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INVESTIGATING PHONATION THROUGH EXCISED HUMAN LARYNGES: RECENT DEVELOPMENTS AND ONGOING WORKS ON AN ANIMATED TESTBENCH

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ABSTRACT

Many aspects of voice production can be investigated by means of *in vivo* observations and experiments on synthetic vocal folds replica. However, either mammals or human excised larynges provide an interesting trade-off between realistic physiological conditions, reproducibility and observability, paving the way to the development in the last years of experimental setups involving excised human larynges. Some of them focus on the control of the laryngeal articulation, in order to enable the simulation of coordinated muscular activations.

We here present the last developments of the excised human larynx test bench hosted at the Medecine Faculty in Marseille, with the motorized animation of some of the intrinsic laryngeal muscles. As an evidence of the possibilities of such experimental devices, we present a quantitative analysis of the contact pressure between vocal folds in presence of a Reinke's edema.

1. INTRODUCTION

From a physical point of view, phonation is seen as the sound production induced by the self-sustained oscillation of the vocal folds in interaction with the airflow in the glottis (see Ref. [1] for a detailed description). This is enabled by the combination of convenient geometry (that speeds up the airflow in the glottal constriction) and elasticity of the tissues (that counteracts the resultant of the air pressure, with possible delay).

In vivo studies are made challenging by the difficult evaluation of the geometric, biomechanical and aerodynamical characteristics of the human larynx from videendoscopic imaging, electroglottographic or even electromyographic measurements through the vocal tract of the subject or his skin. This is even more intricate considering the uncontrolled muscles contraction that hinder repeatability. This paved the road to experiