamental frequency.

2.4.1. Methods

Subjects and CT recordings

The study examined two subjects: a 48 years old female (Subject F) and 35 years old male (Subject M). Both of them were healthy with no voice or hearing problems and were experienced in practicing semi-occluded voice exercises. Before the examination they were informed about potential health risks due to the radiation dose and signed a consent form approved by IRB of the hospital at which the recordings were performed.

The CT recordings were collected at two different occasions. During the recording procedure (for general information on CT method principles see Chapter 1.3.1.2.) both the subjects were lying in horizontal (supine) position (**Figure 33**). Recording times, technique, number of captured images and thickness of the slice are listed in ***Table 2***.

Table 2. Details of CT recording procedure.

	Subject F	Subject M
Device	TOSHIBA AQUILION	LIGHT SPEED VCT GE–64
Recording time [s]	2	3.36
Technique	Helical	Helical
No. of images	181	510
Thickness of the slice [mm]	0.625	0.5

Phonatory tasks and CT measurements

The subjects phonated into a glass resonance tube (27 cm long, 8 mm inner diameter and 9 mm outer diameter; **Figure 33**) and were asked to produce a sustained vowel [a:] at a comfortable pitch and loudness for about 5 minutes. This time duration was chosen based on the studies of Vampola *et al.* (2011) and Guzman *et al.* (2013) who found this period was long enough to self-perceive changes in voice production and resulted in clear alternation of vocal tract shape after the exercise. The CT scanning and following measurements were performed twice before, twice during, and twice after the phonation into the tube.

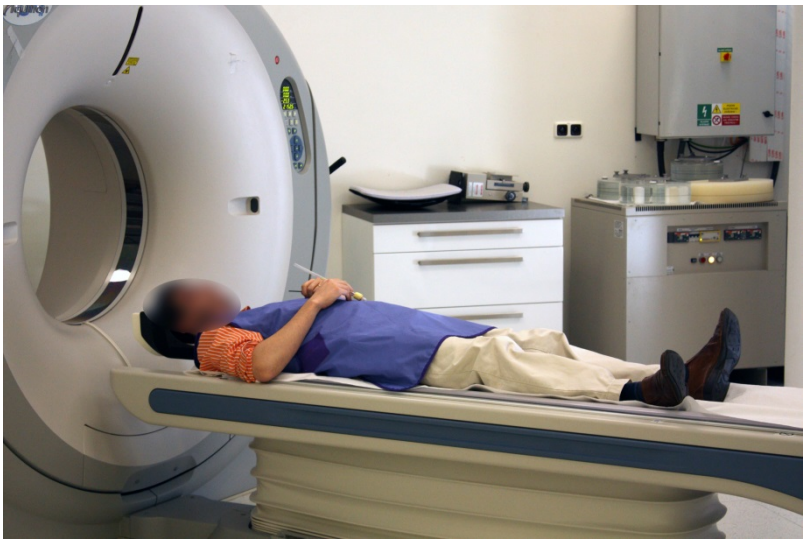


Figure 33. Position of the examined subject holding the glass resonance tube before the CT examination

Free software OsiriX, v. 3.9.4 32-bit (Ratib *et al.*, 2009) was used to visualize the CT data, facilitated an orientation in 3D space and enabled setting the planes to the desired measurement position. A calibrated distance line was inserted into the resulting images which were exported for further analysis. The measurements were performed using ImageJ 1.45s software (freely downloadable).

In order to preserve the same spatial conditions for all the measurements the transverse plane was set to be parallel to the superior VF surface (**Figure 34**, left, a).

For the measurement of the vocal fold lengths, the transverse plane was shifted to reach the level of glottis (**Figure 34**, left, b) which resulted in a similar view as in an anatomical atlas of histological slices by Hirano (1975, section AH9, page 20). The **Figure 34**, right depicts the points used for measurements of particular distances, where the points 1 and 8 correspond to the anterior and posterior commissure. The medially prominent parts of arytenoid cartilages are marked with the numbers 2, 4, 5, and 7. The connection between points 1 and 8 created a glottal axis which intersected (point 3) the half of the distance between vocal processes (i.e. points 2 and 4) and the connection between left and right medially prominent points (5 and 7) of arytenoid cartilages in their posterior part. The membranous length of left vocal fold (LVF) was defined as the distance between points 1 and 2 whereas points 1 and 4 defined the distance corresponding to the length of right vocal fold (RVF). The glottal width (w_1) was measured as the distance between the points 2 and 4.

The methodology for measurements of the vocal fold (VF) thickness and cross-sectional areas was inspired by Hollien and Curtis (1960) and Hollien and Curtis (1962) (see Chapter 1.3.1.1.). The original design was modified in order to obtain better defined measures. The measurements were performed in coronal plane which was perpendicular to the transverse plane. The resulting image (**Figure 35**) was used for measurements of the membranous glottal width (w_2), i.e. distance between the most medial parts of VFs, of the vertical VF thicknesses (**Figure 35**, T1, T2) and cross-sectional areas (**Figure 35**, A1, A2) at 1 and 2 mm from the glottis for both the VFs.

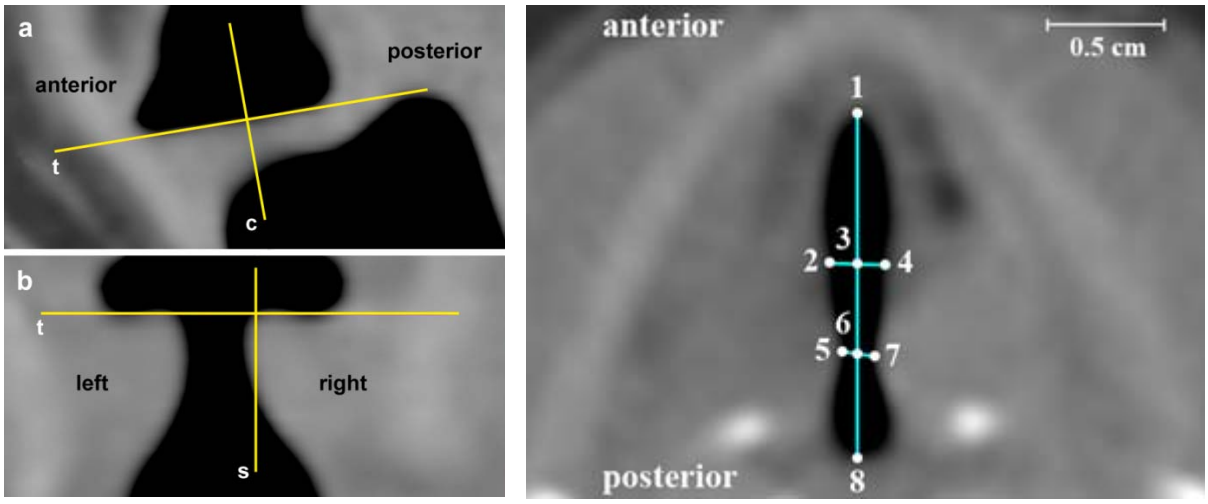


Figure 34. Left – schematics showing the setting of the transverse plane (t) to be parallel to the surface of the VFs: a) sagittal view; b – coronal view (c – coronal plane, s – sagittal plane); Right – points on the transverse plane used for the measurement of the lengths.

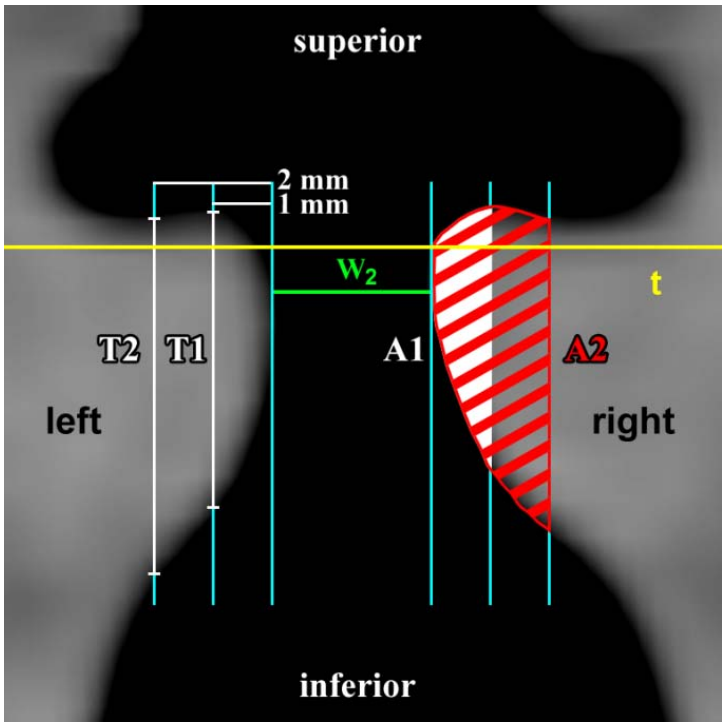


Figure 35. Measurements performed in the coronal plane.

The process of placement of the transverse and coronal planes was performed three times for each of the 6 sets of CT recordings (i.e. two recordings made before, two during, and two after the resonance tube exercise), and thus also the corresponding measurements were performed three times. This allowed calculating a *standard error of the mean* which represented the *measurement uncertainty*. A variability observed due to the task repetition (the phonation was repeated twice before, twice during and twice after the resonance tube exercise), i.e. the *repetition variability*, could then be compared to the measurement uncertainty and the change caused by the resonance tube exercise. The change was considered to be significant when it was larger than the repetition variability and the measurement uncertainty.

2.4.2. Results

Figure 36 to **Figure 38** show the results of the measurements. The X-axis always refers to a particular condition, i.e. recording before, during, or after the resonance tube exercise labelled as “Before”, “Tube”, and “After”. The measured value is shown on the Y-axis. The graphs always contain two mean values for each condition, denoted as minimum and maximum, corresponding to the twice repeated phonations. The maximum mean value (obtained by averaging the three measurements for the respective individual CT recording) is represented by the triangle pointing upwards whereas the minimum mean value is represented by the triangle pointing downwards. The difference between the maximum and minimum mean value quantifies the *repetition variability*. The standard error of the mean (i.e. *measurement uncertainty* based on the three measurements of each of the CT recordings) is then expressed with error spans. The mean values are horizontally interconnected by lines to show evolving trends.

The only significant change before and after the exercise was observed in the vertical thickness T_2 of the female (increase for both VFs, see **Figure 36**) and in the vertical thickness and bulkiness of the male subject. In the male subject, however, the left and right VF showed opposite behaviour (**Figure 37**).

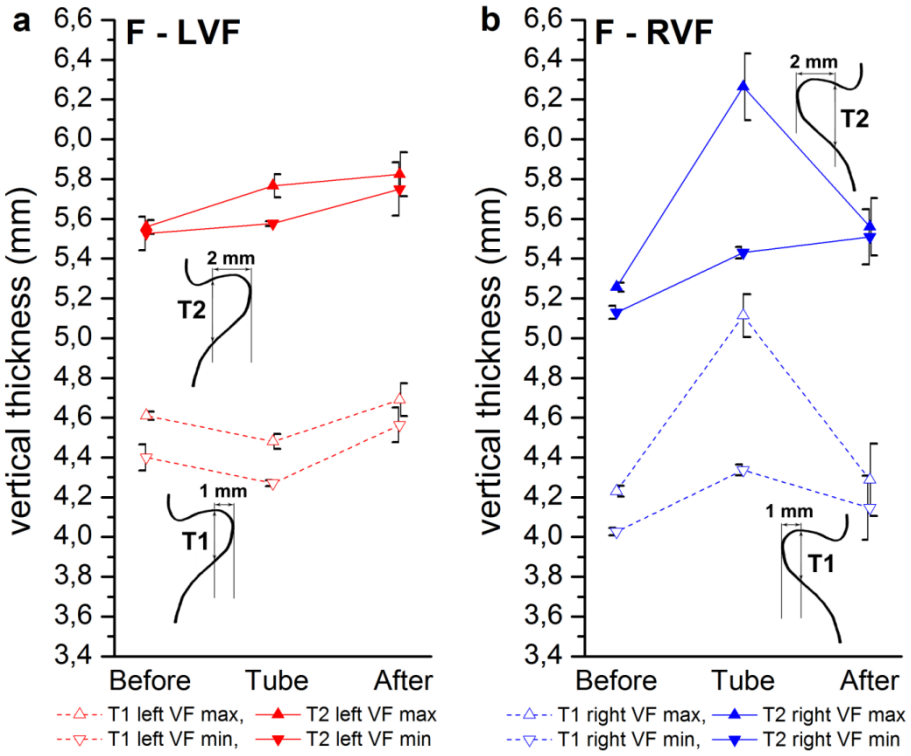


Figure 36. Changes of the thicknesses in Subject F [a – left vocal fold (LVF), b – right vocal fold (RVF)].

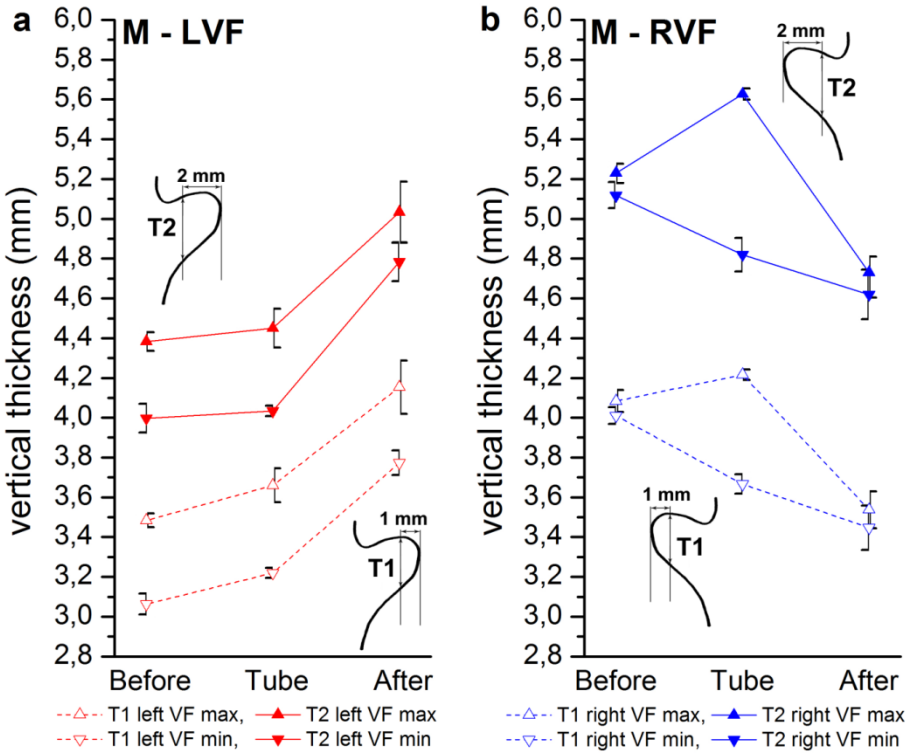


Figure 37. Changes of the thicknesses in Subject M [a – left vocal fold (LVF), b – right vocal fold (RVF)].

In other measured parameters the repetition variability and measurement uncertainty were found larger than the change caused by the resonance tube exercise. This was also the case of the VF membranous length (**Figure 38**) where the mean values did not change significantly. The Subject F had approximately twice shorter VFs than the Subject M. In both subjects, the repetition variability for VF length decreased after the exercise.

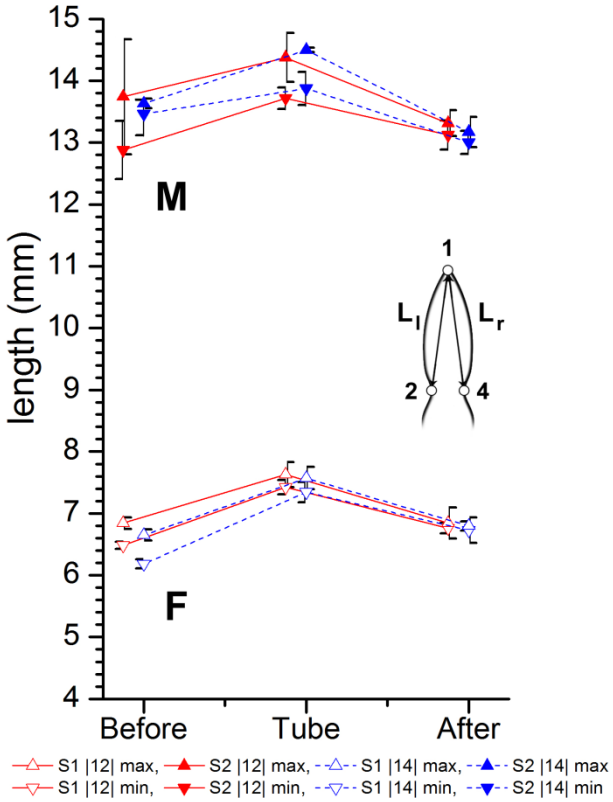


Figure 38. Changes in vocal fold membranous length (L_l – left vocal fold, L_r – right vocal fold) in Subject F (bottom) and Subject M (top).

2.4.3. Discussion

The first hypothesis expected VFs to be more bulged after the resonance tube exercise. Such a change was not found here consistently. The vertical thickness at 2 mm depth increased in Subject F (**Figure 36**), which was the same subject as investigated in Laukkanen *et al.* (2008) and which exhibited the increased TA activity after the tube phonation previously. This trend was, however, not observed in Subject M where the vertical thickness increased in the left vocal fold (LVF) whereas it decreased in the right vocal fold (RVF, **Figure 37**). Therefore, the results did not confirm the notion that VFs are generally more bulged after the phonation into a tube exercise.

The second hypothesis assumed that the glottal width will decrease after the exercise. The large repetition variability in both the subjects did not allow for confirmation or rejection of this hypothesis. In this respect, it is interesting to note that also the literature provides inconsistent results. It was found that an increased resistance introduced by resonance tube may result in an increased effort to produce sound (Laukkanen, 1992; Bele, 2005) and thus also in increased CQ during and after performing the resonance tube exercise as found by Laukkanen (1992). The larger CQ after the resonance tube indicated increased adduction. One would then expect that the glottal width would decrease. Nevertheless, Guzman *et al.* (2013) showed that the CQ decreased thus indicating increased glottal width after the exercise. Interestingly, different effects after the resonance exercise were also

found when compared subjects with normal and hypofunctional voice (Laukkanen *et al.*, 1995; 1998) - in normal subjects the glottal resistance decreased whereas in subjects with hypofunctional voice it increased. The discussed issues indicate that the effect of resonance tube exercise on VF adduction may vary.

The third hypothesis expected no change of VF length. This hypothesis was supported by the data - no significant change of VF length was found in both the subjects. In Subject F the repetition variability was larger than the change from „Before“ to „After“ caused by the actual exercise and in Subject M the measurement uncertainty was larger or of the same magnitude as the change. This indicated that there was no change or it was so small that it could not be reliably detected with this method. Notice, however, that the VF length in Subject F was approximately twice smaller than that in Subject M due to a difference in sex (recall **Figure 38**), which is well in line with the literature (Kahane, 1978; Titze, 1989) and confirms that the measurements are realistic.

Overall, the study did not reveal any systematic changes. This is in contrast with findings from the studies investigating the vocal tract changes induced by the resonance tube exercises (Vampola *et al.*, 2011; Laukkanen *et al.*, 2012; Guzman *et al.*, 2013) where clear changes could be recognized. An increased SPL is one of the resonance tube exercise benefits (Vampola *et al.*, 2011; Guzman *et al.*, 2013). Using finite element model based on MRI recordings, Vampola *et al.* (2011) confirmed the relationship between increased SPL and changes of vocal tract shape after the exercise. The study indicates that systematic changes of voice quality stems from source-filter interaction (Titze, 2006b; 2008) and vocal tract dimension changes rather than from the VF configuration and intrinsic laryngeal muscle adjustments.

2.4.4. Conclusion

No clear systematic changes caused by the phonation into a tube exercise were found in VF vertical thickness, glottal width or in VF length. The thickness of female VFs significantly increased whereas the male ones became more symmetrical. The changes caused by the exercise and the measurement error were mostly smaller or similar to the magnitude of the repetition variability. This indicates that the lack of detection of any systematic trend stemmed from the large repetition variability of glottal adjustment rather than from the limitations of the CT examination method. Nevertheless, larger amount of subjects needs to be examined in order to verify the findings.

The changes of vocal tract shape caused by a phonation into a tube reported in previous studies were more prominent then the changes in VF configuration observed here. Therefore, it appears that the adjustments of the vocal tract resonance and the source–filter interaction phenomena could play more prominent role in the tube phonation exercises than the changes in VF configuration.

Overall conclusion

The first goal of this thesis was to design and build a new subglottal tract with continuously adjustable length. The subglottal tract was successfully manufactured, registered at the Industrial property office of the Czech Republic and used for the experiments devoted to the second goal of this thesis, i.e. validation of the electroglottography as a measure of time-varying vocal fold glottal area during vocal fold vibration. The conducted experiments showed a strong correlation between the electroglottographic signal and the vocal fold contact area, but also revealed discrepancies, which should be addressed in future studies. The third goal of this thesis was to find out whether the therapeutic method phonation into a tube causes distinct systematic changes in the vocal fold adjustments. In this case, the results did not reveal any such changes, in contrast to clear changes in the vocal tract adjustments which were found in related previous studies. These findings indicate that the systematic voice improvement observed after the phonation into a tube is more likely the consequence of the changes in the adjustments of a vocal tract than in the adjustment of the vocal folds. Overall, this dissertation provides new knowledge that can be used for future improvement of methodologies used in voice science and in investigating the results of voice therapy.

Reference list

Alipour, F., Finnegan, E., and Jaiswal, S. (**2013**). "Phonatory Characteristics of the Excised Human Larynx in Comparison to Other Species," J Voice **27**, 441-447.

Alipour, F., and Jaiswal, S. (**2009**). "Glottal Airflow Resistance in Excised Pig, Sheep, and Cow Larynges," Journal of Voice **23**, 40-50.

Alipour, F., Jaiswal, S., and Vigmostad, S. (**2011**). "Vocal Fold Elasticity in the Pig, Sheep, and Cow Larynges," Journal of Voice **25**, 130-136.

Alipour, F., and Scherer, R. C. (**2001**). "Effects of oscillation of a mechanical hemilarynx model on mean transglottal pressures and flows," J Acoust Soc Am **110**, 1562-1569.

Alipour, F., Scherer, R. C., and Finnegan, E. (**1997**). "Pressure-flow Relationships During Phonation as a Function of Adduction," J Voice **11**, 187-194.

Anastaplo, S., and Karnell, M. P. (**1988**). "Synchronized videostroboscopic and electroglottographic examination of glottal opening," J Acoust Soc Am **83**, 1883-1890.

Andrade, P. A. (**2012**). "Analysis of Male Singers Laryngeal Vertical Displacement During the First Passaggio and its Implications on the Vocal Folds Vibratory Pattern," J Voice **26**, 655.e619-e624.

Andrade, P. A., Wood, G., Ratcliffe, P., Epstein, R., Pijper, A., and Svec, J. G. (**2014**). "Electroglottographic study of seven semi-occluded exercises: LaxVox, straw, lip and tongue trills, humming, hand over mouth and tongue trill combined with hand over mouth," J Voice **In press**.

Asher, V. A., Ssaki, C. T., and Gracco, C. (**1996**). "Laryngeal physiology. Normal and Abnormal," in *The Larynx*, edited by M. Fried (Mosby, St. Louis), pp. 45-54.

Austin, S. F., and Titze, I. R. (**1997**). "The effect of subglottal resonance upon vocal fold vibration," Journal of Voice **11**, 391-402.

Awan, S. N., and Awan, J. A. (**2013**). "The Effect of Gender on Measures of Electroglottographic Contact Quotient," Journal of Voice **27**, 433-440.

Baer, T. (**1975**). "Investigation of phonation using excised larynges," in *Massachusetts Institute of Technology*.

Baer, T., Gore, J. C., Boyce, S., and Nye, P. W. (**1987**). "Application of MRI to the analysis of speech production," Magnetic Resonance Imaging **5**, 1-7.

Baer, T., Löfqvist, A., and McGarr, N. S. (**1983a**). "Laryngeal vibrations: A comparison between high-speed filming and giottographic techniques," J Acoust Soc Am **73**, 1304-1308.

Baer, T., Titze, I. R., and Yoshioka, H. (**1983b**). "Multiple simultaneous measures of vocal fold activity.," in *Vocal Fold Physiology*, edited by D. M. Bless, and J. H. Abbs (College-Hill Press, San Diego), pp. 229-237.

Baken, R. J. (**1992**). "Electroglottography," J Voice **6**, 98-110.

Baken, R. J., and Orlikoff, R. F. (**1987**). "Phonatory response to step-function changes in supraglottal pressure," J Voice **1**, 68-76.

Baken, R. J., and Orlikoff, R. F. (**1988**). "Chenges in vocal fundamental frequency at the segmental level: control during voiced fricatives," J Speech Hear Res **31**.

Bakhshaee, H., Moro, C., Kost, K., and Mongeau, L. (**2013**). "Three-Dimensional Reconstruction of Human Vocal Folds and Standard Laryngeal Cartilages Using Computed Tomography Scan Data," J Voice **27**, 769-777.

Bele, I. V. (**2005**). "Artificially lengthened and constricted vocal tract in vocal training methods," Logoped Phoniatr Vocol **30**, 34-40.

Belmont, B., Dodde, R. E., and Shih, A. J. (**2013**). " Impedance of tissue-mimicking phantom material under compression," J. Electr. Bioimp. **4**, 2e12.

Benninger, M. S., Chota, R. L., Bryson, P. C., and Drake, R. L. (**2015**). "Custom Implants for Medialization Laryngoplasty: A Model That Considers Tissue Compression," J Voice **(in press)**, 1-7.

Berjawi, G., Uthman, I., Mahfoud, L., Tanbouzi Husseini, S. T., Nassar, J., Kotobi, A., and Hamdan, A.-L. H. (**2010**). "Cricothyroid Joint Abnormalities in Patients With Rheumatoid Arthritis," J Voice **24**, 732-737.

Berke, G. S., and Gerratt, B. R. (**1993**). "Laryngeal biomechanics: an overview of mucosal wave mechanics," J Voice **7**, 123-128.

Berke, G. S., Moore, D. M., Hantke, D. R., Hanson, D. G., Gerratt, B. R., and Burstein, F. (**1987**). "Laryngeal modeling: Theoretical, in vitro, in vivo," Laryngoscope **97**, 871-881.

Berry, D. A., Herzel, H., Titze, I. R., and Story, B. H. (**1996**). "Bifurcations in excised larynx experiments," J Voice **10**, 129-138.

Berry, D. A., Montequin, D. W., and Tayama, N. (**2001**). "High-speed, digital imaging of the medial surface of the vocal folds," J Acoust Soc Am **110**, 2539-2547.

Bier, S. D., Watson, C. I., and McCann, C. M. (**2014**). "Using the perturbation of the contact quotient of the EGG waveform to analyze age differences in adult speech," J Voice **28**, 267-273.

Bless, D., Hirano, M., and Feder, R. (**1987**). "Videostroboscopic evaluation of the larynx," Ear, Nose and Throat Journal **66**.

Broad, D. J. (**1979**). "The New Theories of Vocal Fold Vibration," Speech and Language **2**, 203-256.

Bushong, S. C., and Clarke, G. (**2015**). *Magnetic Resonance Imaging: Physical and Biological Principles* (Elsevier/Mosby, St. Louis, Missouri).

Colton, R. H., and Conture, E. G. (**1990**). "Problems and Pitfalls of Electroglottography," J Voice **4**, 10-24.

Cooper, D. S. (**1986**). "Research in laryngeal physiology with excised larynges," in *Otolaryngology - head and neck surgery*, edited by C. W. Cummings, J. M. Fredrickson, L. A. Harker, D. E. Schuller, and C. J. Krause (C. V. Mosby., St. Louis and Toronto), pp. 1728-1737.

Cranen, B., and Boves, L. (**1985**). "Pressure Measurements During Speech Production Using Semiconductor Miniature Pressure Transducers - Impact on Models for Speech Production," Journal of the Acoustical Society of America **77**, 1543-1551.

Cranen, B., and Boves, L. (**1987**). "On Subglottal Formant Analysis," Journal of the Acoustical Society of America **81**, 734-746.

Čihák, R. (**1988**). *Anatomie 2, Splanchniologia* (Academia, Praha).

Dejonckere, P. H. (**1981**). "Comparison of two methods of photoglottography in relation to electroglottography," Folia Phoniat **33**, 338-347.

Deliyski, D. D., Petrushev, P. P., Bonilha, H. S., Gerlach, T. T., Martin-Harris, B., and Hillman, R. E. (**2008**). "Clinical implementation of laryngeal high-speed videoendoscopy: challenges and evolution," Folia Phoniatr Logop **60**, 33-44.

Demolin, D., Delvaux, V., Metens, T., and Soquet, A. (**2003**). "Determination of Velum Opening for French Nasal Vowels by Magnetic Resonance Imaging," J Voice **17**, 454-467.

Diano, P. (**2006**). *Atlas lidského těla* (Euromedia Group - Knižní klub, Praha).

Döllinger, M., and Berry, D. A. (**2006a**). "Computation of the three-dimensional medial surface dynamics of the vocal folds," J Biomech **39**, 369-374.

Döllinger, M., and Berry, D. A. (**2006b**). "Visualization and quantification of the medial surface dynamics of an excised human vocal fold during phonation," J Voice **20**, 401-413.

Döllinger, M., Tayama, N., and Berry, D. A. (**2005**). "Empirical eigenfunctions and medial surface dynamics of a human vocal fold," Methods Inf Med **44**, 384-391.

Dufresne, A. M., and Lafreniere, D. (**2000**). "Soft tissue response in the rabbit larynx following implantation of Lactosorb (PLA/PGA copolymer) prosthesis for medialization laryngoplasty," J Voice **14**, 387-397.

Echternach, M., Dippold, S., Sundberg, J., Arndt, S., Zander, M. F., and Richter, B. (**2010**). "High-Speed Imaging and Electroglottography Measurements of the Open Quotient in Untrained Male Voices' Register Transitions," J Voice **24**, 644-650.

Elemans, C. P. H., Spierts, I. L. Y., Muller, U. K., van Leeuwen, J. L., and Goller, F. (**2004**). "Superfast muscles control dove's trill," in *Nature*, p. 146.

Elemans, C. P. H., Zaccarelli, R., and Herzel, H. (**2008**). "Biomechanics and control of vocalization in a non-songbird," Journal of the Royal Society Interface **5**, 691-703.

Elemans, C. H., Spierts, I. L., Hendriks, M., Schipper, H., Müller, U. K., and van Leeuwen, J. L. (**2006**). "Syringeal muscles fit the trill in ring doves (Streptopelia risoria L.)," The Journal of experimental biology **209**, 965-977.

Enflo, L. (**2013**). "Collision Threshold Pressure: A novel measure of voice function. Effects of vocal warm-up, vocal loading and resonance tube phonation in water," in *Department of Clinical and Experimental Medicine* (Linköping University, Linköping, Sweden).

Enflo, L., Sundberg, J., Romedahl, C., and McAllister, A. (**2013**). "Effects on vocal fold collision and phonation threshold pressure of resonance tube phonation with tube end in water," J Speech Lang Hear Res **59**, 1530-1538.

Fabre, P. (**1957**). "Un prodédé electrique percutané d'inscription de l'accolement glottique au cours de la phonation: glottographie de haute fréquence. Premiers résultats.," Bull Acad Natl Med **141**, 66-69.

Fant, G. (**1960**). *Acoustic Theory of Speech Production* (Mouton, The Hague, Netherlands).

Finnegan, E., and Alipour, F. (**2009**). "Phonatory Effects of Supraglottic Structures in Excised Canine Larynges," J Voice **23**, 51-61.

Flanagan, J. L. (**1965**). *Speech analysis, synthesis and perception* (Academic Press, New York).

Flanagan, J. L., and Ishizaka, K. (**1978**). "Computer model to characterize the air flow displacement by the vibrating vocal cords," J Acoust Soc Am **63**, 1559-1565.

Flint, P. W., Corio, R. L., and Cummings, C. W. (**1997**). "Comparison of soft tissue response in rabbits following laryngeal implantation with hydroxylapatite, silicone rubber, and teflon," Ann Otol Rhinol Laryngol **106**.

Fourcin, A. J. (**1974**). "Laryngographic examination of vocal fold vibration," in *Ventilatory and Phonatory Control Systems, edited by B. Wyke* (Oxford University Press, London), pp. 315-333.

Fourcin, A. J. (**1981**). "Laryngographic assessment of phonatory function," ASHA Reports, 116-127.

Fournié, E. (**1866**). *Physiologie de la voix et de la parole* (Delahaye, Paris).

Fujimura, O. (**1981**). "Body-cover theory of vocal fold and its phonetic implications," in *Vocal Fold Physiology*, edited by K. N. Stevens, and M. Hirano (University of Tokyo, Tokyo), pp. 271-288.

Fukuda, H., Saito, S., Kitahara, S., Isogai, Y., Makino, K., Tsuzuki, T., Kogawa, N., and Ono, H. (**1983**). *Vocal Fold Physiology: Contemporary Research and Clinical Issues* (College Hill Press, San Diego).

Gabriel, C., Gabriel, S., and Corthout, E. (**1996**). "The dielectric properties of biological tissues: I. Literature survey," Phys. Med. Biol. **41**, 2231–2249.

Garrett, C. G., Coleman, J. R., and Reinisch, L. (**2000**). "Comparative histology and vibration of the vocal folds: implications for experimental studies in microlaryngeal surgery," Laryngoscope **110**, 814-824.

Gilbert, H. R., Potter, C. R., and Hoodin, R. (**1984**). "Laryngograph as a measure of vocal fold contact area," J Speech Hear Res **27**, 178-182.

Granqvist, S., Simberg, S., Hertegard, S., Holmqvist, S., Larsson, H., Lindestad, P.-Å., Södersten, M., and Hammarberg, B. (**2014**). "Resonance tube phonation in water: High-speed imaging, Electroglottographic and oral pressure observations of vocal fold vibrations - a pilot study," Log Phon Vocol.

Grémy, F., and Guérin, C. (**1963**). "Etude du glottogramme chez l'enfant sourd en cours de reéducation vocale," Ann Otolaryngol Chir Cervicofac **80**, 803-815.

Grimnes, S., and Martinsen, Ø. G. (**2015**). "Passive Tissue Electrical Properties," in *Bioimpedance and Bioelectricity Basics (Third Edition)*, pp. 77-118.

Guzman, M., Laukkanen, A. M., Krupa, P., Horáček, J., Švec, J. G., and Geneid, A. (**2013**). "Vocal tract and glottal function during and after vocal exercising with resonance tube and straw," J Voice **27**, 523.e519-523.e534.

Habermann, G. (**1980**). "Funktionelle Stimmstörungen und ihre Behandlung. [Functional disorders of the voice and their treatment]," Arch. otorhinolaryngol. **227**, 345.

Hála, B., and Sovák, M. (**1941**). *Hlas, řeč, sluch* (Česká grafická Unie, Praha).

Hála, B., and Sovák, M. (**1962**). *Hlas, řeč, sluch - základy fonetiky a logopedie.* (Státní pedagogické nakladatelství, Praha).

Hampala, V. (**2011**). "Vizuální hodnocení videokymografických snímků u hlasových poruch," in *Katedra experimentální fyziky* (Univerzita Palackého v Olomouci, Olomouc), p. 84.

Hampala, V., Garcia, M., Švec, J. G., Scherer, R. C., and Herbst, C. T. (**2015a**). "Relationship Between the Electroglottographic Signal and Vocal Fold Contact Area," J Voice **(in press)**.

Hampala, V., Laukkanen, A.-M., Guzman, M. A., Horáček, J., and Švec, J. G. (**2015b**). "Vocal Fold Adjustment Caused by Phonation Into a Tube: A Double-Case Study Using Computed Tomography," J Voice **in press**.

Hampala, V., Švec, J., Schovánek, P., Mandát, D. (2013). Užitný vzor č. 25585: Modelsubglotického traktu. [Utility model no. 25585: Model of subglottal tract.] Soukup, P.2013-27834(CZ 25505 U1), 1-7. 24-6-2013. Praha, Úřad průmyslového vlastnictví. [Prague, Czech Republic, Industrial property office].

Harless, E. (**1853**). "Die Stimme," in *Handwiirterbuch der Physiologie*, edited by R. Wagner (F Vieweg und Sohn, Braunschweig).

Henrich, N., d'Alessandro, C., Doval, B., and Castellengo, M. (**2004**). "On the use of the derivative of electroglottographic signals for characterization of nonpathological phonation," J Acoust Soc Am **115**, 1321-1332.

Herbst, C. T. (**2011**). "Investigation of glottal configuration in singing (Doctoral dissertation)," in *Department of Biophysics* (Faculty of Science, Palacky University Olomouc, Olomouc), p. 107.

Herbst, C. T. (**2014**). "Glottal Efficiency of Periodic and Irregular In Vitro Red Deer Voice Production," Acta Acoustica united with Acoustica **100**, 724-233.

Herbst, C. T., Fitch, W. T., and Svec, J. G. (**2010a**). "Electroglottographic wavegrams: a technique for visualizing vocal fold dynamics noninvasively," J Acoust Soc Am **128**, 3070-3078.

Herbst, C. T., Howard, D. M., and Schlömicher-Thier, J. (**2010b**). "Using Electroglottographic Real-Time Feedback to Control Posterior Glottal Adduction during Phonation," J. Voice **24**, 72-85.

Herbst, C. T., Lohscheller, J., Švec, J. G., Henrich, N., Weissengruber, G., and Fitch, W. T. (**2014**). "Glottal opening and closing events investigated by electroglottography and super-high-speed video recordings," J Exp Biol **217**, 955-963.

Herbst, C. T., Stoeger, A. S., Frey, R., Lohscheller, J., Titze, I. R., Gumpenberger, M., and Fitch, W. T. (**2012**). "How Low Can You Go? Physical Production Mechanism of Elephant," Science **337**, 595-599.

Herbst, C. T., and Ternström, S. (**2006**). "A comparison of different methods to measure the EGG contact quotient," Log Phon Vocol **31**, 126-138.

Hertegard, S. (**2005**). "What have we learned about laryngeal physiology from high-speed digital videoendoscopy? ," Curr Opin Otolaryngol Head Neck Surg **13**, 152-156.

Hertegård, S., Dahlqvist, A., Laurent, C., Borzacchiello, A., and Ambrosio, L. (**2003**). "Visco-elastic properties of rabbit vocal folds after augmentation," Otolaryngol Head Neck Surg **128**, 401-406.

Hertegård, S., Larsson, H., Nagubothu, S. S., Tolf, A., and Svensson, B. (**2009**). "Elasticity measurements in scarred rabbit vocal folds using air pulse stimulation," Logoped Phoniatr Vocol **34**, 190-195.

Hiramatsu, H., Tokashiki, R., Nakamura, H., Motohashi, R., Sakurai, E., Nomoto, M., Toyomura, F., and Suzuki, M. (**2012**). "Analysis of High-Pitched Phonation Using Three-Dimensional Computed Tomography," J Voice **26**, 548-554.

Hirano, M. (**1974**). "Morphological structure of the vocal cord as a vibrator and its variations," Folia Phoniatr (Basel) **26**, 89-94.

Hirano, M. (**1975**). "Phonosurgery: basic and clinical investigations," Otologia (Fukuoka) **21**, 239-442.

Hirano, M., Gould, W. J., Lambiase, A., and Kakita, Y. (**1980**). "Movements of selected points on a vocal fold during vibration," Folia Phoniatr **32**, 39-50.

Hirano, M., and Kakita, Y. (**1985**). "Cover-body theory of vocal cord vibration," in *Speech science* (College Hill Press San Diego (cit. in Titze, 2000)).

Hirano, M., Kurita, S., and Nakashima, T. (**1981**). "The structure of the vocal folds," in *Vocal Fold Physiology*, edited by K. N. Stevens, and M. Hirano (University of Tokyo Press, Tokyo), pp. 33-43.

Hiroto, I. (**1968**). "Vibration of vocal cords an ultra high-speed cinematographic study," (Department of otolaryngology, Kurume, Japan).

Hollien, H. (**1960**). "Some Laryngeal Correlates of Vocal Pitch," J Speech Lang Hear Res **3**, 52-58.

Hollien, H. (**2014**). "Vocal fold dynamics for frequency change," J Voice **28**, 395-405.

Hollien, H., Coleman, R., and Moore, P. (**1968**). "Stroboscopic laminography of the larynx during phonation," Acta Otolaryngol **65**, 209-215.

Hollien, H., and Colton, R. H. (**1969**). "4 Laminagraphic Studies of Vocal Fold Thickness," Folia Phoniatr. (Basel) **21**, 179-198.

Hollien, H., and Curtis, J. F. (**1960**). "A Laminagraphic Study of Vocal Pitch," J Speech Lang Hear Res **3**, 361-371.

Hollien, H., and Curtis, J. F. (**1962**). "Elevation and Tilting of Vocal Folds as a Function of Vocal Pitch," Folia Phoniatr. (Basel) **14**, 23-26.

Hollien, H., and Moore, G. P. (**1960**). "Measurements of the Vocal Folds During Changes in Pitch," J Speech Lang Hear Res **3**, 157-165.

Holmer, N. G., Kitzing, P., and Lindström, K. (**1973**). "Echo glottography. Ultrasonic recording of vocal fold vibrations in preparations of human larynges.," Acta Otolaryngol **75**, 454-463.

Hounsfield, G. H. (**1973**). "Computerized axial scanning (tomography), I: description of the system," Br J Radiol **46**, 1016-1022.

Hounsfield, G. N. (**1977**). "A Discussion on Recent Developments in Medical Endoscopy and Related Fields," in *Proceedings of the Royal Society of London* (The Royal Society), pp. pp. 281-289.

Howard, D. M. (**1995**). "Variation of Electrolaryngographically Derived Closed Quotient for Trained and Untrained Adult Female Singers," J Voice **9**, 163-172.

Howard, D. M., Lindsey, G. A., and Allen, B. (**1990**). "Toward the Quantification of Vocal Efficiency," J Voice **4**.

Howard, D. M., and Murphy, D. T. (**2008**). *Voice science, acoustics and recording* (Plural Press San Diego, USA).

Chaudhry, I., Mutairi, H., Hassan, E., Afzal, M., and Khurshid, I. (**2014**). "Tracheal Diverticulum: A Rare Cause of Hoarseness of the Voice," Ann Thorac Surg **97**, e29-e31.

Chevrie-Muller, C., and Grémy, C. (**1962**). "Etude de l'electroglottogramme et du phonogramme en periode de "mue vocale"," Ann Otolaryngol Chir Cervicofac **79**, 1035-1044.

Chevrie-Muller, C., Perbos, J., and Guidet, C. (**1983**). "Automated analysis of the electroglottographic signal: application to the study of phonation of the elderly," Bull Audiophonol **16**, 121-144.

Chhetri, D. K., Neubauer, J., and Berry, D. A. (**2012**). "Neuromuscular control of fundamental frequency and glottal posture at phonation onset," J Acoust Soc Am **131**, 1401-1412.

Childers, D. G., Hicks, D. M., Moore, G. P., and Alsaka, Y. A. (**1986**). "A model for vocal fold vibratory motion, contact area and the electroglottogram," J Acoust Soc Am, 1309-1320.

Childers, D. G., Hicks, D. M., Moore, G. P., Eskenazi, L., and Lalwani, A. L. (**1990**). "Electroglottography and vocal fold physiology," J Speech Hear Res **33**, 245-254.

Childers, D. G., and Krishnamurthy, A. K. (**1985**). "A critical review of electroglottography," CRC Crit Rev Biomed Eng **12**.

Childers, D. G., Naik, J. M., Larar, J. N., Krishnamurthy, A. K., and Moore, G. P. (**1983a**). "Electroglottography, speech and ultra-high speed cinematography," in *Vocal fold physiology and biophysics of voice*, edited by I. R. Titze, and R. Scherer (Denver Center for the Performing Arts, Denver), pp. 202-220.

Childers, D. G., Naik, J. M., Larar, J. N., Krishnamurthy, A. K., and Moore, G. P. (**1983b**). "Electroglottography, speech and ultra-high speed cinematography," in *Vocal Fold Physiology and Biophysics of Voice*, edited by I. R. Titze, and R. Scherer (Denver Center for the Performing Arts, Denver).

Childers, D. G., Smith, A. M., and Moore, G. P. (**1984**). "Relationships between electroglottograph, speech, and vocal cord contact," Folia Phoniat **36**, 105-118.

Jiang, J. J., and Titze, I. R. (**1993**). "A Methodological Study of Hemilaryngeal Phonation," Laryngoscope **103**, 872-882.

Jiang, J. J., Zhang, Y., and Ford, C. N. (**2003**). "Nonlinear dynamics of phonations in excised larynx experiments," J Acoust Soc Am **114**, 2198-2205.

Jiang, J. J., Zhang, Y., Kelly, M. P., Bieging, E. T., and Hoffman , M. R. (**2008**). "An automatic method to quantify mucosal waves via videokymography," Laryngoscope **118**, 1504-1510.

Kahane, J. (**1978**). "A morphological study of the human prepubertal and pubertal larynx," American Journal of Anatomy **151**, 11-20.

Kallender, W. A. (**2011**). *Computed Tomography: Fundamentals, System Technology, Image Quality, Applications* (Publicis publishing, Erlangen, Germany).

Kania, R. E., Hans, S., Hartl, D. M., Clement, P., Crevier-Buchman, L., and Brasnu, D. F. (**2004**). "Variability of electroglottographic glottal closed quotients: necessity of standardization to obtain normative values," Archives of otolaryngology--head & neck surgery **130**, 349-352.

Kankare, E., Liu, D., Laukkanen, A. M., and Geneid, A. (**2013**). "EGG and acoustic analyses of different voice samples: Comparison between perceptual evaluation and Voice Activity and Participation Profile," Folia Phoniatr Logop **65**.

Karnell, M. P. (**1989**). "Synchronized videostroboscopy and electroglottography," J Voice, 68-75.

Kitzing, P. (**1977**). "Methode zur kombierten photo- und elektroglottographischen registrierung von stimmlippenschwingungen," Folia Phoniatr **29**.

Kitzing, P. (**1982**). "Photo- and electroglottographical recording of the laryngeal vibratory pattern during different registers," Folia Phoniatr **34**, 234-241.

Kitzing, P. (**1983**). "Simultaneous photo- and electroglottographic measurements of voice strain," in *Vocal Fold Physiology*, edited by I. R. Titze, and R. Scherer (The Denver Center for the Performing Arts, Denver), pp. 221–229.

Kitzing, P., and Löquist, A. (**1975**). "Subglottal and oral air pressures during phonation - preliminary investigation using a miniature transducer system," Medical and Biological engineering, 644-648.

Koike, Y. (**1981**). "Sub- and supraglottal pressure variation during phonation," in *Vocal Fold Physiology*, edited by K. N. Stevens, and M. Hirano (University of Tokyo Press, Tokyo, Japan), pp. 181-189.

Kusuyama, T., Fukuda, H., Shiotani, A., Nakagawa, H., and Kanzaki, J. (**2001**). "Analysis of vocal fold vibration by X-ray stroboscopy with multiple markers," Otolaryngol Head Neck Surg **124**, 317-322.

Lã, F. M. B., and Sundberg, J. (**2015**). "Contact Quotient Versus Closed Quotient: A Comparative Study on Professional Male Singers," J Voice **29**, 148-154.

Ladefoged, P. (**1990**). "The Revised International Phonetic Alphabet," Language **66**, 550-552.

Large, J. (**1972**). "Towards an integrated physiologic-acoustic theory of vocal registers," NATS Bull **29**, 15-25.

Laukkanen, A.-M. (**1992**). "About the so called "resonance tubes" used in Finnish voice training practice: An electroglottographic and acoustic investigation on the effects of this method on the voice quality of subjects with normal voice," Scand J Logoped Phoniatr **17**, 151-161.

Laukkanen, A.-M. (**1995**). "On Speaking Voice Exercises," (University of Tampere, Tampere).

Laukkanen, A.-M., Horacek, J., Krupa, P., and Svec, J. G. (**2012**). "The effect of phonation into a straw on the vocal tract adjustments and formant frequencies. A preliminary MRI study on a single subject completed with acoustic results," Biomed Signal Proces **7**, 50-57.

Laukkanen, A.-M., Lindholm, P., and Vilkman, E. (**1995**). "On the effects of various vocal training methods on glottal resistance and efficiency. A preliminary report," Folia Phoniatr Logop **47**, 324-330.

Laukkanen, A.-M., Lindholm, P., and Vilkman, E. (**1998**). "Vocal exercising and speaking related changes in glottal resistance," Log Phon Vocol **23**, 85-92.

Laukkanen, A.-M., and Titze, I. R. (**2007**). "Can vocal economy in phonation be increased with an artificially lengthened vocal tract? A computer modeling study," Log Phon Vocol **32**, 147-157.

Laukkanen, A.-M., Titze, I. R., Hoffman, H., and Finnegan, E. (**2008**). "Effects of a Semioccluded Vocal Tract on Laryngeal Muscle Activity and Glottal Adduction in a Single Female Subject," Folia Phoniatr Logop **60**, 298-311.

Lecluse, F. L. E. (**1977**). *Electroglottografie* (Dmkkerij Elinkwijk BV, Utrecht).

Lecluse, F. L. E., Brocaar, M. P., and Verschuure, J. (**1975**). "The electroglottography and its relation to glottal activity," Folia Phoniatr. **27**, 217-224.

Li, Z., Bakhshaee, H., Helou, L., Mongeau, L., Kost, K., Rosen, C., and Verdolini, K. (**2013**). "Evaluation of contact pressure in human vocal folds during phonation using high-speed videoendoscopy, electroglottography, and magnetic resonance imaging," in *21st International Congress on Acoustics, ICA 2013 - 165th Meeting of the Acoustical Society of America* (Acoustical Society of America, Montreal, Canada), p. 8.

Mackay, I. (**1978**). *Introducing practical phonetics* (Little, Brown, Boston).

Malcolm, P. N., Saks, A. M., Howlett, D. C., and Ayers, A. B. (**1997**). "Case Report: Magnetic Resonance Imaging (MRI) Appearances of Benign Schwannoma of the Larynx," Clin Radiol **52**, 75-76.

Master, S., Guzman, M., and Dowdall, J. (**2013**). "Vocal economy in vocally trained actresses and untrained female subjects," J Voice **27**, 698-704.

Maytag, L. M., Robitaille, M. J., Rieves, A. L., Madsen, J., Smith, B. L., and Jiang, J. J. (**2013**). "Use of the Rabbit Larynx in an Excised Larynx Setup," Journal of Voice **27**, 24-28.

Mecke, A.-C., Sundberg, J., Granqvist, S., and Echternach, M. (**2012**). "Comparing closed quotient in children singers' voices as measured by high-speed-imaging, electroglottography, and inverse filtering.," J Acoust Soc Am **131**, 435-441.

Miller, N. A., Gregory, J. S., Aspden, R. M., Stollery, P. J., and Gilbert, F. J. (**2014**). "Using Active Shape Modeling Based on MRI to Study Morphologic and Pitch-Related Functional Changes Affecting Vocal Structures and the Airway," J Voice **28**, 554-564.

Molteni, R. (**2013**). "Prospects and challenges of rendering tissue density in Hounsfield units for cone beam computed tomography," OOOO **116**, 105-119.

Moore, G. P., White, F. D., and von Leden, H. (**1962**). "Ultra high speed photography in laryngeal physiology," Journal of Speech and Hearing Disorders **27**, 165-171.

Moore, P., and von Leden, H. (**1958**). "Dynamic variations of the vibratory pattern in the normal larynx," Folia Phoniatr **10**, 205-538.

Műller, J. (**1837**). "Handbuch der Physiologie des Menschen," in *Stimme und Sprache* (Verlag von J Hijlscher, Coblenz).

Műller, J. (**1839**). *Über die Compensation der physischen Kräfte am menschlichen Stimmorgan* (Berlin).

Murugappan, S., Khosla, S., Casper, K., Oren, L., and Gutmark, E. (**2009**). "Flow fields and acoustics in a unilateral scarred vocal fold model," Ann Otol Rhinol Laryngol **118**, 44-50.

Olias, J. (**2004**). *Surgery of the Larynx - Atlas of Surgical Techniques, Dissection Guide (Cirurgia da Laringe - Atlas de Técnicas Cirúrgicas, Guia de Dissecção)* (Círculo Médico, Lisboa, Portugal).

Orlikoff, R. F. (**1998**). "Scrambled EGG: The uses and abuses of electroglottography," Phonoscope **1**, 37-53.

Pedersen, M. F. (**1977**). "Electroglottography compared with synchronized stroboscopy in normal persons," Folia Phoniat **29**, 191-199.

Qiu, Q., Schutte, H. K., Gu, L., and Yu, Q. (**2003**). "An automatic method to quan-tify the vibration properties of human vocal folds via videokymography," Folia Phoniatr **55**, 128-136.

Raj, V., Gopalan, D., Stewart, S., and Dunning, J. (**2011**). "Unusual Cause of Hoarseness of Voice: Giant Pulmonary Artery Aneurysm," Ann Thorac Surg **91**, 285-287.

Ratib, O., Rosset, A., and Heuberger, J. (**2009**). *OsiriX: The Pocket Guide* (OsiriX Foundation).

Regner, M. F., Robitaille, M. J., and Jiang, J. J. (**2010**). "Interspecies comparison of mucosal wave properties using high-speed digital imaging," Laryngoscope **120**, 1188-1194.

Réthi, L. (**1896**). "Experimentelle Untersuchungen űber den Schwingungstypus und den Mechanismus der Stimmbinder bei der Falsettstimme," in *Sitzunosberichte der mathematisch-naturwissenschaftlichen Classe der kaiserlichen Akademie der Wissenschaften* (CV Band, Abtheilung III, Vienna).

Rothenberg, M. (**1973**). "A new inverse-filtering technique for deriving the glottal air flow waveform during voicing," J Acoust Soc Am **53**, 1632-1645.

Rothenberg, M. (**1977**). "Measurement of airflow in speech," J Speech Hear Res **20**, 155-176.

Rothenberg, M. (**1981**). "Some relations between glollal air flow and vocal fold contact area," ASHA Reports **11**, 88-96.

Rothenberg, M., and Mahshie, J. J. (**1988**). "Monitoring vocal fold abduction through vocal fold contact area," Journal of Speech and Hearing Research **31**, 338-351.

Rubin, A. D., Hawkshaw, M. J., Moyer, C. A., Dean, C. M., and Sataloff, R. T. (**2005**). "Arytenoid Cartilage Dislocation: A 20-year Experience," J Voice **19**, 687-701.

Rubin, H. J., and LeCover, M. (**1960**). "Technique of high speed photography of the larynx," Annals of Otology, Rhinology and Laryngology **69(4)**, 1072-1083.

Seeram, E. (**2009**). "Physical Principles of Computed Tomography," in *COMPUTED TOMOGRAPHY: Physical Principles, Clinical Applications, and Quality Control*, edited by J. Wilke, and L. Held (Saunders/Elsevier, St. Louis, Missouri, USA).

Shaw, C. C. (**2014**). *Cone Beam Computed Tomography* (CRC Press, Hoboken).

Shin, H. W., Ahn, Y., Sung, M. W., Kim, K. H., and Kwon, T. K. (**2009**). "Measurement of Cross-Sectional Dimensions of the Cricoid Cartilage: A Computed Tomographic Study," Ann Otol Rhinol Laryngol **118**, 253-258.

Scherer, R. C., Druker, D. G., and Titze, I. R. (**1988**). "Electroglottography and direct measurement of vocal fold contact area," in *Vocal Physiology: Voice Production*, edited by O. Fujimura (Raven Press, Ltd., New York), pp. 279-291.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. (**2012**). "Fiji: an open-source platform for biological-image analysis," Nature methods **9**, 676-682.

Simberg, S., and Laine, A. (**2007**). "The resonance tube method in voice therapy: Description and practical implementations," Logoped Phoniatr Vocol **32**, 165-170.

Singh, S., and Singh, K. (**1976**). *Phonetics: Principles and practices* (Park Press, Baltimore: University).

Sondhi, M. M. (**1975**). "Measurement of the glottal waveform," J Acoust Soc Am **57**, 228-232.

Sovák, M., Courtois, J., Haas, C., and Smith, S. (**1971**). "Observations on the mechanism of phonation investigated by ultraspeed cinefluorgraphy," Folia Phoniatrica **23**, 277-287.

Spahn, M. (**2013**). "X-ray detectors in medical imaging," Nucl Instrum Meth A **731**, 57-63.

Spiess, G. (**1904**). *Kurze Anleitung zur Erlernung einer richtigen Tonbildung in Sprache und Gesang (Aufl. 2). [Brief Guide to Learning a Proper Tone Production in Speech and Singing, 2nd ed]* (Publishing company of Johannes Alt, Frankfurt am Main).

Story, B. H., Hoffman, E. A., and Titze, I. R. (**1996**). "Vocal tract imaging: a comparison of MRI and EBCT," in *SPIE Proceedings, Physiology and Function from Multidimensional Images* (Newport Beach, CA).

Story, B. H., Laukkanen, A. M., and Titze, I. R. (**2000**). "Acoustic Impedance of an Artificially Lengthened and Constricted Vocal Tract," J Voice **14**, 455-469.

Sundberg, J. (**1977**). "The acoustics of the singing voice," Sci Am **236**, 82-91.

Sundberg, J., Schrerer, R. C., Hess, M. M., Müller, F., and Granqvist, S. (**2013**). "Subglottal Pressure Oscillations Accompanying Phonation," Journal of Voice **27**, 411-421.

Svec, J. G. (**1996**). "Studium mechanicko-akustickych vlastností zdroje lidského hlasu," in *Katedra experimentální fyziky; Přírodověděcká fakulta* (Univerzita Palackého v Olomouci, Olomouc, Czech Republic), p. 91.

Svec, J. G., and Schutte, H. K. (**1996**). "Videokymography: high-speed line scanning of vocal fold vibration," J. Voice **10**, 201-205.

Svec, J. G., and Sram, F. (**2011**). "Videokymographic Examination of Voice," in *Handbook of Voice Assessments*, edited by E. P. M. Ma, and E. M. L. Yiu (CA: Plural Publishing, San Diego), pp. 129-146.

Svec, J. G., Sram, F., and Schutte, H. K. (**2007**). "Videokymography in Voice Disorders: What to Look For?," Ann Otol Rhinol Laryngol **116**, 172-180.

Švec, J. G., Schutte, H. K., and Miller, D. G. (**1999**). "On pitch jumps between chest and falsetto registers in voice: data from living and excised human larynges.," J Acoust Soc Am **106**, 1523-1531.

Tang, S., Zhang, C., Wang, S., and Wan, M. (**2015**). "A Preliminary Study for a Slantwise-Placed Electroglottography," J Voice **(in press)**.

Thibeault, S. L., Gray, S. D., Bless, D. M., Chan, R. W., and Ford, C. N. (**2002**). "Histologic and rheologic characterization of vocal fold scarring," J Voice **16**, 69-104.

Thomson, S. L., Mongeau, L., and Frankel, S. H. (**2005**). "Aerodynamic transfer of energy to the vocal folds," J. Acoust. Soc. Am. **118**, 1689-1700.

Titze, I. R. (**1976**). "On the mechanics of vocal fold vibration," J. Acoust. Soc. Am. **60**, 1366-1380.

Titze, I. R. (**1980**). "Comments on the myoelastic - aerodynamic theory of phonation," J Speech Hear Res **23**, 495-510.
Titze, I. R. (**1988**). "The pyhsics of small-amplitude oscillation of the vocal fold," J Acoust Soc Am **83**, 1536-1552.
Titze, I. R. (**1989**). "Physiologic and Acoustic Differences Between Male and Female Voices," J Acoust Soc Am **85**, 1699-1707.
Titze, I. R. (**1990**). "Iterpretation of electroglottographic signal," J Voice **4**, 1-9.
Titze, I. R. (**2000**). *Principles of Voice Production (second printing)* (National Center for Voice and Speech, Iowa City, IA).
Titze, I. R. (**2002a**). "How to use the flow resistant straws," Journal of Singing **58**, 429–430.
Titze, I. R. (**2002b**). "Raising Lung Pressure and Pitch In Vocal Warm-ups: The Use of Flow-Resistant Straws," J Singing **58**, 329-338.
Titze, I. R. (**2006a**). *The Myoelastic Aerodynamic Theory of Phonation* (National Center for Voice and Speech, Denver).
Titze, I. R. (**2006b**). "Voice Training and Therapy With a Semi-Occluded Vocal Tract: Rationale and Scientific Underpinnings," J Speech Lang Hear Res **49**, 448-459.
Titze, I. R. (**2008**). "Nonlinear source–filter coupling in phonation: Theory," J Acoust Soc Am **123**, 2733-2749.
Titze, I. R., Baer, T., Cooper, D., and Scherer, R. (**1984**). "Automatic extraction of glottographic waveform parameters and regression to acoustic and physiologic variables," in *Vocal Fold Physiology: Contemporary Research Clinical Issues*, edited by A. J. Bless (College Hill, San Diego), pp. 146–154.
Titze, I. R., Jiang, J., and Drucker, D. G. (**1988**). "Preliminaries to the body-cover theory of pith control," J Voice **1**, 314-319.
Titze, I. R., Jiang, J. J., and Hsiao, T. Y. (**1993**). "Measurement of mucosal wave propagation and vertical phase difference in vocal fold vibration," Ann Otol Rhinol Laryngol **102**, 28-63.
Titze, I. R., Riede, T., and Popolo, P. (**2008**). "Nonlinear source–filter coupling in phonation: Vocal exercises," J Acoust Soc Am **123**, 1902-1915.
Titze, I. R., and Story, B. H. (**1997**). "Acoustic interactions of the voice source with the lower vocal tract," J Acoust Soc Am **101**, 2234-2243.
Tokuda, I. T., Horácek, J., Švec, J. G., Herzel, H., and 013102. (**2008**). "Bifurcations and chaos in register transitions of excised larynx experiments," Chaos **18**, 013102.
Trendelenburg, W., and Wullstein, H. (**1935**). "Untersuchungen űber die Stimmbandschwingungen," Sitzungsberichte Preuss Akad Wiss Physikal Math Klasse **21**, 399.
Vampola, T., Laukkanen, A.-M., Horacek, J., and Svec, J. G. (**2011**). "Vocal tract changes caused by phonation into a tube: A case study using computer tomography and finite-element modeling," J Acoust Soc Am **129**, 310-315.
Vampola, T., and Svec, J. G. (**2013**). "Finite-element modelling based on CT measurement," Logopedics, phoniatrics, vocology **(in press)**.
Van den Berg, J. (**1958**). "Myoelastic-aerodynamic theory of voice production," J Speech Hear Res **3**, 227-244.
Van den Berg, J. W. (**1962**). "Modem research in experimental phoniatrics," Folia Phoniatr **14**.
Van den Berg, J. W. (**1968a**). "Register problems," Ann. N.Y. Acad. Sci **155**, 129-134.

Van den Berg, J. W. (**1968b**). "Sound production in isolated human larynges," Ann. N.Y. Acad. Sci. **155**, 18-27.

Van den Berg, J. W., and Tan, T. S. (**1959**). "Results of experiments wlth human larynges," Pract Otorhinolaryngol **25**.

Van den Berg, J. W., Vennard, W., Burger, D., and Shervanian, C. C. (**1960**). "Voice production. The vibrating larynx. (Instructional film)," (University of Groningen).

Vasconcelos, M. J. M., Ventura, S. M. R., Freitas, D. R. S., and Tavares, J. M. R. S. (**2011**). "Towards the Automatic Study of the Vocal Tract From Magnetic Resonance Images," J Voice **25**, 732742.

Ventura, S. M. R., Freitas, D. R. S., Ramos, I. M. A. P., and Tavares, J. M. R. S. (**2013**). "Morphologic Differences in the Vocal Tract Resonance Cavities of Voice Professionals: An MRI-Based Study," J Voice **27**, 132-140.

Weiss, O. (**1914**). "Űber die Entstehung der Vokale. II. Abhandlung Die organge im ausgeschnittenen Kehlkopfe," Arch Exp Klin Phonet **1**.

Wolfram, W., and Johnson, R. (**1982**). *Phonological analysis: Focus on American English* (Center for Applied Linguistics and Harcourt Brace Jovanovich, Washington, DC).

Yumoto, E., Kadota, Y., and Kurokawa, H. (**1993a**). "Infraglottic aspect of canine vocal fold vibration: effect of increase of mean airflow rate and lengthening of vocal fold," J Voice **7**, 311-318.

Yumoto, E., Kadota, Y., and Kurokawa, H. (**1993b**). "Tracheal view of vocal fold vibration in excised canine larynxes.," Arch Otolaryngol Head Neck Surg **119**, 73-78.

Yumoto, E., Kadota, Y., and Kurokawa, H. (**1995**). "Thyroarytenoid Muscle Activity and Infraglottic Aspect of Canine Vocal Fold Vibration," Arch Otolaryngol Head Neck Surg **121**, 759-764.

Yumoto, E., Oyamada, Y., Nakano, K., Nakayama, Y., and Yamashita, Y. (**2004**). "Three-dimensional characteristics of the larynx with immobile vocal fold," Arch Otolaryngol Head Neck Surg

130, 967-974.

Zhang, Y., Krausert, C. R., Kelly, M. P., and Jiang, J. J. (**2009**). "Typing vocal fold vibratory patterns in excised larynx experiments via digital kymography," Ann Otol Rhinol Laryngol **118**, 598-605.

Zhang, Y., Reynders, W. J., Jiang, J. J., and Tateya, I. (**2007**). "Determination of phonation instability pressure and phonation pressure range in excised larynges," J Speech Lang Hear Res **50**, 611-620.

Zhang, Z. Y., Neubauer, J., and Berry, D. A. (**2006**). "The influence of subglottal acoustics on laboratory models of phonation," Journal of the Acoustical Society of America **120**, 1558-1569.

Zuna, I., and Poušek, L. (**2002**). *Úvod do zobrazovacích metod v lékařské diagnostice* (Vydavatelství ČVUT, Praha).

Supplement A: Paper I

Užitný vzor No. 25585: Model subglottického traktu
Utility model No. 25585: Model of a subglottal tract

V. HAMPALA, J. G. ŠVEC, P. SCHOVÁNEK, D. MANDÁT

Úřad průmyslového vlastnictví / Industrial property office
Praha / Prague

(2013)

ČESKÁ REPUBLIKA
ÚŘAD PRŮMYSLOVÉHO VLASTNICTVÍ

OSVĚDČENÍ

O ZÁPISU UŽITNÉHO VZORU

J. Kratochvíl

Josef Kratochvíl
předseda
Úřadu průmyslového vlastnictví

Úřad průmyslového vlastnictví

zapsal podle § 11 odst. 1 zákona č. 478/1992 Sb., v platném znění, do rejstříku

UŽITNÝ VZOR

číslo

25585

na technické řešení uvedené v přiloženém popisu.

V Praze dne 24.6.2013

Za správnost:

Ing. Jan Mrva
vedoucí oddělení rejstříků

UŽITNÝ VZOR

(11) Číslo dokumentu:

25585

(13) Druh dokumentu: **U1**

(51) Int. Cl.:

G09B 23/14 (2006.01)
G09B 23/28 (2006.01)

(19) ČESKÁ REPUBLIKA

ÚŘAD PRŮMYSLOVÉHO VLASTNICTVÍ

(21) Číslo přihlášky: **2013 - 27834**
(22) Přihlášeno: **24.04.2013**
(47) Zapsáno: **24.06.2013**

(73) Majitel:
Univerzita Palackého, Olomouc, CZ

(72) Původce:
Hampala Vít Mgr., Uherské Hradiště, CZ
Švec Jan RNDr. Ph.D., Olomouc, CZ
Schovánek Petr RNDr., Lipník nad Bečvou, CZ
Mandát Dušan Mgr. Ph.D., Kokory, CZ

(74) Zástupce:
Ing. Petr Soukup, Vídeňská 8, Olomouc, 77200

(54) Název užitného vzoru:
Model subglotického traktu

CZ 25585 U1

Model subglotického traktu

Oblast techniky

Technické řešení spadá do oblasti biofyzikálních zařízení určených zejména k vytváření či zkoumání zvuku a týká se modelu subglotického traktu s nastavitelnými akustickými rezonancemi pro napodobení akustických vlastností vokálního traktu různých živočišných druhů.

Dosavadní stav techniky

Hlas je zvuk tvořený vlivem kmitání hlasivek, který je dále upraven rezonancemi v dutinách vokálního traktu. Velmi důležitým rezonančním prostorem, který významnou měrou ovlivňuje kmitání hlasivek a podílí se tak na vytváření hlasu, je subglotický trakt nacházející se pod hlasivkami, přičemž akustické vlastnosti subglotického traktu se liší dle živočišného druhu.

První pokusy o produkci lidského hlasového projevu byly uskutečněny již v 18. století, kdy jednak Ch. G. Kratzenstein sestrojil zařízení simulující samohlásky pomocí rezonanční trubky připojené na varhany a jednak Wolfgang von Kempelen ve své knize „Mechanismus der Sprache menschlichen nebst Beschreibung einer sprechenden Maschine“ prezentoval podrobný popis akustického stroje. Různé modely a zařízení simulující část vokálního ústrojí jsou známy například ze spisů US 2041487, US 2056295 A, US 3978286 A, US 5121434 A, WO 9418669 A1, EP 0287104 A1. Experimenty s tvorbou hlasu na preparátech hrtanu jsou popsány například v publikacích „M. Sovák: "Kmitání hlasivek ve světle laryngostroboskopie, Česká akademie věd a umění (Praha)" a „E. Vilkman: "An apparatus for studying the role of the cricothyroid articulation in the voice production of excised human larynges", Folia Phoniatr (Basel) 39 (4):169-177, 1987” nebo v „Horáček J., Švec J. G., Veselý J., "Bifurcation phenomena in vocal fold vibrations - experiments in vitro", In: Zolotarev I, editor, Proceedings Interaction and Feedbacks '2003, Prague: Institute of Thermomechanics AS ČR, 2003, p. 51-60”, dále v „Tokuda I. T., Horáček J., Švec J. G., Herzel H.: "Comparison of biomechanical modeling of register transitions and voice instabilities with excised larynx experiments", J Acoust Soc Am 2007; 122(1):519-31.“, nebo také v „Tokuda I. T.,Horáček J., Švec J. G., Herzel H.: "Bifurcations and chaos in register transitions of excised larynx experiments", Chaos 2008; 18(1): article no. 013102“, a rovněž i v „Sidlof P., Švec J. G., Horáček J., Veselý J., Klepáček I., Havlík R.: "Geometry of human vocal folds and glottal channel for mathematical and biomechanical modeling of voice production", J Biomech 2008; 41(5): 985-95“. Taktéž jsou známy publikace, kde jsou popsány modely hlasivek, například „Horáček J., Sidlof P., Uruba V., Veselý J., Radolf V., Bula V.: "Coherent structures in the flow inside a model of the human vocal tract with self-oscillating vocal folds", Acta Technica 2010; 55(4): 327-43” nebo v „Horáček J., Uruba V., Radolf V., Vesely J., Bula V.: "Airflow visualization in a model of human glottis near the self-oscillating vocal folds model", Applied and Computational Mechanics 2011; 5:21-8“ anebo v „Kniesburges S., Thomson S. L., Barney A., Triep M., Sidlof P., Horáček J., et al.: "In Vitro experimental investigation of voice production", Current Bioinformatics 2011; 6(3):305-22“. Nevýhodou řešení uvedených v těchto publikacích je skutečnost, že subglotická oblast je realizována tak, že vnitřní rezonanční prostor je neměnným prostorem a nelze jej tedy nastavovat.

Z publikace „Zhang, Z. Y., J. Neubauer, and D. A. Berry "The influence of subglottal acoustics on laboratory model sofphonation", Journal of the Acoustical Society of America 120.3 (2006): 1558-69“ je znám model vokálního systému s nastavitelnou délkou subglotického traktu. Základní délka subglotického traktu činí 32 cm s možností prodloužení 5 cm pomocí segmentové trubice, přičemž pro větší rozsah je model subglotického traktu vybaven U-trubicí prodlužující celkovou délku traktu z 60 na 325 cm. Nevýhodou tohoto řešení je skutečnost, že užitím dlouhé U-trubice je znemožněno napodobení fyziologických podmínek v lidském organismu, například rezonanční frekvence v rozmezí 500 až 600 Hz. Nevýhodou segmentované trubice je nepraktická manipulace, přičemž segmentovaná trubice umožňuje pouze skokovou změnu délky subglotického traktu.

Úkolem předkládaného technického řešení je představit konstrukční uspořádání délkově nastavitelného modelu subglotického traktu, který plynulým prodloužením či zkrácením rezonančních prostor a přesnějším nastavením délky U-trubice umožňuje lépe napodobit fyziologické podmínky organismu a akustické vlastnosti vokálního systému.

Podstata technického řešení

Stanoveného cíle je do značné míry dosaženo technickým řešením, kterým je model subglotického traktu napojený přes ohřívací a zvlhčovací jednotku na zdroj vzduchu a tvořený tělesem ve formě oboustranně otevřeného dutého válce, přičemž těleso je jednak ze strany zadního čela vsazeno do spodního uzávěru, jednak ze strany předního čela je upevněno v nástavci a jednak je opatřeno přívodním otvorem pro přívod vzduchu do vnitřního rezonančního prostoru, kde podstata řešení spočívá v tom, že nástavec sestává jednak z úzké výstupní hubice určené pro nasazení preparátu a jednak ze široké vstupní hubice uzpůsobené k upevnění tělesa, přičemž jednak ve vnitřním prostoru vstupní hubice nástavce je uložena vnitřní redukční vložka vytvořená ve tvaru oboustranně otevřeného dutého válce s kónicky tvarovanou redukční dutinou a jednak v rezonančním prostoru je ve směru podélné osy uložen píst.

Je výhodné, když píst sestává z válcovité pístové hlavice opatřené jednak obvodovým vybráním pro uložení O-kroužkového těsnicího členu přiléhajícího k vnitřní stěně tělesa a jednak středovým lůžkem pro upevnění tyčového pístového těla, přičemž pístové tělo je přes spodní uzávěr vyvedeno vně model subglotického traktu a svojí vně situovanou koncovou částí je vsazeno do manipulační matice.

Ve výhodném provedení redukční vložka obvodově přiléhá k vnitřnímu průměru vstupní hubice nástavce, přičemž vstupní otvor tvarově odpovídá vnitřnímu průměru tělesa a výstupní otvor má stejný průměr jako je vnitřní rozměr výstupní hubice.

V optimálním případě je těleso ze strany bočního čela vybaveno alespoň jedním výpustním otvorem a do tělesa je v místě přívodního otvoru vsazena přívodní trubice.

Rovněž je výhodné, když okolo vnějšího obvodu tělesa je umístěna alespoň jedna prstencová upevňovací matice a do spodního uzávěru je zabudován alespoň jeden aretační šroub.

Nový a vyšší účinek technického řešení spočívá v tom, že akustický model subglotického traktu poskytuje možnost ovládání změny délky rezonančních prostor pro kontrolu rezonančních frekvencí, které mají přímý vliv na kmitání hlasivek, čímž je umožněna kontrola nad důležitým aspektem experimentů mající za následek zvýšení kvality naměřených výsledků. S využitím konstrukčního prvku s plynule měnitelnou délkou rezonančního prostoru, například pístu, je možné provádět experimenty na hrtanech různých živočišných druhů.

Objasnění výkresů

Konkrétní příklady provedení technického řešení jsou schematicky znázorněny na připojených výkresech, kde:

obr. 1 je blokové schéma experimentální sestavy vokálního traktu,
obr. 2 je podélný řez modelem subglotického traktu, a
obr. 3 je podélný explodovaný řez modelem subglotického traktu.

Výkresy, které znázorňují představované technické řešení, a následně popsané příklady konkrétního provedení v žádném případě neomezují rozsah ochrany uvedený v definici, ale jen objasňují podstatu technického řešení.

Příklady uskutečnění technického řešení

Experimentální sestava vokálního traktu znázorněná na obr. 1 je tvořena zdrojem 1 vzduchu ve formě vzduchové pumpy či láhve stlačeného vzduchu, který je přes ohřívací a zvlhčovací jednotkou 2 propojen s modelem 3 subglotického traktu, na němž je upevněn preparát 4 hrtanu.

Model 3 subglotického traktu, znázorněný na obr. 2, je tvořen tělesem 31 ve formě oboustranně otevřeného dutého válce, které je ze strany zadního čela 311 vsazeno do spodního uzávěru 32 ve formě víka a ze strany předního čela 312 je rozebíratelně upevněno v dutém dvoustupňově tvarovaném nástavci 33. Nástavec 33 sestává jednak z úzké výstupní hubice 331, určené pro nasazení zde neznázorněného preparátu 4, a jednak z široké vstupní hubice 332, jejíž vnitřní průměr odpovídá vnějšímu průměru tělesa 31. Ve vnitřním prostoru 3321 vstupní hubice 332 je uložena vnitřní redukční vložka 34 vytvořená rovněž ve tvaru oboustranně otevřeného dutého válce, která jednak obvodově přiléhá k vnitřnímu průměru vstupní hubice 332 a jednak je opatřena kónicky tvarovanou redukční dutinou 341. Redukční vložka 34, určená pro plynulý přechod mezi vnitřním průměrem tělesa 31 a vnitřním průměrem výstupní hubice 331 nástavce 33, je vytvarována tak, že vstupní otvor 342 tvarově odpovídá vnitřnímu průměru tělesa 31 a výstupní otvor 343 má stejný průměr, jako je vnitřní rozměr výstupní hubice 331. Těleso 31 je dále ze strany bočního čela 313 vybaveno jednak spodním výpustním otvorem 315 a jednak přívodním otvorem 314 pro vedení zvlhčeného a ohřátého vzduchu do vnitřního rezonančního prostoru 38 modelu 3 subglotického traktu, přičemž do přívodního otvoru 314 je vsazena přívodní trubice 35 a v rezonančním prostoru 38 je ve směru podélné osy uložen píst 36. Samotný píst 36 sestává z válcovité pístové hlavice 361 opatřené jednak obvodovým vybráním 3611 pro uložení O-kroužkového těsnicího členu 362, který těsně přiléhá k vnitřní stěně tělesa 31, a jednak středovým lůžkem 3612 pro upevnění tyčového pístového těla 363. Pístové tělo 363 je přes spodní uzávěr 32 vyvedeno vně model 3 subglotického traktu, přičemž svojí vně situovanou koncovou částí je vsazeno do manipulační matice 364. Pro uchycení modelu 3 subglotického traktu k neznázorněnému vnějšímu objektu je okolo vnějšího obvodu tělesa 31 umístěna prstencová upevňovací matice 37 a pro polohování modelu 3 subglotického traktu je do spodního uzávěru 32 zabudován aretační šroub 39.

V průběhu experimentu je přívodní trubicí 35 naveden ohřátý a zvlhčený vzduch z ohřívací a zvlhčovací jednotky 2 do rezonančního prostoru 38 modelu 3 subglotického traktu. Vzduch poté rezonančním prostorem 38 plynule postupuje až do výstupní hubice 331 nástavce 33, kde vzduch opouští model 3 subglotického traktu a vstupuje do preparátu 4 hrtanu. Případnému úniku vzduchu mezi preparátem 4 hrtanu a nástavcem 33 brání vhodně řešený neznázorněný stahovací prvek. Procházející vzduch rozkmitá hlasivky v preparátu 4 hrtanu, které se tak stanou zdrojem akustického signálu, jež vybudí subglotické rezonanční prostory 38 modelu 3 subglotického traktu. Úprava akustických vlastností modelu 3 subglotického traktu, tedy zvýšení či snížení frekvence rezonančních maxim, se provádí pomocí pístu 36, kdy pomocí vnější manipulační matice 364 dochází v podélném směru k posunutí pístové hlavice 361, čímž dochází ke zkrácení či prodloužení vnitřního rezonančního prostoru 38. Aby preparát 4 hrtanu během experimentu nevysychal, je potřeba vzduch zvlhčovat pomocí fyziologického roztoku, přičemž ve spodní části trubicovitého tělesa 31 dochází k hromadění zkondenzovaného fyziologického roztoku a vody. Z tohoto důvodu obsahuje těleso 31 spodní výpustní otvor 315, sloužící jako výpusť.

Popsané provedení není jedinou možnou konstrukcí podle technického řešení, když průměr výstupní hubice 331 nástavce 33 může mít různou velikost v závislosti na velikosti preparátů 4 hrtanu, které se mohou lišit šířkou průdušnice. Pro uchycení modelu 3 subglotického traktu k neznázorněnému vnějšímu objektu může být okolo vnějšího obvodu tělesa 5 umístěna více než jedna prstencová upevňovací matice 37.

Rovněž pro polohování modelu 3 subglotického traktu může být použit více než jeden aretační šroub 39.

Průmyslová využitelnost

Model subglotického traktu je využitelný v oblasti biofyziky a lékařství, například pro studium kmitání hlasivek, a pro napodobování akustických vlastností subglotického traktu při experimentech s vypreparovanými hrtany.

NÁROKY NA OCHRANU

1. Model (3) subglotického traktu napojený přes ohřívací a zvlhčovací jednotku (2) na zdroj (1) vzduchu a tvořený tělesem (31) ve formě oboustranně otevřeného dutého válce, přičemž těleso (31) je jednak ze strany zadního čela (311) vsazeno do spodního uzávěru (32), jednak ze strany předního čela (312) je upevněno v nástavci (33) a jednak je opatřeno přívodním otvorem (314) pro přívod vzduchu do vnitřního rezonančního prostoru (38), **vyznačující se tím**, že nástavec (33) sestává jednak z úzké výstupní hubice (331), určené pro nasazení preparátu (4), a jednak ze široké vstupní hubice (332), uzpůsobené k upevnění tělesa (31), přičemž jednak ve vnitřním prostoru (3321) vstupní hubice (332) nástavce (33) je uložena vnitřní redukční vložka (34), vytvořená ve tvaru oboustranně otevřeného dutého válce s kónicky tvarovanou redukční dutinou (341), a jednak v rezonančním prostoru (38) je ve směru podélné osy uložen píst (36).

2. Model (3) subglotického traktu podle nároku 1, **vyznačující se tím**, že píst (36) sestává z válcovité pístové hlavice (361), opatřené jednak obvodovým vybráním (3611) pro uložení O-kroužkového těsnicího členu (362) přiléhajícího k vnitřní stěně tělesa (31) a jednak středovým lůžkem (3612) pro upevnění tyčového pístového těla (363), přičemž pístové tělo (363) je přes spodní uzávěr (32) vyvedeno vně model (3) subglotického traktu a svojí vně situovanou koncovou částí je vsazeno do manipulační matice (364).

3. Model (3) subglotického traktu podle nároků 1 a 2, **vyznačující se tím**, že redukční vložka (34) obvodově přiléhá k vnitřnímu průměru vstupní hubice (332) nástavce (33), přičemž vstupní otvor (342) tvarově odpovídá vnitřnímu průměru tělesa (31) a výstupní otvor (343) má stejný průměr jako je vnitřní rozměr výstupní hubice (331).

4. Model (3) subglotického traktu podle nároků 1 až 3, **vyznačující se tím**, že těleso (31) je ze strany bočního čela (313) vybaveno alespoň jedním výpustním otvorem (315).

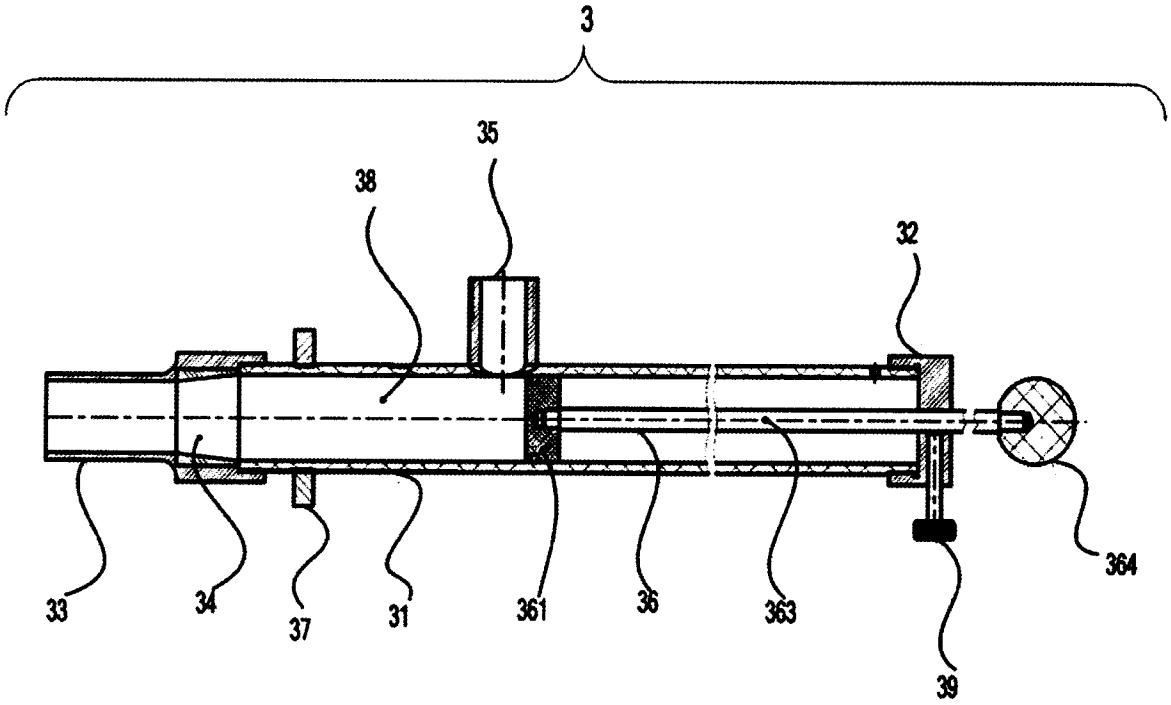
5. Model (3) subglotického traktu podle nároků 1 až 4, **vyznačující se tím**, že do tělesa (31) je v místě přívodního otvoru (314) vsazena přívodní trubice (35).

6. Model (3) subglotického traktu podle nároků 1 až 5, **vyznačující se tím**, že okolo vnějšího obvodu tělesa (31) je umístěna alespoň jedna prstencová upevňovací matice (37).

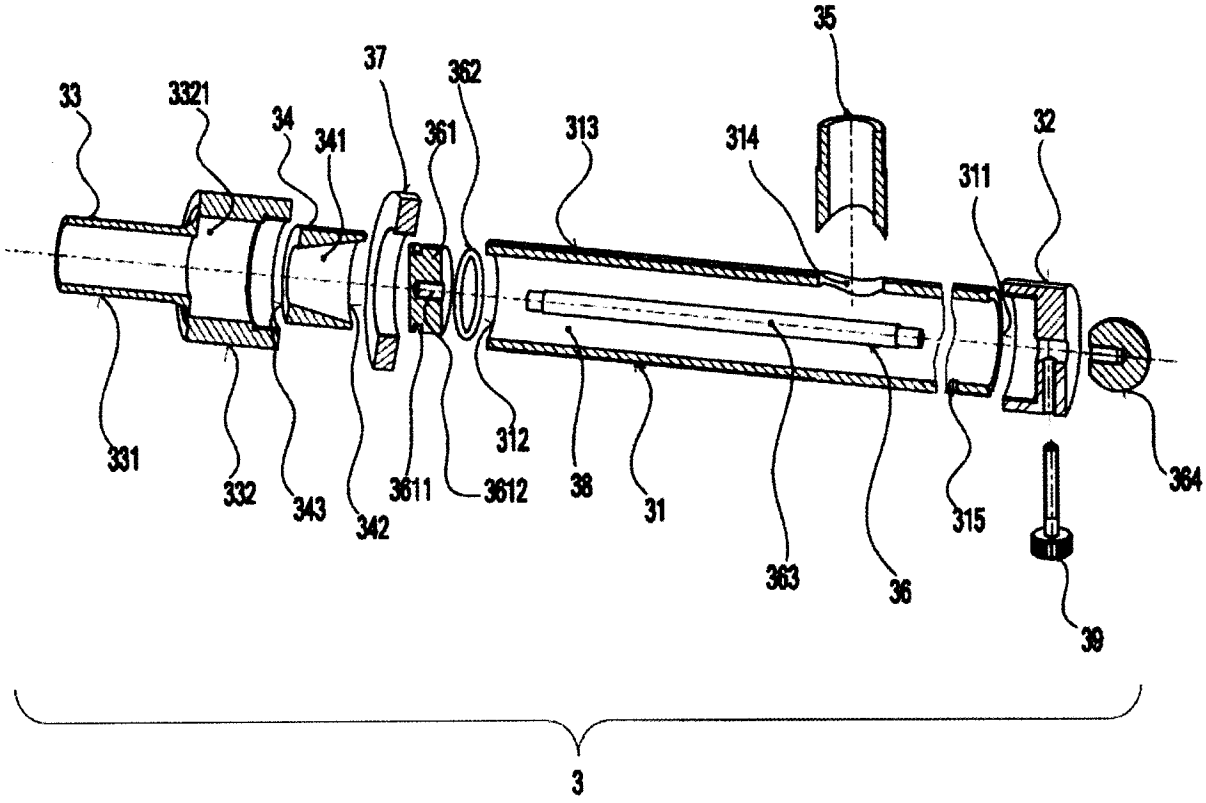
7. Model (3) subglotického traktu podle nároků 1 až 6, **vyznačující se tím**, že do spodního uzávěru (32) je zabudován alespoň jeden aretační šroub (39).

3 výkresy

OBR. 1



OBR. 2



OBR. 3

Konec dokumentu

Supplement B: Paper II

Relationship Between the Electroglottographic Signal and Vocal Fold Contact Area

V. HAMPALA, M. GARCIA, J. G. ŠVEC, R. C. SCHERER, C. T. HERBST

Journal of Voice

(2015, in press)

Relationship Between the Electroglottographic Signal and Vocal Fold Contact Area

*Vít Hampala, †Maxime Garcia, *Jan G. Švec, ‡Ronald C. Scherer, and *Christian T. Herbst, *Olomouc, Czech Republic, †Vienna, Austria, ‡Bowling Green, Ohio

Summary: Objective. Electroglottography (EGG) is a widely used noninvasive method that purports to measure changes in relative vocal fold contact area (VFCA) during phonation. Despite its broad application, the putative direct relation between the EGG waveform and VFCA has to date only been formally tested in a single study, suggesting an approximately linear relationship. However, in that study, flow-induced vocal fold (VF) vibration was not investigated. A rigorous empirical evaluation of EGG as a measure of VFCA under proper physiological conditions is therefore still needed.

Methods/design. Three red deer larynges were phonated in an excised hemilarynx preparation using a conducting glass plate. The time-varying contact between the VF and the glass plate was assessed by high-speed video recordings at 6000 fps, synchronized to the EGG signal.

Results. The average differences between the normalized [0, 1] VFCA and EGG waveforms for the three larynges were 0.180 (±0.156), 0.075 (±0.115), and 0.168 (±0.184) in the contacting phase and 0.159 (±0.112), −0.003 (±0.029), and 0.004 (±0.032) in the decontacting phase.

Discussions and conclusions. Overall, there was a better agreement between VFCA and the EGG waveform in the decontacting phase than in the contacting phase. Disagreements may be caused by nonuniform tissue conductance properties, electrode placement, and electroglottograph hardware circuitry. Pending further research, the EGG waveform may be a reasonable first approximation to change in medial contact area between the VFs during phonation. However, any quantitative and statistical data derived from EGG should be interpreted cautiously, allowing for potential deviations from true VFCA.

Key Words: Electroglottography–Vocal fold contact area–Excised hemilarynx–High-speed imaging.

INTRODUCTION

Electroglottography (EGG) is a noninvasive method to assess the vibratory behavior of the vocal folds (VFs) during voice production, introduced 6 decades ago by Fabre.[1] For acquiring the electroglottographic signal, two electrodes are placed at either side of the thyroid cartilage at the level of the VFs, and a low-amperage[2] frequency-modulated (0.3–5 MHz) current is passed between them. Variations in vocal fold contact area (VFCA) during the glottal cycle introduce variations in the electrical admittance across the larynx, resulting in variation in the current between the two electrodes. These variations of the electrical admittance are proportional to the resulting EGG waveform.[3–6]

Experimental research[7–15] suggests that there is a relation between kinematic events of the oscillating VFs and stereotypical landmarks in the EGG waveform (Figure 1). These findings were corroborated by modeling studies.[16–20]

Because of its low cost and noninvasive nature, electroglottography is an attractive alternative to direct (and thus more invasive) methods for observing VF vibration, such as videostrobolaryngoscopy,[21] videokymography,[22] or high-speed laryngeal videoendoscopy,[23–27] leading to an increasing number of publications using EGG as a primary data acquisition method in the recent past.[28–36] EGG-based studies are conducted under the assumption that the acquired EGG signal closely represents the relative VFCA during phonation. This is particularly crucial for quantitative methods analyzing the EGG waveform, for example, for calculating the EGG contact quotient[37,38] or the EGG contact index.[39]

Surprisingly, the putative direct relation between the EGG waveform and VFCA has, to the knowledge of the authors, to date only been formally investigated in a single study,[40] suggesting an approximately linear relationship between the change in VFCA and the EGG signal. This study investigated two canine hemilarynx specimens, using a mechanical arm to drive the VF against a conductive glass pane resulting in a sinusoidal signal at 10–30 Hz. Flow-induced VF vibration was not used. Furthermore, the study was conducted with videostroboscopy, that is, an aliasing technique that only provides an illusion of vibration, usually at a rate of 1 Hz, documented by 25 video frames per second. This suggests that, strictly speaking, a formal empirical evaluation of EGG as a measure of relative VFCA under more physiological conditions is lacking.

To address this issue, this study was concerned with the question whether the EGG waveform is a reliable physiological correlate of the relative VFCA during flow-induced sustained VF oscillation, using an excised hemilarynx setup, a conducting glass plate, and high-speed video (HSV) recording.

Accepted for publication March 30, 2015.
This article will be presented at the 44th Annual Symposium: Care of the Professional Voice, Philadelphia, PA, May 2015.
From the *Voice Research Lab, Department of Biophysics, Faculty of Science, Palacký University, Olomouc, Czech Republic; †Laboratory of Bio-Acoustics, Department of Cognitive Biology, University of Vienna, Vienna, Austria; and the ‡Department of Communication Sciences and Disorders, 200 Health and Human Services, Bowling Green State University, Bowling Green, Ohio.
Address correspondence and reprint requests to Vít Hampala, Katedra biofyziky, Přírodovědecká fakulta, Univerzita Palackého v Olomouci, Šlechtitelů 586/11, 783 71 Olomouc, Holice. E-mail: vit.hampala@gmail.com
Journal of Voice, Vol. ■, No. ■, pp. 1-11
0892-1997/$36.00

http://dx.doi.org/10.1016/j.jvoice.2015.03.018

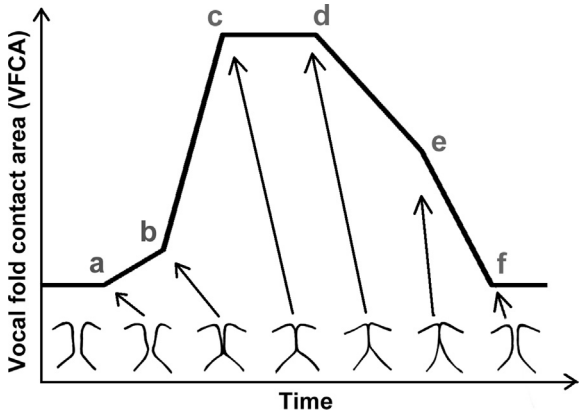


FIGURE 1. Landmarks in the EGG waveform according to Baken and Orlikoff[4] and Berke et al[20]: a—initial contact of the lower vocal fold (VF) margins; b—initial contact of the upper VF margins; c—maximum VF contact reached; d—decontacting phase initiated by separation of the lower VF margins; e—upper margins start to separate; and f—glottis is open, the contact area is minimal.

METHODS

Larynx specimens and experimental setup

The larynges investigated in this experiment came from three female red deer (*Cervus elaphus*) specimens. The choice of species was justified by results of a recently published study, suggesting that the EGG signal from a red deer larynx is comparable with that of humans and canines.[41] The deer were culled during an authorized third party hunt near Allentsteig, Lower Austria. The larynges were immediately harvested on site and were transported in ice boxes to the Department of Cognitive Biology, University of Vienna, where they were flash-frozen using liquid nitrogen[42] and then stored at −80°C for further use.

Each larynx was slowly thawed immediately before the experiment and cleaned (ie, the hyoid bone and epiglottis were removed as well as surplus extrinsic muscle tissue). For creating the hemilarynx, the left half of the thyroid cartilage, the left arytenoid cartilage, and a part of the cricoid cartilage were removed. This incision formed an L-shaped mortice allowing the larynx to be glued to a conductive glass plate (Figure 2) using dental cement (Kukident Super-Haftcreme complete; Reckitt Benckiser Deutschland GmbH, Mannheim, Germany). Another (nonconductive) glass plate was perpendicularly glued to the conductive glass plate, forming an L-shaped structure. To ensure good visibility of the VF from the top-view camera, the soft tissue above the VF (ie, the ventricular fold) was removed. The trachea was cut at the fourth tracheal ring, and the larynx was vertically mounted on an air supply tube as described in a previous publication[43] (Supplementary materials). The adduction of the VF was facilitated by use of a prong as described by Titze.[42]

The schematic of the experimental setup for obtaining the electroglottographic (EGG) signal is illustrated in Figure 2E. The EGG electrode attachment to the conductive glass plate and the hemilarynx, respectively, is shown in Figure 2A–D. The glass plate used in this experiment was covered with a layer of titanium-tin oxide, having a sheet resistance of 40 Ohms per square.

Self-sustained VF vibration was established by blowing warmed and humidified air through the hemiglottis. Subglottal pressure was varied via computer-controlled pressure sweeps in the range of 0–5 kPa (about 0–50 cm H_2O).

Data acquisition

The EGG signal was acquired with a Glottal Enterprises EG2-1000 electroglottograph (Glottal Enterprises, Syracuse, NY).

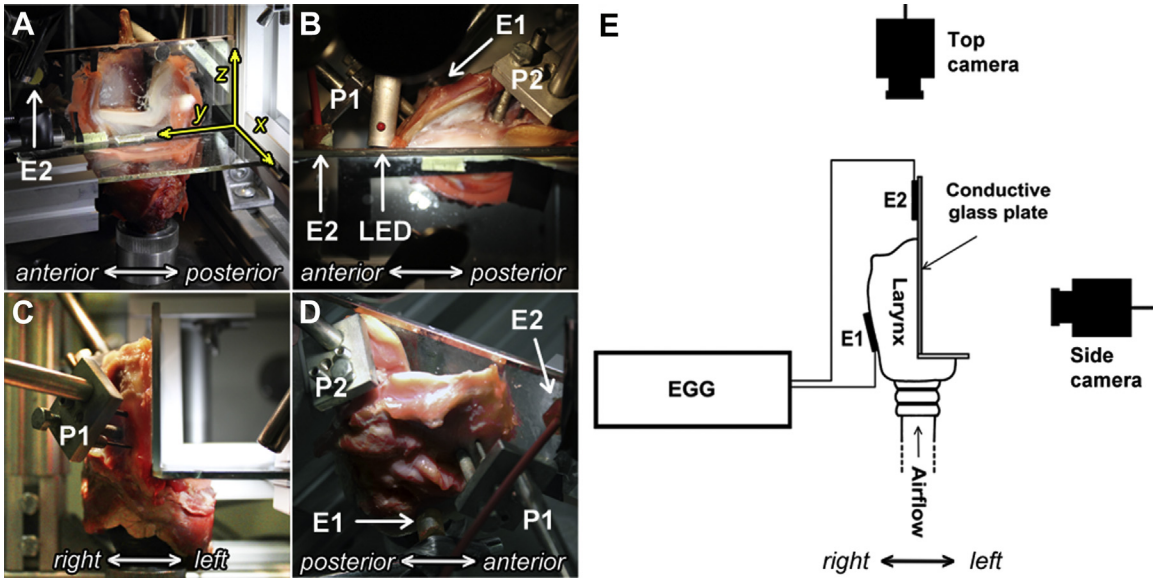


FIGURE 2. Hemilarynx setup with conductive glass plate used in this study: (**A**) side view through the glass. The orientation of the x, y, and z axis (eg, Equation 1) was adopted from Titze[42]; (**B**) top view; (**C**) anterior view; (**D**) anterolateral view (*right* side of hemilarynx attached to glass plate); and (**E**) schematic illustration of the experimental setup. E1—EGG electrode attached to muscles around the thyroid cartilage; E2—EGG electrode attached to the glass plate; LED—LED diode used for synchronization; P1—prong stabilizing the thyroid cartilage; P2—prong adducting VF.

The device's high-pass filter was set to 2 Hz, thus minimizing signal distortion introduced by the filter. To avoid clipping of the EGG signal, custom-built electrodes with a reduced diameter of 11 mm (Glottal Enterprises, Syracuse, NY) were used, and a 100-Ohm resistor was inserted in series into the wire of one of the electrodes (Martin Rothenberg, personal communication). One electrode was placed on the muscle tissue around the thyroid cartilage (Figure 2, label E1), and the other one was clipped onto the conductive layer of the glass plate (Figure 2, label E2). Spectra 360 electrode gel (Parker Laboratories, Inc., Fairfield, NJ) was applied to both electrodes.

The EGG signal was recorded with a Dewe-43 A/D data acquisition card (Dewetron, Munich, Germany) at a sampling frequency of 44 100 Hz.

Synchronized HSV recordings were acquired at a rate of 6000 fps from two positions (top view and side view), using two cameras: a MotionBLITZ LTR1 portable camera (Mikrotron, Unterschleissheim, Germany) for the side view and a MotionBLITZ Cube7 camera (Mikrotron, Unterschleissheim, Germany) for the top view. Illumination was provided by three devices: a Cymo 7300.03 300W Xenon Light Source (Cymo, Groningen, The Netherlands), a custom-built array of twelve 5W MR16 LED bulbs (SLV Elektronik GmbH, Übach-Palenberg, Germany) powered by a 12-V car battery, and one standard flexible one-bulb LED table lamp. Both camera images contained a 10-mm scale used for spatial calibration of the recordings.

The average subglottal pressure was measured with a Keller PR-41X pressure sensor (Keller, Winterthur, Switzerland) positioned 32 cm upstream from the VFs and captured with a LabJack U6 data acquisition card (LabJack Corporation, Lakewood, CO, USA).

The synchronization between all used data acquisition systems was achieved via a transistor-transistor-logic (TTL) signal generated by the LabJack U6 data acquisition card. This TTL signal, which consisted of pulses of approximately 20 ms duration, encoding the running recording time, was routed through an IC555 timer circuit (pulse rise time $\leq$ 15 ns) and was recorded simultaneously by all recording devices in dedicated channels. In the MotionBLITZ LTR1 portable camera system (ie, the side view camera), the TTL signal was electronically encoded in one pixel in the top left part of the recorded image, and that signal was later extracted with custom-built software to aid in aligning the camera data with the other signals. In the case of the MotionBLITZ Cube7 camera (ie, the camera providing the top view), the TTL signal was used to drive a LED visible in the acquired video data (labeled "LED" in Figure 2). The LED signal was extracted from the respective video data with custom-built software. The maximum synchronization error was calculated to be 0.167 ms, that is, the time delay between two consecutive video frames at a video frame rate of 6000 fps. For more details on the synchronization approach, see the supplementary material in a previous publication.[43]

Data analysis

For the three larynges used in this experiment, a set of five, seven, and one recording(s) were obtained, respectively. Small variations in light position greatly influenced the quality of the side-video recording showing the VFCA. Therefore, these video recordings were inspected without knowledge of the corresponding EGG signal before data analysis. For each larynx, the selected sequence was where the outline of VFCA was optimally visible throughout three consecutive glottal cycles of periodic vibration. For each of the three larynges, one resulting sequence (denoted as L1, L2, and L3 for the remainder of this article) was thus subjected to further analysis.

The synchronized video recordings from both cameras (top view and side view) were extracted and merged into one video. Because the contours of the VFCA were not always clearly delineated, a manual instead of an automatic image analysis method was used. The VFCA contours were manually annotated by three experimenters (authors V.H., M.G., and C.T.H.) using custom-built scripts within the FIJI[44] image processing framework. All the experimenters annotated the same video frames within the three merged recordings, covering one VF oscillation cycle per recording, without previous knowledge of the respective EGG signals. On average, the manual annotation of one full vibratory cycle took about two full working days per experimenter.

The main purpose of the top view camera was to clearly identify the anterior-posterior (A-P) extension of VF contact and to use this information when annotating the VFCA from the side view HSV data. Because the raw video data from the two cameras did not share common spatial dimensions and zoom factors, a method was devised to spatially relate the top view HSV data to the side view video data. Before video annotation, a common base point along the A-P dimension, carefully chosen to be clearly present in both camera views, was defined for each larynx (denoted y_{0T} for the *top view* and y_{0S} for the *side view*, Figure 3). Using this information, the relation between points in the *top view* (y_T) and the *side view* (y_S) along the A-P dimension (ie, along the y-axis as established in Figure 2A), expressed in pixels, was defined as:

$$y_S = y_{0S} - \frac{\lambda_S(y_{0T} - y_T)}{\lambda_T}[px], \quad (1)$$

where λ_T and λ_S are the equivalent measured distances of 5 mm expressed in pixels for *the top and the side view*, respectively. The process of relating the extensions of the VF contact along the A-P dimension (as seen in the *top view*) to the *side view* is illustrated in Figure 3.

The annotation process was conducted in two iterations. After the first iteration, the experimenters discussed their independently obtained VFCA contours to achieve a consensus if possible and then revised their annotations where appropriate during the second iteration. Every second video frame was annotated in the decontacting phase of the cycle, but every frame was annotated in the contacting phase because VF contacting generally takes place over a shorter time frame.

After the second annotation iteration, disagreements between the experimenters were resolved as follows: A pixel within the side-view video was considered to be contacting with the glass plate when at least two experimenters included that pixel in their suggested VFCA measure. This criterion was applied to all pixels in each video frame. Thus counting all the pixels

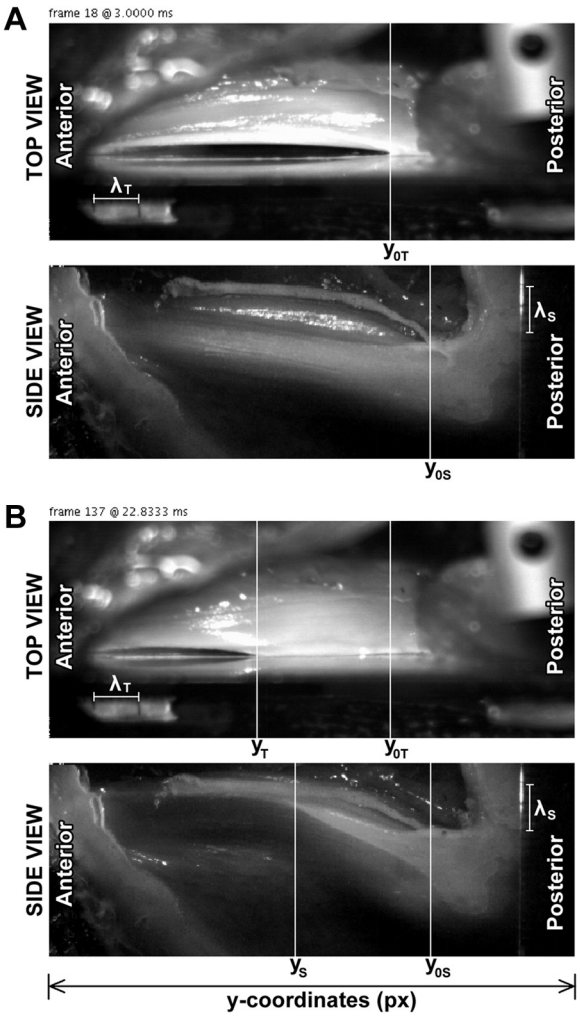


FIGURE 3. Illustration of coordinate translation between the camera top and side views. First, a baseline measure was taken (**A**) on the y-axis coordinate (as defined in Figure 2A) in both the top and the side view (y_{0T} and y_{0S}, respectively), based on clear landmarks (ie, the most anterior vocal fold contact in the posterior glottis at the moment of minimum vocal fold contact). Then, other vocal fold contact landmarks along the y-axis coordinates of the top view were marked (**B**, top) in subsequent video frames (y_T), and their corresponding position was calculated and annotated in the side view (y_S; **B**, bottom) on the basis of Equation 1, using the scales in both the top and the side view (λ_T and λ_S, respectively).

considered to be "contacting the glass" within a video frame resulted in the overall VFCA measure ($VFCA_{OVR}$, expressed in pixels) for each analyzed video frame in each sequence.

An objective quality criterion for the agreement among the experimenters within each video frame was established by using a custom standard deviation σ_{CUSTOM}, viz,

$$\sigma_{CUSTOM} = \sqrt{\frac{\sum_{i=1}^{n} (VFCA_i - VFCA_{OVR})^2}{n}}, \quad (2)$$

where n = 3, that is, the number of experimenters. σ_{CUSTOM} is then related to $VFCA_{OVR}$ by a custom coefficient of variation X, viz,

$$X = \frac{\sigma_{CUSTOM}}{\max(VFCA_{OVR})} \quad (3)$$

If the criterion X were >0.05 (ie, >5% of the total value range of $VFCA_{OVR}$ of the glottal cycle), the $VFCA_{OVR}$ of the respective video frame was flagged as unreliable.

RESULTS

VFCA measurements

VFCA measurement data for the three larynges (L1, L2, and L3) are shown in Figure 4 and Supplementary movies M1–M3. One glottal cycle was analyzed per each larynx. The *left panels* of Figure 4A–C depict VFCA for consecutive frames as measured by the three experimenters (E1–E3, *gray points*) and the resulting overall VFCA ($VFCA_{OVR}$, *black points*). Because of insufficient illumination in the video, the first few video frames of the contacting phase of L2 were not analyzed.

Overall, there was a good agreement between the three experimenters, with the exception of the late decontacting phase in larynx L1, where experimenter E3 measured higher values of VFCA. The right panels of Figure 4A–C show the quality criterion X (Equation 3) for larynges L1–L3. The threshold of 0.05 (*dashed line*) was only exceeded in larynx L1 in three of the 48 analyzed video frames, caused by the disagreement between the experimenters in the VFCA measures of the decontacting phase.

Comparison of VFCA and EGG waveforms

The relationship between the normalized (to the range between 0 and 1) EGG waveform (EGG_{norm}) and the normalized overall VFCA ($VFCA_{OVRnorm}$) for the three larynges L1–L3 is shown in Figure 5. In all three larynges, the EGG_{norm} waveform precedes the $VFCA_{OVRnorm}$ during the closing phase of the cycle (Figure 5, *left panels*). This is reflected by the large difference between EGG_{norm} and $VFCA_{OVRnorm}$ at the beginning of the glottal cycle for all three larynges (Figure 5, *middle and right panels*, and Table 1). The maximum disagreement in the contacting phase was about 0.42, 0.40, and 0.54 in the three larynges (Table 1), amounting to about half the possible value range [0, 1] (Figure 6).

In the relatively longer decontacting phase, larynges L2 and L3 showed a good agreement (ie, differences mostly smaller than 5% of the normalized total amplitude) between EGG_{norm} and $VFCA_{OVRnorm}$ (Figure 5 and Table 1). In contrast, data from larynx L1 exhibited a considerable disagreement (ie, differences ranging from 0% to 33% of the normalized total amplitude) between EGG_{norm} and $VFCA_{OVRnorm}$. When averaging over time, the difference between these two normalized signals was about 16% of the possible value range of [0, 1] in larynx L1 (Table 1).

When correlating EGG_{norm} and $VFCA_{OVRnorm}$ (*right panels* in Figure 5), an ideal agreement between these two signals would result in a linear correlation with a slope of 1 (*dashed line* in the *right panels* of Figure 5). This condition is fulfilled by the data from the decontacting phase of larynges L2 and L3 but not for the decontacting phase of larynx L1 and for the contacting phase of all three larynges (*black circles* with superimposed plus signs in Figure 5).

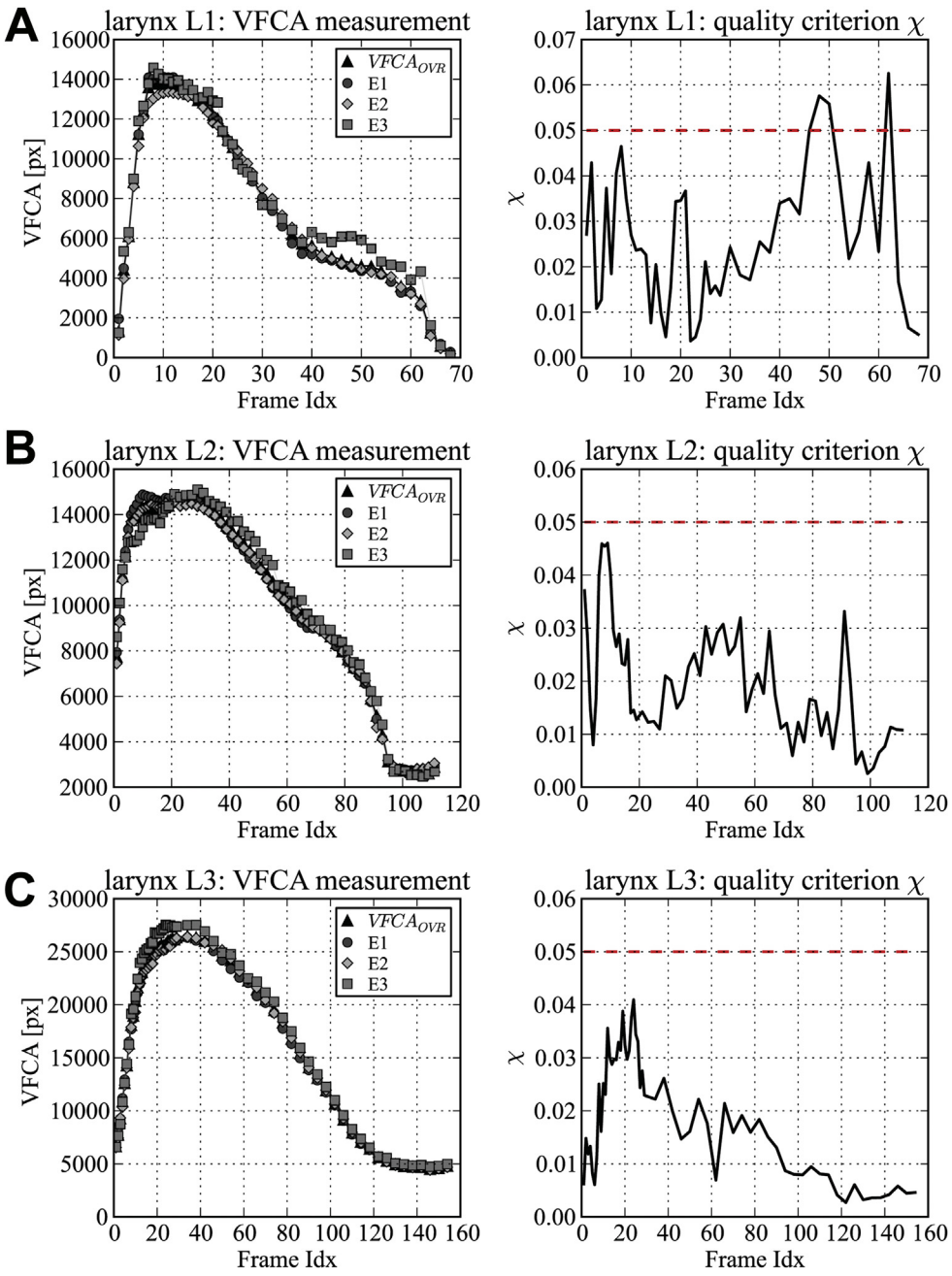


FIGURE 4. (**A**–**C**): Results from VFCA measures for the three larynges (L1–L3), respectively. *Left*—VFCA annotation results from the three experimenters (E1, E2, and E3), with $VFCA_{OVR}$ superimposed (see text); *right*—quality criterion (*dashed line*) for expressing reliable $VFCA_{OVR}$ measurements for all annotated frames.

DISCUSSION

This study investigated whether the EGG signal is an exact representation of the relative VFCA during flow-induced self-sustaining VF oscillation. On the basis of the collected data, this research question cannot be answered conclusively. On the one hand, there was good agreement between EGG_{norm} and $VFCA_{OVRnorm}$ during a major portion of the glottal cycle in two of the three larynges, supporting the relatively linear relationship between the EGG waveform and VFCA found by Scherer et al.[40] This was contrasted with noteworthy discrepancies between these two signals in larynx L1 and in the contacting phase in all three larynges.

Systematic disagreement between EGG and VFCA in the contacting phase

The systematic disagreement between EGG_{norm} and $VFCA_{OVRnorm}$ in the contacting phase in all three larynges was brought about by a temporal offset between the two signals and by a steeper signal slope in the EGG signal. A synchronization issue between the EGG signal and the HSV recording was ruled out by careful post hoc assessment of the synchronization between these two systems (cf supplementary materials in the study by Herbst[41]), confirming the estimated synchronization error of ± 0.167 ms, that is, the duration of one video frame at 6000

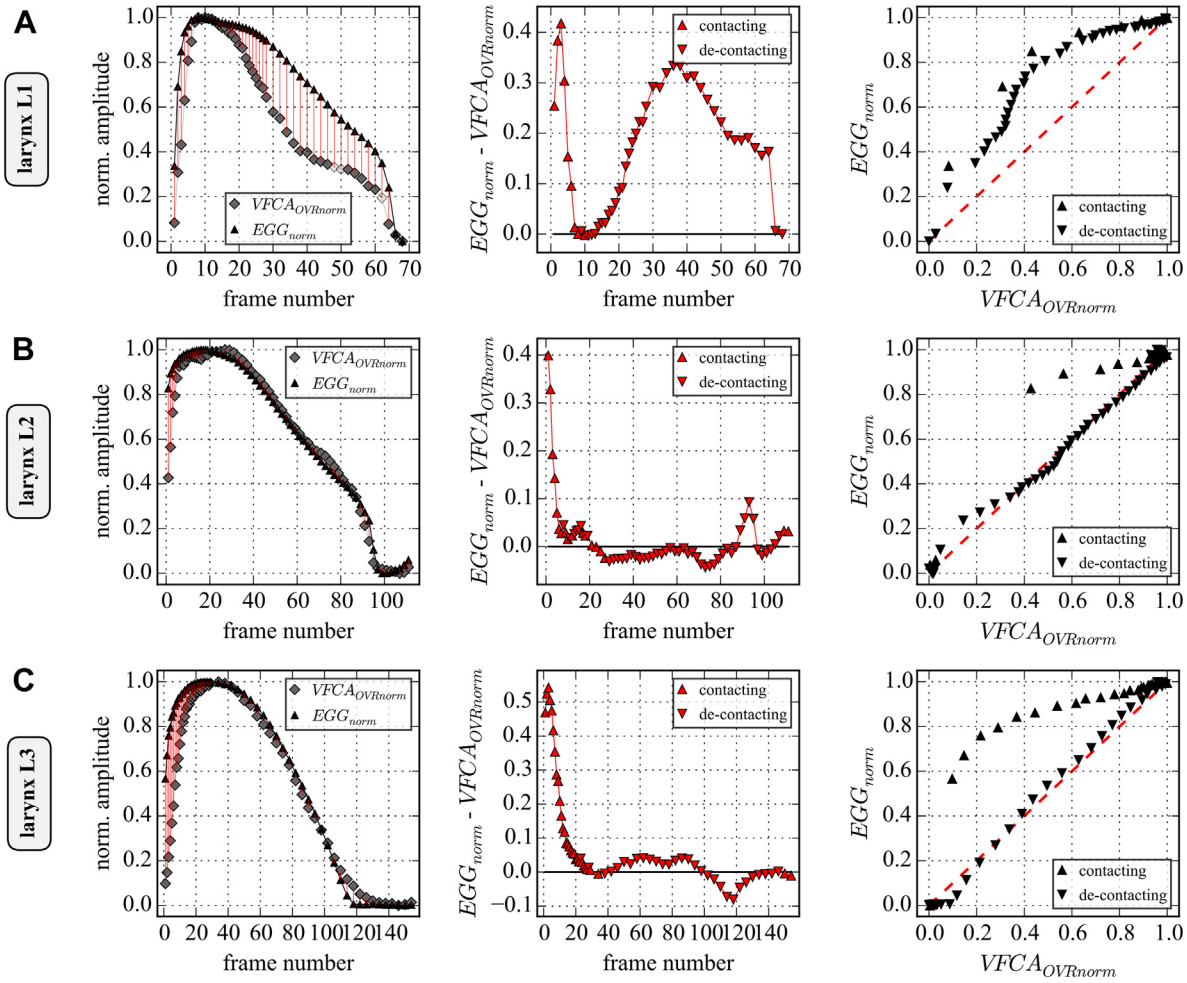


FIGURE 5. (**A**–**C**): Comparison and relations between normalized EGG (EGG_{norm}) and VFCA ($VFCA_{OVRnorm}$) waveforms for the three larynges L1, L2, and L3. *Left panels*: EGG_{norm} (*black triangles*) and $VFCA_{OVRnorm}$ (*dark gray and light gray diamonds* for video frames where the quality criterion *X* was below and above 0.05, respectively). *Middle panels*: difference between EGG_{norm} and $VFCA_{OVRnorm}$ for each analyzed video frame. *Right panels*: relation between $VFCA_{OVR}$ and EGG waveform.

fps. Examination of the difference between EGG_{norm} and $VFCA_{OVRnorm}$ (*middle panels* in Figure 5) reveals a time shift of about five video frames in larynges L1 and L2 and about 10 video frames in larynx L3 over which the major disagreement between the two signals occurs. This clearly shows that this difference cannot be explained by the synchronization error alone. As a potential alternative explanation, capacitance of the electroglottograph's hardware circuits might have caused this phenomenon. The EGG device used in this study internally applies a 4000-Hz low-pass signal filter before putting out the EGG signal (personal communication with Martin Rothenberg, Glottal Enterprises), as well as a high-pass filter with a controllable cutoff frequency (see Methods). These circuits might introduce systematic waveform changes into the recorded EGG signal.

Overall disagreement between EGG and VFCA in larynx L1

The considerable overall disagreement between EGG and VFCA in larynx L1 might have been caused by a number of reasons.

The time-varying VFCA was assessed by three experimenters, and their judgments were not always unanimous (Figure 4). In particular, in larynx L1, there was a slightly greater disagreement during the decontacting phase. However, this intrarater disagreement caused the quality criterion *X* to exceed the defined threshold of 0.05 only after frame 48, whereas the disagreements between EGG and VFCA in larynx L1 already start around frame 20. Furthermore, the intrarater disagreement amounted to about 10% of the $VFCA_{OVR}$ in the respective frames, whereas the disagreement between EGG_{norm} and $VFCA_{OVRnorm}$ ranged from 20% to 30% (Figure 5A, *middle panel*). Finally, inspection of Supplementary movie M1 suggests that the HSV recording was taken with optimal lighting conditions and that there was a good match between the visible VFCA and the estimated $VFCA_{OVR}$, ruling out human error in the estimation of that measure.

To rule out data for larynx L1 having come from an atypical glottal cycle during the recording, the acquired material was subjected to an additional test: Three more glottal cycles of that recording (extracted at offsets of 0.02 seconds, 1.4

TABLE 1.
Average, Standard Deviation, and Absolute Maximum of Differences Between EGG_{norm} and $VFCA_{OVRnorm}$ in Larynges L1–L3 in the Contacting and Decontacting Phases, Respectively

Part of Glottal Cycle	Measure	Larynx L1	Larynx L2	Larynx L3
Entire glottal cycle	Number of video frames	68	111	154
	Number of frames analyzed	48	65	60
Contacting phase	Number of frames analyzed	9	18	30
	Average difference	0.180	0.075	0.168
	Standard deviation	0.156	0.115	0.184
	Absolute maximum	0.418	0.399	0.542
Decontacting phase	Number of frames analyzed	39	47	30
	Average difference	0.159	−0.003	0.004
	Standard deviation	0.112	0.029	0.032
	Absolute maximum	0.334	0.093	0.081

seconds, and 8 seconds from the analyzed cycles) were annotated by experimenter E1. The newly acquired VFCA waveforms did not differ substantially from the data acquired for larynx L1 by all three experimenters, suggesting that the data presented in Figure 4A represents typical vibratory patterns of larynx L1.

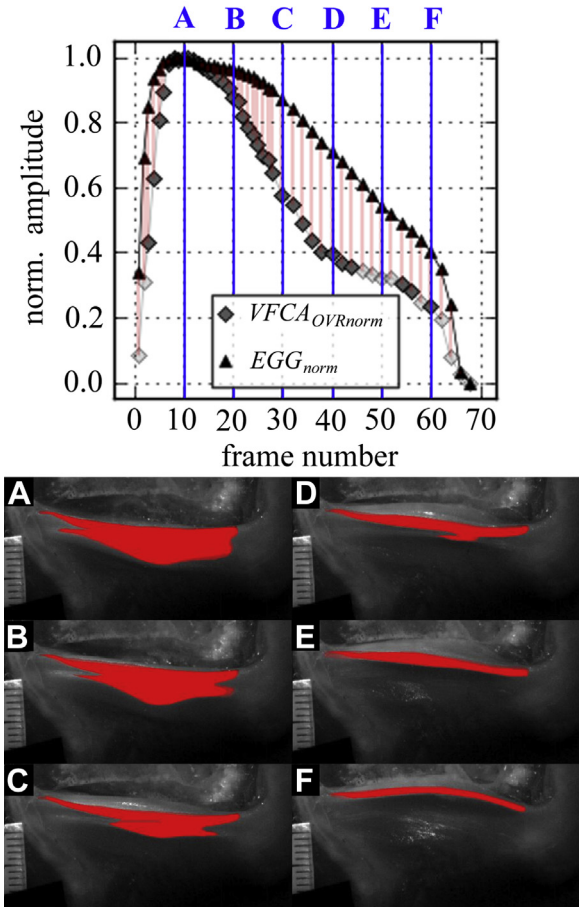


FIGURE 6. Development of the VFCA shape during the glottal cycle in the problematic larynx L1. *Top*: EGG_{norm} (*black triangles*) and $VFCA_{OVRnorm}$ (*gray diamonds*); *Bottom* (**A**–**F**): high-speed video images (side-view camera) with superimposed $VFCA_{OVR}$, extracted at every 10th video frame during the glottal cycle.

In principle, the discrepancy of EGG and VFCA in larynx L1 may have been induced by nonuniform conductance properties of the used conducting glass plate. However, larynx L1 was the first specimen investigated in the project, and the glass plate used was in pristine condition as provided by the Joint Laboratory of Optics, Palacky University, Olomouc, the Czech Republic. Thus, the influence of a potential glass plate conductance issue was considered to be unlikely.

The observed disagreement between EGG and VFCA could have also been brought about by nonlinear conductivity patterns: Inspection of the available video material revealed that the discrepancy between EGG and VFCA arose when the inferior portion of the VF lost contact (Supplementary movie M1). This observation gave rise to the speculation that the superior and the inferior VF section might have had different conductivity properties, thus contributing differently to the overall VFCA.

This notion was tested quantitatively by dividing VFCA of larynx L1 into a superior and an inferior portion ($VFCA_{UPPER}$ and $VFCA_{LOWER}$, respectively), by considering a clearly visible sulcus-like horizontal groove in the investigated VF as the demarcation line between the two areas (Supplementary movie M1 and Figure 6). On the basis of the two partial VFCA time series, the weighted VFCA was calculated as

$$VFCA_{WEIGHTED} = f \cdot VFCA_{UPPER} + (1 - f) \cdot VFCA_{LOWER} \quad \text{(Eq. 4)}$$

by varying the weighting factor f in the range of [0, 1] in steps of 0.05. Each of the resulting $VFCA_{WEIGHTED}$ time series was normalized and compared with the normalized EGG signal, and the sum of the squared difference between these two time series was calculated. This least-squares error approach suggested a near perfect fit for $f \approx 0.8$ (Figure 7). In that scenario, the lower VF portion's contribution to the overall VFCA is only a quarter of the upper VF portion.

Such a phenomenon could potentially be explained in relation to the placement of the EGG electrodes: In EGG, the current across the larynx follows an electric field having its maximum density along the most direct path between the electrodes.[19] The further an electric field line is removed from that

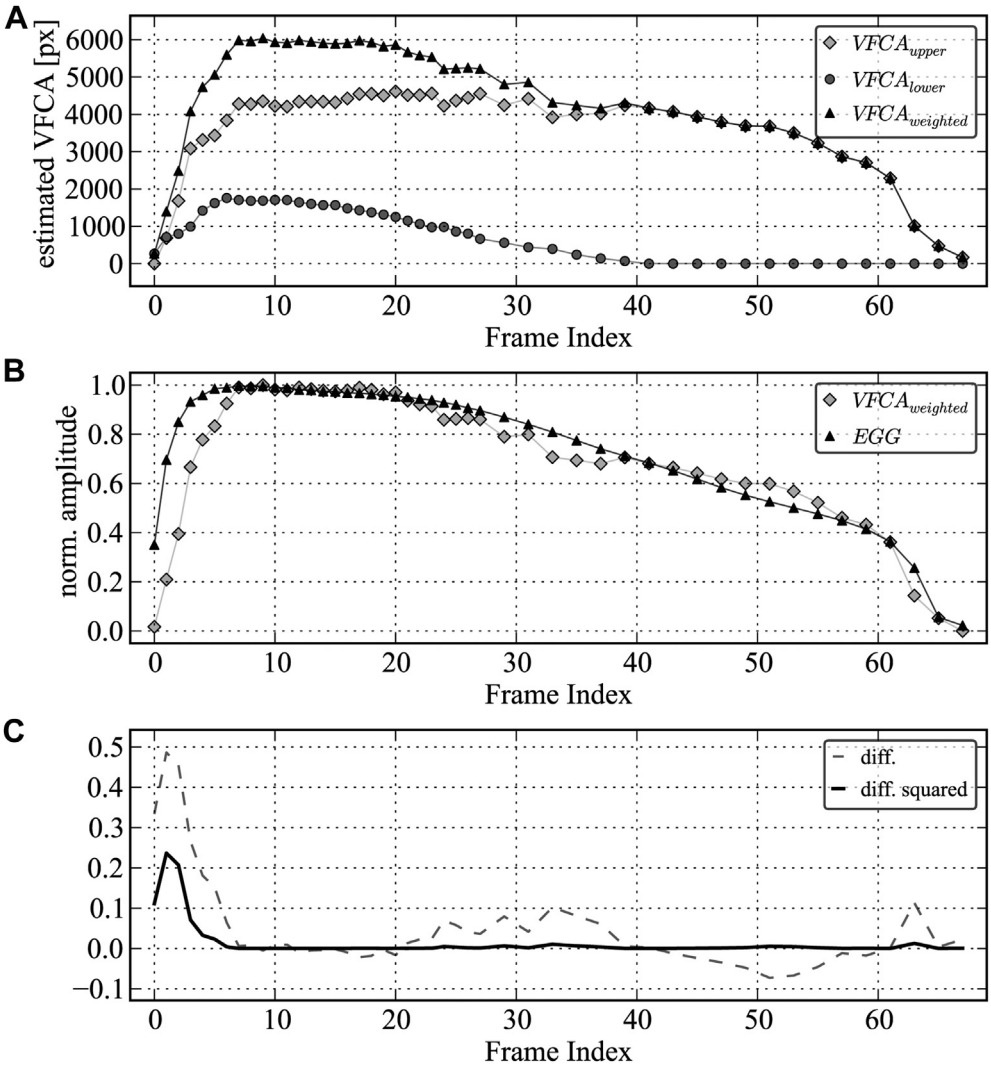


FIGURE 7. Assessment of hypothetical contribution of lower and upper vocal fold portion to the overall EGG signal—illustration of best fit scenario (see text). (**A**) contribution of the superior ($VFCA_{UPPER}$, *light gray rhombs*) and inferior ($VFCA_{LOWER}$, *dark gray circles*) vocal fold portion to the overall weighted vocal fold contact area ($VFCA_{WEIGHTED}$, *black triangles*); (**B**) comparison of $VFCA_{WEIGHTED}$ (*gray rhombs*) and actual EGG waveform (*black triangles*); (**C**) difference (*dashed curve*) and squared difference (*solid curve*) between the *curves* depicted in **B**.

most direct path, the longer it gets, thus increasing the impedance along that particular path. In the setup used for this study, the electrode attached to the glass plate was positioned anteriorly and above the level of glottis, to not obstruct the top camera view (Figure 2). Such a configuration would most probably cause the superior VF portion to contribute more strongly to the overall EGG signal because the most direct electrical field line would be found there. This notion is well in line with previous observations that changes of electrode placement *in vivo* are likely to influence the shape of the resulting EGG waveform.[6,45,46] Interestingly, approximately identical EGG electrode placements were maintained for each of the investigated larynges. Yet, the substantial differences between EGG and VFCA over the glottal cycle were only found in larynx L1, an argument that is not in favor of the notion that EGG electrode placement had a great influence on the acquired EGG signal. Further research is needed to investigate this assumption.

Previous research has shown that mucus bridges between the VFs could introduce artifacts into the EGG signal, particularly immediately after the opening instant.[47–49] As the mucus glands are not actively secreting in an excised larynx preparation, the influence of mucus bridges, as seen *in vivo*, can therefore be ruled out. During the experiments, the larynges were regularly moistened with saline solution, to slow down the dehydration process. Uneven distribution of the excess liquid could have potentially had an influence on the overall conductivity of the tissue and consequently on the EGG signal. However, the larynges have been checked for excess liquid before each trial run in an attempt to minimize the potential influence of the presence of saline solution on the tissue.

Finally, the different conductivity properties in various regions along the VFCA could be brought about by nonuniform tissue properties. Cartilage might have lower conductivity than muscle and other tissue.[19] In case of a pathology, lesions that contain less water or less conductive material may locally alter tissue admittance properties. For frequency-modulated currents <10 MHz, tissue impedance is typically dominated by the conductivity of the body electrolytes,[50] and it varies under compression, caused by an exodus of conductive fluids from the compressed regions.[51] Such effects may occur as the VFs collide during oscillation, either with each other *in vivo* or with the conductive glass plate in the hemilarynx setup. It is therefore conceivable that VF conductivity undergoes spatiotemporal variation, thus introducing nonlinear relations between the actual VFCA and the acquired EGG signal.

Practical implications

Because of its noninvasive nature, electroglottography is a popular method for assessing VF vibration *in vivo*. The EGG signal can be subjected to either qualitative analysis, such as assessing the presence of a "knee" in the EGG waveform as a putative indicator of VF bulging,[52] or to quantitative analysis. A widely used quantitative parameter derived from the EGG signal is the EGG contact quotient CQ_{EGG}. Also known as the "duty cycle" of VF vibration,[53] it estimates the relative duration of VF contact during a glottal cycle. This parameter has been widely used, for example, to assess VF adduction,[54] vocal registers in speech and singing,[55] differences between trained and untrained singers,[56] the effect of voice therapy on patients with mutational dysphonia,[57] indications of voice quality after surgery,[58] glottal configurations in singing,[59] differences in singing styles,[60] effects and principles of vocal exercises,[32–34,61–63] and other areas of interest in vocal production.

Various approaches for estimating the CQ_{EGG} have been used,[37] either using a "criterion level,"[39,53,64,65] the first derivative of the EGG signal,[38,66] or a combination of these two.[56,67] These methods rely on the assumption that the EGG signal is a correct representation of the relative VFCA. Any error introduced during the data acquisition stage would inescapably make the computed quantitative analysis data less accurate, and thus also any inferential statistics that would be based thereupon. Knowing the relation (and its potential error) between VFCA and the acquired EGG signal is therefore paramount for assessing the validity of past, present, and future studies involving quantitative EGG analysis.

Unfortunately, neither the initial study by Scherer et al[40] nor this study could report a perfect correlation between EGG and VFCA (although a relatively strong relation of sorts was found in both studies). Also, owing to the complexity of the experimental setup, neither of these two studies had a large enough sample size that would allow a report of reliable error margins for relative VFCA measurement with EGG, particularly with respect to quantitative analysis parameters such as the CQ_{EGG}. The potential presence of such a measurement error might for instance in part explain the observed disagreement between glottal landmarks from EGG and HSV,[41] or between the EGG contact quotient and the closed quotient derived from inverse filtered airflow,[68] HSV,[69] or videokymography.[37] Consequently, until such data are available, quantitative EGG analysis data should be interpreted with care.

Acknowledgments

The research has been supported by an "APART" grant received from the Austrian Academy of Sciences (C.T.H.), the European Social Fund project OP VK CZ.1.07/2.3.00/30.0004 "POST-UP" (C.T.H., J.G.Š.), the Technology Agency of the Czech Republic project no. TA04010877 (J.G.Š., V.H.), Palacky University student's project IGA_PrF_2014_029 (V.H.), and the University of Vienna (M.G.). The data were acquired at the Bioacoustics Laboratory, Department of Cognitive Biology, University of Vienna, Austria. The authors are very grateful to Mag. Riccardo Hofer (University of Vienna) for his contributions to the excised larynx experimental setup and RNDr. Petr Schovánek (Palacký University) for providing the conducting glass plates.

V.H. created the hemilarynx preparations and the hemilarynx setup, contributed to data acquisition and analysis, and wrote the initial version of the article. M.G. aided in creating the hemilarynx preparations and contributed to data acquisition and analysis. R.S. and J.G.S. contributed to the critical interpretation of the results and significantly contributed to writing the article. C.T.H. conceived and supervised the experiment, created the excised larynx setup, contributed to data acquisition and analysis, wrote all software used in data processing, analysis, and figure generation, and contributed significantly to writing the article.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jvoice.2015.03.018.

REFERENCES

1. Fabre P. Un prodédé electrique percutané d'inscription de l'accolement glottique au cours de la phonation: glottographie de haute fréquence. Premiers résultats. *Bull Acad Natl Med*. 1957;141:66–69.
2. *IEC 60601-1-Medical Electrical Equipment—Part 1: General Requirements for Basic Safety and Essential Performance*. Geneva, Switzerland: International Electrotechnical Commission; 2011.
3. Fourcin A, Abberton E. First application of a new laryngograph. *Med Biol Illus*. 1971;21:172–182.
4. Baken RJ, Orlikoff RF. *Clinical Measurement of Speech and Voice*. 2nd ed. Singular Publishing, Thompson Learning; 2000.
5. Kitzing P. *Electroglottography. Diseases of the Larynx*. New York: Arnold; 2000:127–138.
6. Baken RJ. Electroglottography. *J Voice*. 1992;6:98–110.
7. Fant G, Ondrackova J, Lindqvist-Gauffin J, Sonesson B. Electrical glottography. *STL-QPSR*. 1966;7:15–21.
8. Fourcin AJ. Laryngographic examination of vocal fold vibration. In: Wyke B, ed. *Ventilatory and Phonatory Control Systems*. London: Oxford University Press; 1974:315–333.
9. Fourcin AJ. Laryngographic assessment of phonatory function. *ASHA Reports*. 1981;11:116–127.
10. Lecluse FLE, Brocaar MP, Verschuure J. The electroglottography and its relation to glottal activity. *Folia Phoniatr*. 1975;27:217–224.
11. Kitzing P. Photo- and electroglottographical recording of the laryngeal vibratory pattern during different registers. *Folia Phoniatr*. 1982;34:234–241.

12. Baer T, Titze IR, Yoshioka H. Multiple simultaneous measures of vocal fold activity. In: Bless DM, Abbs JH, eds. *Vocal Fold Physiology.* San Diego: College-Hill Press; 1983:229–237.
13. Baer T, Lofquist A, McGarr NS. Laryngeal vibrations: a comparison between high-speed filming and glottographic techniques. *J Acoust Soc Am.* 1983;73:1304–1308.
14. Gilbert HR, Poller CR, Hoodin R. Laryngograph as a measure of vocal fold contact area. *J Speech Hear Res*. 1984;27:178–182.
15. Childers DG, Krishnamurthy AK. A critical review of electroglottography. *Crit Rev Biomed Eng*. 1985;12:131–162.
16. Rothenberg M. Some relations between glollal air flow and vocal fold contact area. *ASHA Reports*. 1981;11:88–96.
17. Titze IR. Parameterization of the glottal area, glottal flow, and vocal fold contact area. *J Acoust Soc Am.* 1984;75:570–580.
18. Childers DG, Hicks DM, Moore GP, Alsaka YA. A model for vocal fold vibratory motion, contact area, and the electroglottogram. *J Acoust Soc Am*. 1986;80:1309–1320.
19. Titze IR. Interpretation of electroglottographic signal. *J Voice*. 1990;4:1–9.
20. Berke GS, Moore DM, Hantke DR, Hanson DG, Gerratt BR, Burstein F. Laryngeal modeling: theoretical, in vitro, in vivo. *Laryngoscope*. 1987; 97(7 Pt 1):871–881.
21. Bless D, Hirano M, Feder R. Videostroboscopic evaluation of the larynx. *Ear Nose Throat J*. 1987;66.
22. Svec JG, Schutte HK. Videokymography: high-speed line scanning of vocal fold vibration. *J Voice*. 1996;10:201–205.
23. Rubin HJ, LeCover M. Technique of high speed photography of the larynx. *Ann Otol Rhinol Laryngol*. 1960;69:1072–1083.
24. Moore GP, White FD, von Leden H. Ultra high speed photography in laryngeal physiology. *J Speech Hear Disord*. 1962;27:165–171.
25. Deliyski DD, Hillman RE. State of the art laryngeal imaging: research and clinical implications. *Curr Opin Otolaryngol Head Neck Surg*. 2010;18: 147–152.
26. Deliyski DD, Petrushev PP, Bonilha HS, Gerlach TT, Martin-Harris B, Hillman RE. Clinical implementation of laryngeal high-speed videoendoscopy: challenges and evolution. *Folia Phoniatr Logop*. 2008;60:33–44.
27. Hertegard S. What have we learned about laryngeal physiology from high-speed digital videoendoscopy? *Curr Opin Otolaryngol Head Neck Surg*. 2005;13:152–156.
28. Herbst CT, Howard DM, Schlömicher-Thier J. Using electroglottographic real-time feedback to control posterior glottal adduction during phonation. *J Voice*. 2010;24:72–85.
29. Enflo L. *Collision Threshold Pressure: A Novel Measure of Voice Function. Effects of Vocal Warm-Up, Vocal Loading and Resonance Tube Phonation in Water*. Linköping, Sweden: Linköping University; 2013.
30. Kankare E, Liu D, Laukkanen AM, Geneid A. EGG and acoustic analyses of different voice samples: comparison between perceptual evaluation and voice activity and participation profile. *Folia Phoniatr Logop*. 2013;65:98–104.
31. Andrade PA. Analysis of male singers laryngeal vertical displacement during the first passaggio and its implications on the vocal folds vibratory pattern. *J Voice*. 2012;26:655.e19–655.e24.
32. Andrade PA, Wood G, Ratcliffe P, Epstein R, Pijper A, Svec JG. Electroglottographic study of seven semi-occluded exercises: LaxVox, straw, lip and tongue trills, humming, hand over mouth and tongue trill combined with hand over mouth. *J Voice*. 2014;5:589–595.
33. Granqvist S, Simberg S, Hertegard S, et al. Resonance tube phonation in water: high-speed imaging, electroglottographic and oral pressure observations of vocal fold vibrations—a pilot study. *Logoped Phoniatr Vocol*. 2014.
34. Guzman M, Laukkanen AM, Krupa P, Horáček J, Švec JG, Geneid A. Vocal tract and glottal function during and after vocal exercising with resonance tube and straw. *J Voice*. 2013;27:523.e19–523.e34.
35. Master S, Guzman M, Dowdall J. Vocal economy in vocally trained actresses and untrained female subjects. *J Voice*. 2013;27:698–704.
36. Li Z, Bakhshaee H, Helou L, et al, eds. *Evaluation of Contact Pressure in Human Vocal Folds During Phonation Using High-Speed Videoendoscopy, Electroglottography, and Magnetic Resonance Imaging*. Montreal, Canada: Acoustical Society of America; June 2013. 21st International Congress on Acoustics, ICA 2013-165th Meeting of the Acoustical Society of America.
37. Herbst CT, Ternström S. A comparison of different methods to measure the EGG contact quotient. *Log Phon Vocol*. 2006;31:126–138.
38. Henrich N, d'Alessandro C, Doval B, Castellengo M. On the use of the derivative of electroglottographic signals for characterization of nonpathological phonation. *J Acoust Soc Am*. 2004;115:1321–1332.
39. Orlikoff RF. Assessment of the dynamics of vocal fold contact from the electroglottogram: data from normal male subjects. *J Speech Hear Res*. 1991;34:1066–1072.
40. Scherer RC, Druker DG, Titze IR. Electroglottography and direct measurement of vocal fold contact area. In: Fujimura O, ed. *Vocal Fold Physiology, Vol2: Voice production, Mechanisms and Functions*. New York: Ed. Raven Press; 1988:279–290.
41. Herbst CT. Glottal Efficiency of Periodic and Irregular In Vitro Red Deer Voice Production. *Acta Acustica united with Acoustica*. 2014;100:724–733.
42. Titze IR. *The Myoelastic Aerodynamic Theory of Phonation*. Denver: National Center for Voice and Speech; 2006:424.
43. Herbst CT, Stoeger AS, Frey R, et al. How low can you go? Physical production mechanism of elephant. *Science*. 2012;337:595–599.
44. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012;9:676–682.
45. Colton RH, Conture EG. Problems and pitfalls of electroglottography. *J Voice*. 1990;4:10–24.
46. Tang S, Zhang C, Wang S, Wan M. A preliminary study for a slantwise-placed electroglottography. *J Voice*. 2015;29:129.e19–129.e27.
47. Childers DG, Naik JM, Larar JN, Krishnamurthy AK, Moore GP. Electroglottography, speech, and ultra-high speed cinematography. In: Titze IR, Scherer R, eds. *Vocal Fold Physiology and Biophysics of Voice*. Denver: Denver Center of Performing Arts; 1983:202–220.
48. Childers DG, Hicks DM, Moore GP, Alsaka YA. A model for vocal fold vibratory motion, contact area, and the electroglottogram. *J Acoust Soc Am*. 1986;80:1309–1320.
49. Golla ME, Deliyski DD, Orlikoff RF, Moukalled H. Objective comparison of the electroglottogram to synchronous high-speed images of vocal-fold contact during vibration. In: Manfredi C, ed. *Proceedings of the 6th International Workshop on Models and Analysis of Vocal Emissions for Biomedical Applications MAVEBA*. Firenze, Italy: Firenze University Press; 2009: 141–144.
50. Grimnes S, Martinsen ØG. *Passive Tissue Electrical Properties. Bioimpedance and Bioelectricity Basics (Third Edition)*. 3rd ed. 2015;77–118.
51. Belmont B, Dodde RE, Shih AJ. Impedance of tissue-mimicking phantom material under compression. *J Electr Bioimp*. 2013;4:2–12.
52. Awan SN, Krauss AR, Herbst CT. An examination of the relationship between electroglottographic (EGG) contact quotient, EGG decontacting phase profile, and acoustical spectral moments. *J Voice*. 2015; http://dx.doi.org/10.1016/j.jvoice.2014.10.016.
53. Rothenberg M, Mahshie JJ. Monitoring vocal fold abduction through vocal fold contact area. *J Speech Hear Res*. 1988;31:338–351.
54. Scherer R, Titze IR. The abduction quotient related to vocal quality. *J Voice*. 1987;1:246–251.
55. Henrich N, d'Alessandro C, Doval B, Castellengo M. Glottal open quotient in singing: measurements and correlation with laryngeal mechanisms, vocal intensity, and fundamental frequency. *J Acoust Soc Am*. 2005;117: 1417–1430.
56. Howard DM. Variation of electrolaryngographically derived closed quotient for trained and untrained adult female singers. *J Voice*. 1995;9:163–172.
57. Lim J-Y, Lim SE, Choi SH, Kim JH, Kim K-M, Choi H-S. Clinical characteristics and voice analysis of patients with mutational dysphonia: clinical significance of diplophonia and closed quotients. *J Voice*. 2007;21:12–19.
58. Choi H-S, Chung SM, Lim J-Y, Kim H-S. Increasing the closed quotient improves voice quality after type I thyroplasty in patients with unilateral vocal cord paralysis: analysis using SPEAD program. *J Voice*. 2008;22: 751–755.
59. Herbst CT, Ternstrom S, Svec JG. Investigation of four distinct glottal configurations in classical singing—a pilot study. *J Acoust Soc Am*. 2009;125: EL104–EL109.
60. Barlow C, LoVetri J. Closed quotient and spectral measures of female adolescent singers in different singing styles. *J Voice*. 2010;24:314–318.

61. Guzman M, Rubin A, Muñoz D, Jackson-Menaldi C. Changes in glottal contact quotient during resonance tube phonation and phonation with vibrato. *J Voice*. 2013;27:305–311.
62. Dargin TC, Searl J. Semi-occluded vocal tract exercises: aerodynamic and electroglottographic measurements in singers. *J Voice*. 2015;29: 155–164.
63. Ogawa M, Hosokawa K, Yoshida M, Iwahashi T, Michiko H, Inohara H. Immediate effects of humming on computed electroglottographic parameters in patients with muscle tension dysphonia. *J Voice*. 2014;28: 733–741.
64. Sapienza CM, Stathopoulos ET, Dromey C. Approximations of open quotient and speed quotient from glottal airflow and EGG waveforms: effects of measurement criteria and sound pressure level. *J Voice*. 1998;12:31–43.
65. Scherer R, Vail V, Rockwell B. Examination of the laryngeal adduction measure EGGW. In: Bell-Berti F, Raphael LJ, eds. *Producing Speech: Contemporary Issues: for Katherine Safford Harris: American Institute of Physics*. New York: ATI Press; 1995:269–289.
66. Childers D, Hicks DM, Eskanazi L, Moore GP, Lalwani AL. Electroglottography and vocal fold physiology. *J Speech Hear Res*. 1990;33:245–254.
67. Howard DM, Lindsey GA, Allen B. Toward the quantification of vocal efficiency. *J Voice*. 1990;4:205–212.
68. La P, Sundberg J, eds. *Effect of Subglottal Pressure Variation on the "Closed Quotient"—Comparing Data Derived From Electroglottograms and From Flow Glottograms*. Philadelphia, PA: The Voice Foundation; 2012. The Voice Foundation's 41st Annual Symposium: Care of the Professional Voice.
69. Echternach M, Dippold S, Sundberg J, Arndt S, Zander MF, Richter B. High-speed imaging and electroglottography measurements of the open quotient in untrained male voices' register transitions. *J Voice*. 2010;24: 644–650.

Supplement C: Paper III

Vocal fold adjustment caused by phonation into a tube: A double case study using computed tomography

V. HAMPALA, A.-M. LAUKKANEN, M. A. GUZMAN, J. HORÁČEK, J. G. ŠVEC

Journal of Voice

(2015, in press)

Vocal Fold Adjustment Caused by Phonation Into a Tube: A Double-Case Study Using Computed Tomography

*Vít Hampala, †Anne-Maria Laukkanen, ‡Marco A. Guzman, §Jaromír Horáček, and *,||Jan G. Švec, *Olomouc and §||Prague, Czech Republic, †‡Santiago, Chile

Summary: Objectives. Phonation into a tube is a widely used method for vocal training and therapy. Previous studies and practical experience show that the phonation becomes easier and louder after such an exercise. The purpose of this study was to find out whether there are systematic changes in the vocal fold adjustment after the exercise.

Methods. Two volunteer subjects (1 male and 1 female) without voice disorders were examined with computed tomography (CT). Both produced a sustained vowel [a:] at comfortable pitch and loudness before and after the tube phonation and a vowel-like phonation into the tube. Computed tomography (CT) scans were obtained before, during, and after the exercise, twice for each condition. The gathered CT images were used for measurements of vertical vocal fold thickness, bulkiness, length, and glottal width.

Results. No prominent trends common to both subjects were found in vocal fold adjustment during and after the phonation into the tube. Variability observed under the same conditions was usually of the same magnitude as the changes before and after the tube phonation.

Conclusions. Changes in vocal tract configuration observed after the resonance tube exercises in previous related studies were more prominent than the changes in vocal fold configuration observed here.

Key Words: Vocal folds–Resonance tube–Computed tomography.

INTRODUCTION

Phonation into a tube is a useful method widely used for vocal training and therapy. It belongs to a wider group of semi-occluded vocal exercises that take advantage of a semi- or full closure of the vocal tract.[1] Other commonly used exercises of this type are tongue trills, nasals, and voiced fricatives.[2] Humming into various small glass tubes started to be used in the beginning of 20th century by Spiess[3] for improving vocal function. Tube phonation has been used, for example, for treatment of hypernasality.[4,5] Method of phonation into glass tubes, so called resonance tubes, has also been used for decades in Finnish voice and speech training and therapy where it has become popular.[6,7] Sovijärvi[8] was at first interested in testing different kinds of glass tubes in the children with hypernasality, but soon he started to use the tubes also with adult singers who had voice problems. According to his observations, phonation into the tubes improved voice quality of the patients with functional phonasthenia, laryngeal paresis, and vocal fold nodules.[6,9] This method has also been used for vocal care and further vocal training in healthy and normophonic subjects using their voice extensively (ie, singers or teachers) because the voice is perceived as being louder and feels to be easier to produce after such an exercise.[7]

Laukkanen et al[10] showed that sound pressure level (SPL) slightly increased after the tube phonation. This was also observed by Vampola et al[11] who calculated SPL values using finite element modeling method. Despite number of studies published about resonance tubes, the exact mechanism of their functioning has not been fully understood. Basically, there are three possibilities of adjustment caused by the exercises, such as (1) change in the voice source (vocal folds), (2) change in the vocal tract (filter), and (3) change caused by the interaction between the voice source and the filter.

To investigate the *changes in the voice source*, Laukkanen[12] analyzed electroglottographic signals of vibrating vocal folds and showed that the quasi-open quotient decreased during and after the exercise, which was possibly related to change in adduction of vocal folds. Speed quotient rose indicating more rapid collisions of the vocal folds. Furthermore, Laukkanen et al[7] studied muscle activities in a single female subject via electromyography (EMG) and found that the ratio of thyroarytenoid (TA) versus cricothyroid muscle activity increased. According to Hirano[13] and Yumoto et al,[14] increased activity of TA muscle makes the vocal fold thicker and bulged. In addition, according to Chhetri et al[15] who investigated neuromuscular mechanisms for modulating glottal posture in canine larynx, TA activation has been shown to close the mid-membranous glottis. So far, however, there has not been any conclusive evidence of specific consistent changes in vocal fold adjustments caused by the semi-occluded voice exercises.

Changes in the vocal tract and size of its cavities caused by the resonance tube exercise were examined by Vampola et al.[16] The study reported that the velum raised and closed the nasopharyngeal port. In addition, cross-sectional areas of vocal tract (nasal cavities excluded) expanded and its total volume became

Accepted for publication October 9, 2014.

This study was presented at the 10th Pan European Voice Conference PEVOC; August 21–24, 2013; Prague, Czech Republic.

From the *Department of Biophysics, Faculty of Science, Voice Research Lab, Palacky University, Olomouc, Czech Republic; †Department of Communication Sciences and Disorders, University of Chile, Santiago, Chile; ‡Department of Otolaryngology, Las Condes Clinic, Santiago, Chile; §Department of Dynamics and Vibrations, Institute of Thermomechanics, Academy of Sciences of the Czech Republic, Prague, Czech Republic; and the ||Voice Centre Prague, Medical Healthcom, Ltd., Prague, Czech Republic.

Address correspondence and reprint requests to Vít Hampala, Department of Biophysics, Faculty of Science, Voice Research Lab, Palacky University, Olomouc, 17. listopadu 12, 77146 Olomouc, Czech Republic. E-mail: vit.hampala@upol.cz

Journal of Voice, Vol. ■, No. ■, pp. 1-10

0892-1997/$36.00

http://dx.doi.org/10.1016/j.jvoice.2014.10.022

considerably larger. Similar results have been reported by Guzman et al.[17]

Interaction between the voice source and the filter has been found to be able to change the vibration properties of the vocal folds solely owing to the changes in the supraglottal tract.[18,19] The use of the resonance tube creates a constriction and this reduction of cross-sectional area increases the vocal tract resistance. Simultaneously, the tube elongates the vocal tract causing the first formant frequency to go down.[20] This increases the inertive reactance of the vocal tract resulting in an increased source-filter interaction.[2] The resonance tube also increases supraglottal pressure that tends to decrease transglottal pressure (ie, the difference between the subglottal pressure and the supraglottic pressure).[1,5] Sufficiently low transglottal pressure makes phonation more economical in terms of preserving the vocal folds from powerful collisions and provides a sensation of maximal outcome achieved with minimal effort.[2,5]

In 1960s, Hollien et al[21–26] published an original methodology for measurement of vocal fold anatomical dimensions *in vivo* using X-ray laminagrams. The contours of the laryngeal tract and vocal folds were outlined in the X-ray images and a system of lines and points was designed to measure the length, thickness, and surface tilting of the vocal folds related to changes in fundamental frequency (F_0). Present study applies the methodology of Hollien et al[21–26] to the modern examination method of computed tomography (CT) imaging (Methods section). The purpose of this study was to find out whether there are systematic changes in the vocal fold adjustment caused by the phonation into a tube. To do that, vocal fold geometry before, during, and after the phonation into the resonance tube was measured and compared.

Based on the studies of Laukkanen et al,[7] who observed increased activity of TA muscle after the tube phonation, the following three hypotheses were formulated and investigated here: (1) the vocal folds are going to be more bulged and thicker, (2) the glottal width is going to decrease, and (3) the length of the vocal folds is not going to change. The first two hypotheses stem from the knowledge on the effect of the TA activity: The TA muscle has been shown to bulge the vocal fold[13] and to shift the vocal fold margin more medially.[13,14] The third hypothesis considers that the phonations before and after the tube phonation are both requested to be produced at comfortable pitch, thus not requiring considerable vocal fold length adjustments.

MATERIALS AND METHODS

Subjects and CT recordings

Two vocally healthy subjects were investigated: subject F—a female, aged 48 years, and co-author A.M.L. and subject M—a male, aged 35 years, and co-author M.A.G. Both subjects had no voice or hearing problems and were experienced in semi-occluded exercises. The subjects were informed about potential risks related to the radiation dose during CT examination and signed a consent form. The recordings were performed at two different occasions using a CT device (Light Speed VCT GE–64 and Toshiba Aquilion). The subjects were placed in supine position. The recording time for subject F was 2 seconds. During this time, 181 images with the resolution of 512 × 512 pixels were collected. The thickness of each slice was 0.625 mm. For subject M, the recording time was 3.36 seconds yielding 510 images with the resolution of 512 × 512 pixels and slice thickness of 0.5 mm. For both subjects, the scanning covered an area from below the larynx up to the bottom of nasal cavities.

Phonatory tasks and CT measurements

The subjects were asked to produce a sustained vowel [a:] at a comfortable pitch and loudness before and after phonating into the resonance tube. The resonance tube training exercise was performed for about 5 minutes into 27-cm long resonance tube (glass) with the inner and outer diameters of 8 and 9 mm. The duration of 5 minutes was previously found long enough for sensing the changes in voice production and causing clear changes in the vocal tract configuration after the exercise.[16,17] To investigate the variability of the glottal configuration during the individual phonations, the CT scanning was performed twice before, twice during, and twice after the phonation into the tube. The freely downloadable software *OsiriX* (version 3.9.4, 32-bit; Osirix, Pixmeo, Switzerland)[27] was used for setting the planes to the desired position and for inserting a calibrated distance line into the image. For further analysis, the gathered images were exported and processed with the *ImageJ* 1.45s software (National Institutes of Health, Maryland, USA), which provided an environment for the final measurements of lengths, thicknesses, and areas, using the calibrated distance line.

To measure the relevant lengths of the vocal folds, it was necessary to set the transverse plane (Figure 1A) to be parallel to the upper surface of both vocal folds (Figure 1B). Then, the transverse plane was shifted to reach the level of glottis (Figure 2) similar to the one as shown by Hirano in a collection

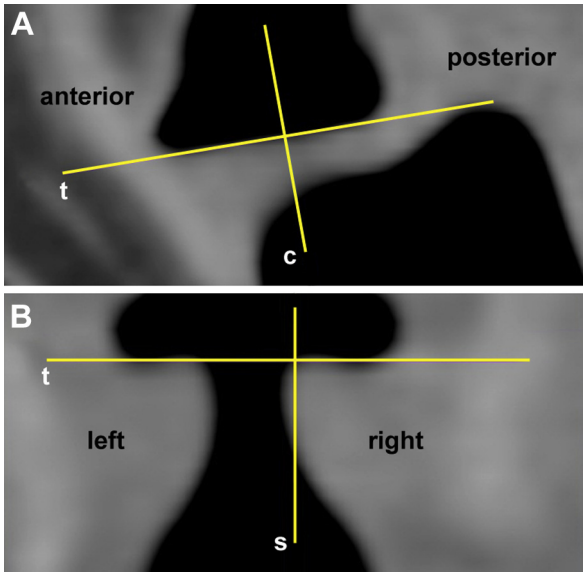


FIGURE 1. The sagittal (**A**) and coronal (**B**) slices demonstrating how the transverse plane was adjusted to be parallel to the upper surface of the vocal folds: t, transverse plane; c, coronal plane; s, sagittal plane.

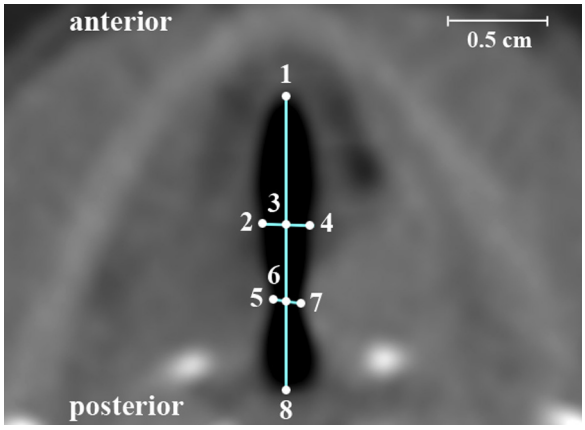


FIGURE 2. The CT transverse plane of the vocal folds and the points marked as one to eight used for glottal measurements.

of histological slices (Hirano,[13] section AH9, page 20). The resulting image was used to measure the distances between the points marked on the Figure 2. Herein, the points 1 and 8 correspond to the anterior and posterior commissures and create an axis, which intersects the apex of the anterior commissure and the middle of the distance between the vocal processes. The medially prominent parts of the arytenoid cartilages were marked as the points 2, 4, 5, and 7. Finally, the points 3 and 6 were recognized as the intersections between the axis of glottis and lines connecting points 2, 4 and 5, 7.

The width of glottis, thicknesses, and areas of the vocal folds were measured in the coronal plane perpendicularly to the vocal fold surface. These measurements were first attempted to be done in the mid-membranous part of the vocal folds, but herein, an unusual cross-sectional shape of the vocal folds was found in subject M. Therefore, the coronal plane was placed more posteriorly at the ratio of 0.86 of a reference vocal fold length, that is, the distance between the anterior apex of the lowest part of the laryngeal ventricle and the vocal processes (Figure 3). At this position, the left vocal fold (LVF) of subject M showed standard shape with a well-defined upper vocal fold surface, allowing the cross-sectional shape measurements to be performed here. The plane adjustments were done in the following way: (1) The transverse plane was set to be parallel to the upper surface of the vocal folds (Figure 1A); (2) The mid-sagittal plane was rotated to intersect the apex of the anterior commissure and the midpoint between vocal processes. This kept the coronal plane perpendicular to the axis of the glottis (Figure 3B, c_1); (3) The coronal plane was moved to the position fulfilling the 0.86 ratio of the reference vocal fold length (Figure 3B, c_2). This coronal plane (Figure 4) was used for measurement of the vocal fold thicknesses at 1- and 2-mm distances (Figure 4; T1 and T2) from the glottis, and of the cross-sectional vocal fold areas (Figure 4; A1 and A2). The process of location of the planes and the corresponding measurements of the vocal fold morphology were performed three times for each of the CT scans. The three measurements were used to determine the *measurement uncertainty* for each single phonation, which was expressed as the *standard error of the mean*. This measurement uncertainty could then be compared with the variability

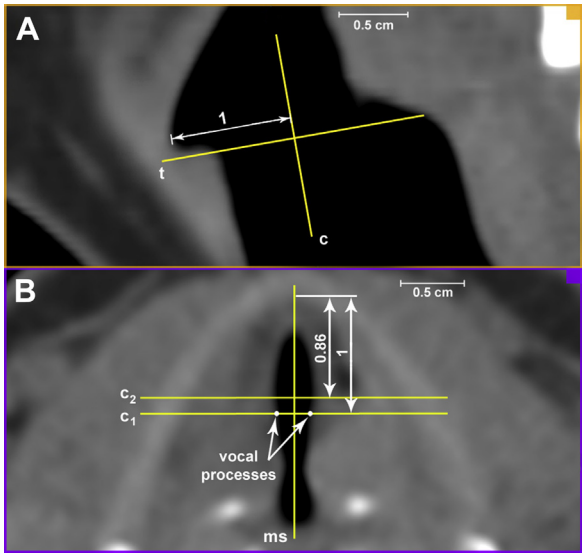


FIGURE 3. The CT mid-sagittal (**A**) and the transverse (**B**) slice demonstrating the adjustment of the coronal plane c. Labeling: c_1, position of the coronal plane at the level of vocal processes; c_2, position of the coronal plane used for the measurement of vocal fold vertical thickness and cross-sectional area; ms, position of the mid-sagittal plane; t, position of the transverse plane. The distance labeled by number 1 indicates the reference vocal fold length, that is, the normalized distance between the anterior apex of the lowest part of the laryngeal ventricle and the vocal processes.

observed during the task repetition (called *repetition variability* further on) and with the changes caused by the resonance tube exercises. The change caused by the exercise was considered significant when it was greater than the repetition variability and measurement uncertainty. Glottal width was measured

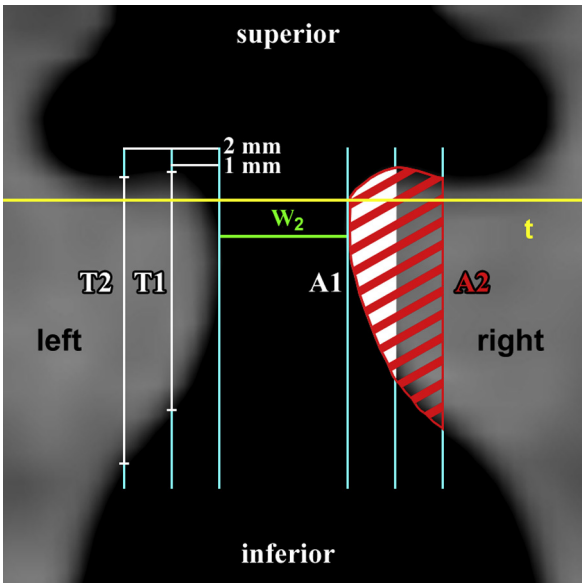


FIGURE 4. Scheme of the CT coronal plane used for the measurement of the vertical thicknesses (T1, T2), areas (A1, A2), and the glottal width w_2; t, transverse plane.

here as a distance between the most medial parts of the vocal folds (Figure 4, w_2). Because of an unusual shape of the upper surface of the right vocal fold (RVF) in subject M, the transverse plane (Figure 4, t) was used as the upper boundary for the measurements of T1, T2, A1, and A2 in this subject.

RESULTS

The results are shown in five graphs (Figures 5–9) showing the changes in the vocal fold adjustment related to the resonance tube exercise. The X-axis shows the states before ("Before"), during ("Tube"), and after ("After") the phonation into a tube. The measured distances (ie, length, vertical thickness) or areas are displayed on the Y-axis. Because each recording of "Before," "Tube," and "After" was made twice, the graphs always contain two mean values (labeled as "maximum" and "minimum") for each of the conditions, which indicate the *repetition variability*. The mean values were obtained from three repeated measurements of the same phonation. The spans of the error bars show the *standard error of the mean* for each of the measurements (based on the three measurement repetitions) revealing the *measurement uncertainty*. The mean values are interconnected horizontally among the different states to illustrate the changes caused by the resonance tube exercise.

Vertical thickness

The vertical thicknesses T1 and T2 of the vocal folds (measured at the depth of 1 and 2 mm) in subject F are shown in Figure 5. For better clarity, the LVF and RVF are shown separately. The results reveal that:

1. The LVF was generally thicker than the RVF before the exercise (4.40–4.61 mm vs 4.03–4.23 mm for T1; 5.53–5.56 mm vs 5.13–5.26 mm for T2, respectively) as well as after the exercise (4.56–4.69 mm vs 4.15–4.29 mm for T1 and 5.71–5.75 mm vs 5.51–5.56 mm for T2).
2. The thickness T2 (at 2-mm depth) was about 1 mm larger than the thickness T1 (at 1-mm depth) for both the vocal folds.
3. The measurement uncertainty (note the error bars indicating the standard error) of the determined mean thickness T1 was on average ±0.05 mm for the LVF and ±0.09 mm for the RVF; for T2, it was ±0.07 and ±0.09 mm, respectively.
4. The repetition variability was on average 0.19 mm, which was mostly larger than the measurement uncertainty (especially LVF in Figure 5). Particularly large repetition variability (0.8 mm) was observed during the tube phonation in RVF.
5. The thickness differences for T1 between "Before" and "After" conditions were smaller than the repetition variability, thus indicating no significant change. The average thickness T2, however, increased from "Before" to "After" by 0.24 mm in LVF and 0.34 mm in RVF. This increase was slightly larger than the repetition variability and the measurement uncertainty, thus indicating significant change.

Figure 6 illustrates the results from the measurement of the vertical thicknesses in the male subject M, in the same way as done in Figure 5. The results show that:

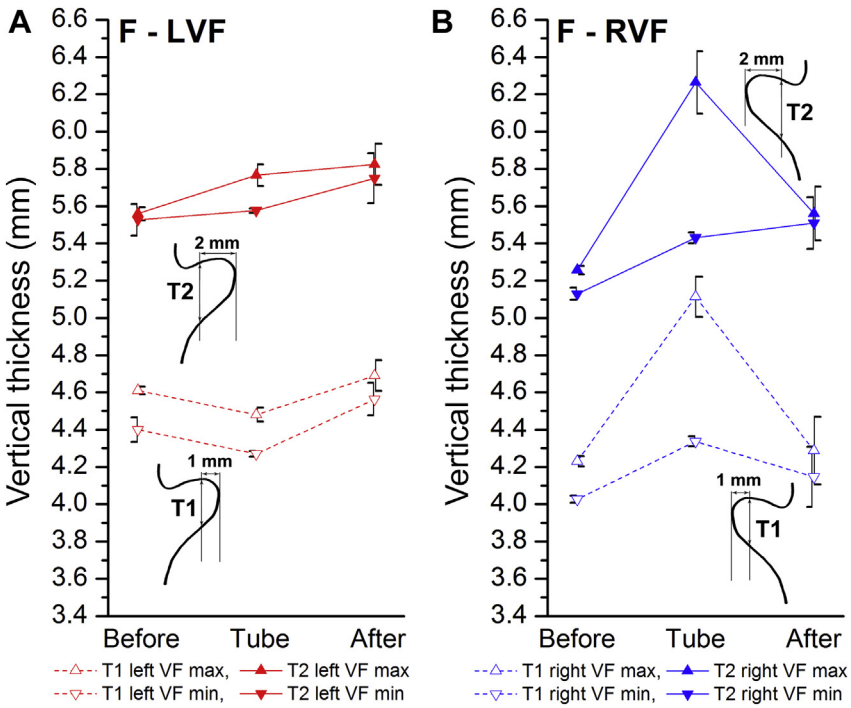


FIGURE 5. Changes in left vocal fold (**A**, LVF) and right vocal fold (**B**, RVF) vertical thickness in subject F measured at 1- and 2-mm depth (T1 and T2).

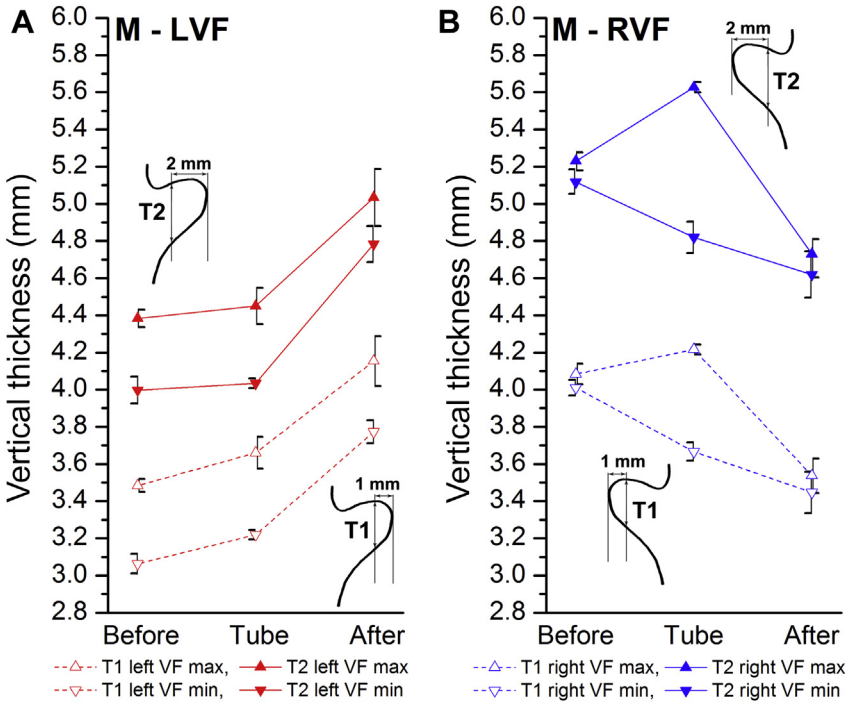


FIGURE 6. Changes in the left and right vocal fold (**A**, LVF; **B**, RVF) vertical thicknesses in subject M measured at 1- and 2-mm depth (T1 and T2).

1. The RVF was markedly thicker than LVF before the exercise (4.01–4.08 mm vs 3.06–3.48 mm for T1 and 5.12–5.23 mm vs 4.00–4.38 mm for T2, respectively) but became slightly thinner after (3.45–3.54 mm vs 3.77–4.15 mm for T1 and 4.62–4.71 mm vs 4.78–5.03 mm for T2, respectively).
2. Similarly to subject F, the thickness T2 (at 2-mm depth) was about 1 mm larger than the thickness T1 (at 1 mm depth) in both vocal folds.
3. The measurement uncertainty was mostly below ±0.1 mm (±0.06 mm for T1 and ±0.08 mm for T2, on average).

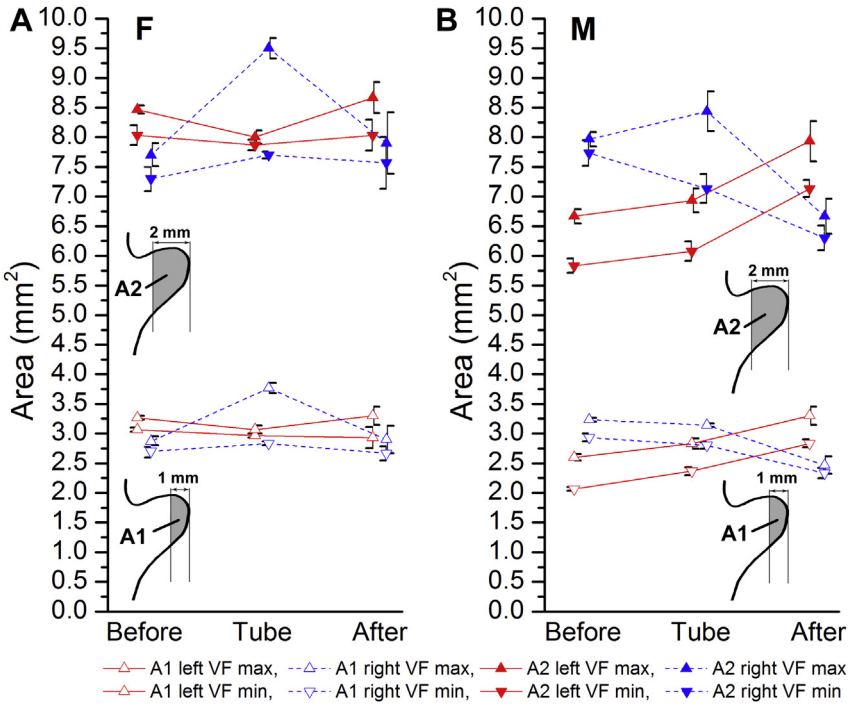


FIGURE 7. Changes in vocal fold cross-sectional areas bounded by the vertical lines at 1- and 2-mm distances for the subjects F (**A**) and M (**B**) and both the vocal folds (left, *solid lines*, right, *dashed lines*).

4. The repetition variability was around 0.4 mm (see particularly the LVF in Figure 6A), which was considerably larger than the measurement uncertainty.
5. The changes of vocal fold thickness caused by the exercise were about 0.6 mm for both T1 and T2. These were found significant because they were larger than the repetition variability and the measurement uncertainty. The changes were, however, not uniform: The LVF became significantly thicker, whereas the RVF became significantly thinner after the exercise than before. As a result, the LVF and RVF became considerably more symmetric after the exercise than before.

Cross-sectional areas

Figure 7 shows the "bulkiness" of the vocal folds measured via cross-sectional areas A1 and A2 (1 and 2 mm from the vocal fold margin) and their changes for both the subjects. Subject F is shown on the left (Figure 7A) and subject M on the right (Figure 7B). The results for subject F show that:

1. The LVF (red) was slightly bulkier than RVF (blue) both before and after the exercise.
2. Area A2 was about 2.7 times larger than A1 for both the vocal folds.
3. The measurement uncertainty was on average about ±0.1 mm^2 for A1 and ±0.21 mm^2 for A2.
4. The repetition variability was on average 0.33 mm^2 for A1 and 0.62 mm^2 for A2, which was larger than the measurement uncertainty.
5. The changes caused by the exercise were on average less than 0.1 mm^2 for A1 and, less than 0.2 mm^2 for A2, which was smaller than the repetition variability and the measurement uncertainty; thus, no significant change in vocal fold bulkiness could be detected for this subject.

The results for subject M (Figure 7B) show that:

1. The LVF (red) was less bulky than RVF (blue) before the exercise, but the opposite became true after the exercise. This is reflected in both the areas A1 and A2.
2. Similarly, as in subject F, the area A2 was about 2.7 times larger than the area A1 for both the vocal folds.
3. The measurement uncertainty was on average about ±0.08 mm^2 for A1 and ±0.21 mm^2 for A2.
4. The repetition variability was on average 0.37 mm^2 for A1 and 0.66 mm^2 for A2, which was larger than the measurement uncertainty.
5. The bulkiness changes caused by the exercise were, on average, 0.7 mm^2 for A1 and 1.3 mm^2 for A2. These changes are larger than the repetition variability as well as than the measurement uncertainty and can therefore be considered significant. However, the RVF and LVF showed changes in opposite directions.

Glottal width

Figure 8 depicts the changes of the glottal widths w_1 and w_2. The width w_1 is the distance measured in the transverse plane between the points 2 and 4 representing medial peaks of the vocal processes (hereafter "cartilaginous glottal width"). The width w_2 is a minimal distance between the vocal folds

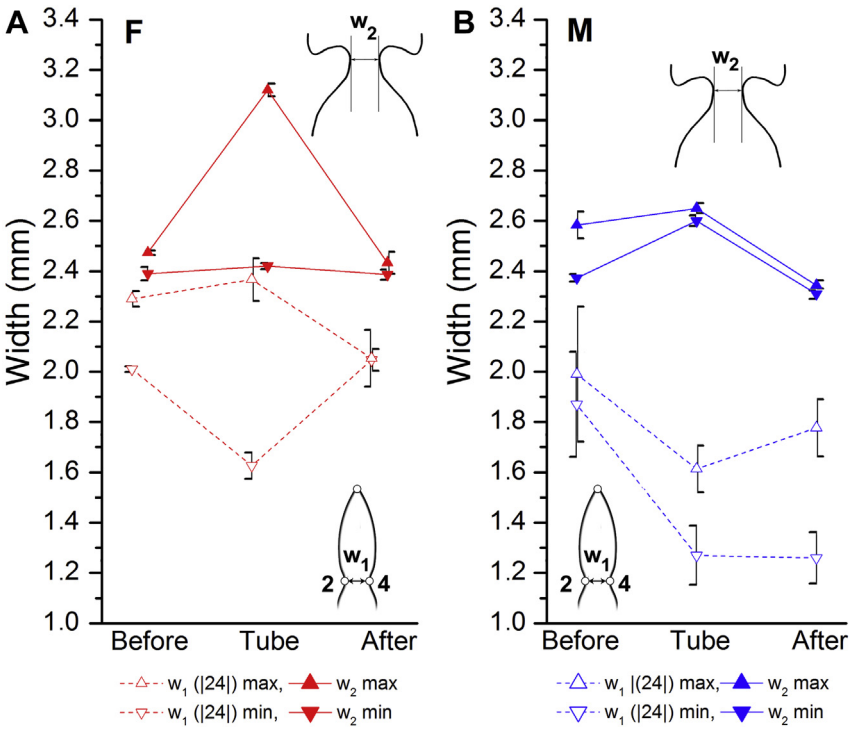


FIGURE 8. Glottal widths w_1 (empty symbols, *dashed lines*) and w_2 (filled symbols, *solid lines*) for subject F (**A**) and subject M (**B**).

measured in the coronal plane in the membranous part of the glottis (hereafter "membranous glottal width").

For the female subject F, Figure 8A shows that:

1. The membranous glottal width w_2 was larger (average size: ~2.54 mm) than the cartilaginous glottal width w_1 (average size: ~2.05 mm) both before and after the exercise.
2. For both glottal widths, the repetition variability changed: it became smaller after the exercise (0.006 mm for w_1 and 0.047 mm for w_2) than before the exercise (0.28 mm for w1 and 0.083 mm for w_2) and it was largest during the exercise (0.74 mm for w_1, 0.70 mm for w_2).
3. For both glottal widths, the repetition variability was larger than the measurement uncertainty (0.056 mm and 0.02 mm on average for w_1 and w_2) before and during the exercise but not after the exercise.
4. The changes from before to after the exercise were smaller than the repetition variability and the measurement uncertainty; thus, no significant change in glottal width was found for neither w_1 nor w_2 in subject F.

For the male subject M, Figure 8B shows that:

1. Similarly to subject F, the membranous glottal width w_2 (average size: 2.48 mm) was larger than the cartilaginous glottal width w_1 (average size: 1.63 mm) both before and after the exercise.
2. The repetition variability differed greatly: it decreased after the exercise for w_2 (0.21 mm before vs 0.03 mm after) but increased for w_1 (0.12 mm before vs 0.52 mm after).
3. The repetition variability of w_2 was larger than the measurement uncertainty (on average ±0.1 mm) before the exercise but not during and after the exercise. The repetition variability of w_1 was smaller than the measurement uncertainty (on average ±0.13 mm) before the exercise but not during and after the exercise.
4. The changes from before to after the exercise were smaller than the repetition variability or the measurement uncertainty; thus, no significant change in glottal width was found for neither w_1 nor w_2 in subject M. A decreasing trend was found for the cartilaginous width w_1 from before to after (1.87–1.99 mm vs 1.26–1.78 mm).

Vocal fold length

Figure 9 shows the changes of the membranous vocal fold lengths measured in both of the subjects as the distances between the anterior commissure (Figure 9, schematic, point 1) and vocal processes (Figure 9, schematic, points 2 and 4) in the transverse plane. The figure shows that:

1. The female vocal fold length (~6.7 mm) was about half the size of the male one (~13.5 mm).
2. In both the subjects, repetition variability (F: 0.23 mm, M: 0.45 mm, on average) was similar in size to the measurement uncertainty (female: ±0.13 mm, male: ±0.29 mm, on average).
3. There were no clear changes from before to after the exercise: the changes were smaller than both the repetition variability and the measurement uncertainty. Nevertheless, the vocal folds tended to be slightly longer (~7.5 mm in female and ~14.1 mm in male) during the exercise than before or after.

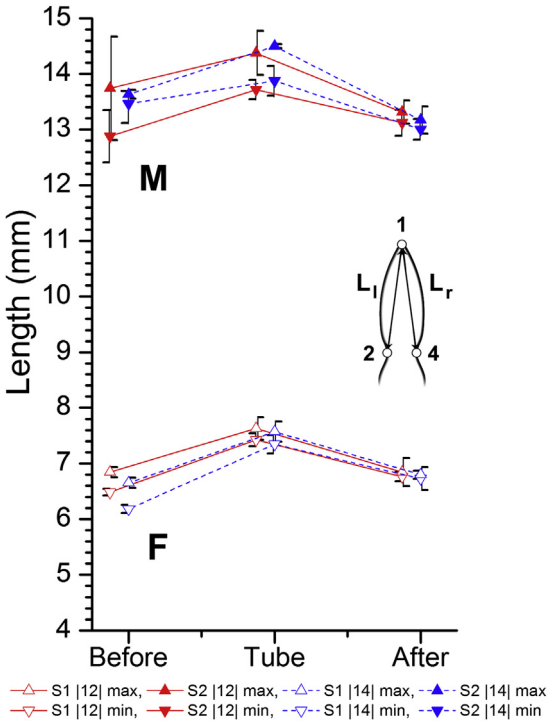


FIGURE 9. The membranous lengths of the left (L_l [|12|]; red symbols, *solid lines*) and right (L_r [|14|]; blue symbols, *dashed lines*) vocal fold. The values for the female subject F are shown as empty and for the male subject M as filled triangles. The membranous length corresponds to the distance between the anterior commissure and the vocal process. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

Besides of the lengths L_l and L_r, also other vocal fold lengths were measured in the transverse plane (such as |15|, |17|, |18|, |24|, and |57| according to Figure 2), but these did not bring any further information on the vocal fold changes, and therefore are not presented here.

DISCUSSION

The first hypothesis assumed that the vocal folds become more bulged and thicker after the resonance tube exercise. This was assumed because of an increased TA muscle activity reported previously in a single-subject pilot EMG study of Laukkanen et al.[7] Such a change was not uniquely observed here. Although there was some increase of the vertical thickness at the 2-mm depth in subject F (Figure 5), who was identical to the one used in the study of Laukkanen et al,[7] this trend was not found

in subject M. Subject M showed an increase of the vertical thickness and vocal fold area of the LVF but a decrease of the RVF (Figures 6 and 7B). Moreover, the measured vocal fold areas did not verifiably increase in subject F after the exercise (Figure 7A). These results therefore did not confirm the vocal folds to be more bulged and thicker after the phonation into a tube.

The second hypothesis expected the glottal width to decrease. This hypothesis was based on the finding that the phonation into a tube causes an increased resistance at lips that could result in an increased effort to produce a sound during the exercise.[5,12] Laukkanen[12] reported increased closed quotient (CQ) during and after the resonance tube exercise, which could be related to an increased adduction leading to a decreased glottal width (w_1) at the vocal processes. However, the results obtained here did not show any clear trend toward a smaller glottal width w_1 in subject F (Figure 8A)—the repetition variability observed before the exercise was much larger than the overall changes stimulated by the exercise. In subject M, there was a tendency toward smaller w_1 after the exercise, but the change was not convincing owing to high repetition variability and higher measurement uncertainty (Figure 8B). In summary, no clear changes of w_1 were observed here, but the measurement uncertainty does not allow the hypothesis to be fully rejected based on our data.

According to Titze[2,28] and Laukkanen et al,[7] the narrow constriction at the lips also increases the mean intraglottal pressure, which reduces an impact stress and tends to separate the vocal folds. This should have been projected in Figure 8, where the membranous glottal width w_2 was expected to increase after the exercise. Our data, however, do not show any such increase for neither of the subjects. On the contrary, there was some tendency for the width w_2 to decrease in subject M, although not significantly because of the repetition variability. Inconsistency of the results can also be found in the literature: Guzman et al[17] reported that CQ decreased after the exercise, which was exactly the opposite of what Laukkanen[12] had showed. Moreover, a lower glottal resistance was observed after exercising in normal voiced subjects, whereas increased resistance was found in a patient with hypofunctional voice quality (eg, Laukkanen et al[10,29]). Various trends in glottal adduction changes therefore appear to be rather usual after semioccluded voice exercises.

The third hypothesis expected no change of the vocal fold length because no change of F_0 was required. Objective measurements done previously confirmed only small changes of F_0 after the tube phonation—4 Hz decrease in subject M[17] and 15 Hz decrease in subject F.[16] Overall, the length measurements seem to be in accordance with literature: the membranous vocal fold length in the female subject F was approximately twice smaller than in the male subject M, as expected owing to the sex differences.[30,31] As hypothesized, no significant change of the vocal fold length was found in neither of the two subjects. Detailed analysis showed that in subject F, the nonsignificance was owing to the repetition variability before the exercise (~0.5 mm), which was larger than the length changes before and after the exercise. For subject M, the nonsignificance was more owing to the measurement uncertainty: the length changes before and after the exercise were of the same magnitude as the measurement uncertainty on the RVF (±0.3 mm, Figure 9A, blue) and smaller than the measurement uncertainty on the LVF (±1 mm before the exercise, Figure 9A, red). These data suggest that either the vocal fold length did not change considerably from "before" to "after," or the change was so small that it could not be detected with this measurement method. The same measurements nevertheless allowed detecting an unexpected trend of the vocal folds to be longer during the tube phonation exercise than before or after the exercise in both subjects. The reason for this trend remains to be investigated.

Although no clear uniform trends in glottal configuration adjustments were observed here from before to after the tube phonation, acoustic investigations showed changes in voice quality and increased SPLs in the same subjects after the resonance tube phonation exercises. These were already reported in related studies[16,17] and are therefore only briefly summarized here. The SPL increased for both the subjects especially in higher frequency regions (speaker's formant). For subject F, the overall change of SPL caused by change in the vocal tract dimensions was also confirmed with the simulation, which showed 3-dB increase.[16] Because the simulation used the same source signal, it indicated that an important role in the SPL increase is played by the vocal tract.[16]

Indeed, the lack of clear systematic trends in vocal fold adjustment observed here contrasts with the clearly identifiable and much more prominent changes of the vocal tract (ie, expansion of its cavities, closure of the nasopharyngeal port, etc.) visible in the same CT recordings of the same subjects as analyzed here and in their MRI recordings performed on the same occasion. These vocal tract changes were reported in the related studies of Vampola et al,[16] Guzman et al,[17] and Laukkanen et al.[32] It is known that the vocal fold behavior can be considerably influenced by the interaction with the vocal tract.[2,18,19,33] The systematic changes in voice quality and vocal fold vibration reported in these subjects in the previous studies[16,17,32] seem therefore more likely to be caused by vocal tract changes and source-filter interaction effects rather than by the laryngeal muscle adjustments.

As far as the limitations of the study are concerned, the subjects underwent the examinations in a supine position because of the CT equipment requirements. This may have caused some changes in the configuration of the phonatory apparatus.[34] Nevertheless, the subjects subjectively reported that the resonance tube exercises in supine position did not prevent them in achieving voice quality improvements similar to those achieved in the upright position. Furthermore, acoustic comparisons of the phonations in supine and upright position done in a separate study revealed similar changes of voice quality parameters (eg, formants shifts), caused by the tube phonation in both positions.[32] Logically, because supine position was used for all the phonations, similar gravitational influences on the phonatory apparatus can be expected to occur before, during, and after the tube phonation and the changes in glottal configuration after the tube phonation may be expected to be owing to other

factors. These findings and considerations indicate that the supine position should not have any critical negative effect and may be used for evaluation of the tube phonation effects when upright position is not possible. Some limitation may be posed here also by the unusual geometry of the RVF in subject M mentioned in the Methods section. However, because structural asymmetry of the larynx is frequently found in normal subjects[35–39] and subject M did not report any vocal problems, the data were kept here.

The CT images of the vocal folds were obtained during sustained phonations with the acquisition time of 2 and 3.36 seconds. This caused the captured vocal fold shape to be averaged during vibration over a large number of vibratory cycles. How much were the resulting vocal fold shapes affected by the averaging process remains unclear and also poses a limitation of this study. However, because the averaging process was the same for all the conditions, it should not have any major effect on the reported comparisons before and after the tube phonations.

Only two subjects were examined in this study. This limitation is owing to radiation hazards, which do not allow examining large number of subjects and relies on volunteers, who are motivated to undergo the examination, such as the two researchers-coauthors of this study. Although examinations of more subjects would be desirable to have a better insight into the vocal fold adjustments caused by the tube phonation, this has not been targeted here for ethical reasons. However, because technological improvements of less-invasive imaging techniques (such as micro-MRI) are progressing (eg, Chen et al[40] and Delyiski & Hillman[41]), hopefully a larger study may become possible in future. A more accurate technique is requisite because the standard CT devices used here were at their limits for such precise measurements.

In conclusion, the results presented here did not show any systematic trends in the adjustment of the vocal folds that occurred after the phonation into a tube and were common to both of the subjects. Importantly, variability observed under the same conditions was mostly larger than the changes before and after the tube phonation. As such, the data indicate that the subjects did not adjust the vocal folds exactly the same way when repeating similar phonations. Such variability was seen here under all the conditions—before, during, and after the tube phonation.

There may be other factors, which were not investigated here, such as the optimum glottal width predicted from modeling studies of voice production.[42,43] These studies suggest that for an optimal voice production, the vocal folds should be neither hyperadducted nor hypoadducted, thus requiring different types of glottal adjustment for people using their vocal folds suboptimally (abduction for pressed voices vs more adduction for breathy voices). The tendency for less variability after the exercise was observed here for the vocal fold length and membranous glottal width (Figures 8 and 9); these adjustments can, however, be expected to become more apparent in subjects with voice disorders who were not targeted here. Further studies may be directed toward investigating whether the variability is smaller after the exercise than before. Although these gross glottal adductory changes are likely to be important in voice disorders,[44–46] our study together with results from other studies indicate that great accuracy of glottal configuration may not be critical: appropriate vocal tract adjustments may improve the voice quality and positively influence also the glottal behavior regardless of its exact configuration.[47,48] However, further studies with more subjects (with and without voice disorders) are needed to clarify the effects of the semi-occluded voice exercises in more detail.

CONCLUSIONS

1. No prominent uniform changes from before to after the exercise were found for vocal fold vertical thickness, bulkiness, glottal width, or vocal fold length.
2. The only significant change before and after the exercise was observed in the vertical thickness T_2 of the female (increase for both vocal folds) and vertical thickness and bulkiness of the male subject (but here the LVF and RVF showed opposite behavior).
3. The changes from before to after the exercise were of the same magnitude or smaller than the repetition variability before the exercises.
4. The measurement uncertainty was mostly smaller or similar to the repetition variability. This indicates that the lack of detection of any systematic changes in vocal fold configuration caused by the exercise is more likely owing to the inherent variability of glottal adjustment than because of the measurement uncertainty of the method used.
5. There was some tendency for smaller repetition variability after the resonance tube exercise for the vocal fold length and membranous glottal width. Study with larger number of subjects and potentially also with more precise measurement technology is needed to see whether the repetition variability decreases after the resonance tube exercises.
6. Changes in vocal tract configuration observed in previous studies after the resonance tube exercises were more prominent than the changes in vocal fold configuration observed here. Vocal tract resonance and vocal fold-vocal tract interaction phenomena therefore seem to play a more dominant role in the resonance tube exercises than the changes in vocal fold configuration.

Acknowledgments

The research has been supported by the Academy of Finland grant No. 134868 (A.M.L.) and in the Czech Republic by the Palacky University student's projects IGA_PrF_2013_017 and IGA_PrF_2014_029 (V.H.), by the European Social Fund Projects OP VK CZ.1.07/2.3.00/20.0057 (J.G.S.), OP VK CZ.1.07/2.4.00/17.0009 (V.H. and J.G.S.), and by the grant GA ČR P101/12/1306 (J.H.).

REFERENCES

1. Laukkanen A-M. *On Speaking Voice Exercises*. Tampere, Finland: University of Tampere; 1995.
2. Titze IR. Voice Training and therapy with a semi-occluded vocal tract: rationale and scientific underpinnings. *J Speech Lang Hear Res*. 2006;49: 448–459.
3. Spiess G. *Anleitung zur Erlernung einer richtigen Tonbildung in Sprache und Gesang (Aufl. 2)* [*Brief Guide to Learning a Proper Tone Production in Speech and Singing, 2nd ed*]. Frankfurt am Main, Germany: Publishing company of John Alt; 1904.
4. Habermann G. Funktionelle Stimstörungen und ihre Behandlung [Functional disorders of the voice and their treatment]. *Arch Otorhinolaryngol*. 1980;227:345.
5. Bele IV. Artificially lengthened and constricted vocal tract in vocal training methods. *Logoped Phoniatr Vocol*. 2005;30:34–40.
6. Simberg S, Laine A. The resonance tube method in voice therapy: description and practical implementations. *Logoped Phoniatr Vocol*. 2007;32: 165–170.
7. Laukkanen A-M, Titze IR, Hoffman H, Finnegan E. Effects of a semioccluded vocal tract on laryngeal muscle activity and glottal adduction in a single female subject. *Folia Phoniatr Logop*. 2008;60:298–311.
8. Sovijärvi A. *Die Bestimmung der Stimmkategorien mittels Resonanzröhren*. [*The Determination of Voice Categories by Means of Resonance Tubes*]. 5. Int. Kongr. Phon. Wiss., Münster 1964. Basel: S. Karger; 1965:532–535.
9. Sovijärvi A. Nya metoder vid behandlingen w. [New methods for treatment of voice disorders]. *Nordisk Tidskrift for Tale og Stemme*. 1969;3:121–131.
10. Laukkanen A-M, Lindholm P, Vilkman E. On the effects of various vocal training methods on glottal resistance and efficiency. A preliminary report. *Folia Phoniatr Logop*. 1995;47:324–330.
11. Vampola T, Laukkanen A-M, Horacek J, Svec JG. Finite element modelling of vocal tract changes after voice therapy. *Appl Comput Mech*. 2011;5: 77–88.
12. Laukkanen A-M. About the so called "resonance tubes" used in Finnish voice training practice: an electroglottographic and acoustic investigation on the effects of this method on the voice quality of subjects with normal voice. *Scand J Logop Phoniatr*. 1992;17:151–161.
13. Hirano M. Phonosurgery: basic and clinical investigations. *Otologia (Fukuoka)*. 1975;21:239–442.
14. Yumoto E, Kadota Y, Kurokawa H. Thyroarytenoid muscle activity and infraglottic aspect of canine vocal fold vibration. *Arch Otolaryngol Head Neck Surg*. 1995;121:759–764.
15. Chhetri DK, Neubauer J, Berry DA. Neuromuscular control of fundamental frequency and glottal posture at phonation onset. *J Acoust Soc Am*. 2012; 131:1401–1412.
16. Vampola T, Laukkanen A-M, Horacek J, Svec JG. Vocal tract changes caused by phonation into a tube: a case study using computer tomography and finite-element modeling. *J Acoust Soc Am*. 2011;129:310–315.
17. Guzman M, Laukkanen AM, Krupa P, Horáček J, Švec JG, Geneid A. Vocal tract and glottal function during and after vocal exercising with resonance tube and straw. *J Voice*. 2013;27:523.e19–523.e34.
18. Titze IR. Nonlinear source–filter coupling in phonation: theory. *J Acoust Soc Am*. 2008;123:2733–2749.
19. Titze IR, Riede T, Popolo P. Nonlinear source–filter coupling in phonation: vocal exercises. *J Acoust Soc Am*. 2008;123:1902–1915.
20. Story BH, Laukkanen AM, Titze IR. Acoustic impedance of an artificially lengthened and constricted vocal tract. *J Voice*. 2000;14:455–469.
21. Hollien H. Some laryngeal correlates of vocal pitch. *J Speech Lang Hear Res*. 1960;3:52–58.
22. Hollien H, Curtis JF. A laminagraphic study of vocal pitch. *J Speech Lang Hear Res*. 1960;3:361–371.
23. Hollien H, Curtis JF. Elevation and tilting of vocal folds as a function of vocal pitch. *Folia Phoniatr (Basel)*. 1962;14:23–26.
24. Hollien H, Moore GP. Measurements of the vocal folds during changes in pitch. *J Speech Lang Hear Res*. 1960;3:157–165.
25. Hollien H, Colton RH. Four laminagraphic studies of vocal fold thickness. *Folia Phoniatr (Basel)*. 1969;21:179–198.
26. Hollien H. Vocal fold dynamics for frequency change. *J Voice*. 2014;28: 395–405.
27. Ratib O, Rosset A, Heuberger J. *OsiriX: The Pocket Guide*. Geneva, Switzerland: OsiriX Foundation; 2009.
28. Titze IR. Raising lung pressure and pitch in vocal warm-ups. *J Sing*. 2002; 58:329–338.
29. Laukkanen A-M, Lindholm P, Vilkman E. Vocal exercising and speaking related changes in glottal resistance. *Logoped Phoniatr Vocol*. 1998;23: 85–92.
30. Kahane J. A morphological study of the human prepubertal and pubertal larynx. *Am J Anat*. 1978;151:11–20.
31. Titze IR. Physiologic and acoustic differences between male and female voices. *J Acoust Soc Am*. 1989;85:1699–1707.
32. Laukkanen A-M, Horacek J, Krupa P, Svec JG. The effect of phonation into a straw on the vocal tract adjustments and formant frequencies. A preliminary MRI study on a single subject completed with acoustic results. *Biomed Signal Process*. 2012;7:50–57.
33. Hanamitsu M, Kataoka H. Effect of artificially lengthened vocal tract on vocal fold oscillation's fundamental frequency. *J Voice*. 2004;18:169–175.
34. Stone M, Stock M, Bunin K, et al. Comparison of speech production in upright and supine position. *J Acoust Soc Am*. 2007;122:532–541.
35. Bonilha HS, O'Shields M, Gerlach TT, Deliyski DD. Arytenoid adduction asymmetries in persons with and without voice disorders. *Logoped Phoniatr Vocol*. 2009;34:128–134.
36. Lindestad P-Å, Hertegård S, Björck G. Laryngeal adduction asymmetries in normal speaking subjects. *Logoped Phoniatr Vocol*. 2004;29:128–134.
37. Hirano M, Kurita S, Yukizane K, Hibi S. Asymmetry of the laryngeal framework—a morphologic study of cadaver larynges. *Ann Otol Rhinol Laryngol*. 1989;98:135–140.
38. Friedrich G, Kainz J. Morphometrie des Kehlkopfes an Horizontalschnitten. Basisdaten für die quantitative Auswertung moderner Bildgebender Verfahren [Morphometry of the larynx in horizontal sections. Reference data for quantitative analysis of recent imaging techniques]. *Laryngol Rhinol Otol*. 1988;67:269–274.
39. Friedrich G, Kainz J, Anderhuber F. Der Einfluss der Schildknorpelkonfiguration auf Asymmetrien des dorsalen Kehlkopfeingangspfeilers und deren Bedeutung für die Stimmfunktion. [The effect of the configuration of the thyroid cartilage on the asymmetry of the dorsal glottis and its significance for vocal function]. *HNO*. 1988;36:241–250.
40. Chen T, Chodara AM, Sprecher AJ, et al. A new method of reconstructing the human laryngeal architecture using micro-MRI. *J Voice*. 2012;26:555–562.
41. Deliyski DD, Hillman RE. State of the art laryngeal imaging: research and clinical implications. *Curr Opin Otolaryngol Head Neck Surg*. 2010;18: 147–152.
42. Titze IR, Schmidt SS, Titze MR. Phonation threshold pressure in a physical model of the vocal fold mucosa. *J Acoust Soc Am*. 1995;97:3080–3084.
43. Lucero JC. Relation between the phonation threshold pressure and the prephonatory glottal width in a rectangular glottis. *J Acoust Soc Am*. 1996;100: 2551–2554.
44. Verdolini K, Druker DG, Palmer PM, Samawi H. Laryngeal adduction in resonant voice. *J Voice*. 1998;12:315–327.
45. Isshiki N. Mechanical factors responsible for hoarseness. In: Hurme P, ed. *Vox Humana. Studies Presented to Aato Sonninen on the Occasion of His Sixtieth Birthday.* Jyväskylä, Finland: Institute of Finnish Language and Communication, University of Jyväskylä; 1982:94–102.
46. Guzman M, Castro C, Testart A, Muñoz D, Gerhard J. Laryngeal and pharyngeal activity during semioccluded vocal tract postures in subjects diagnosed with hyperfunctional dysphonia. *J Voice*. 2013;27:709–716.
47. Titze IR. The pyhsics of small-amplitude oscillation of the vocal fold. *J Acoust Soc Am*. 1988;83:1536–1552.
48. Titze IR. Acoustic interpretation of resonant voice. *J Voice*. 2001;15: 519–528.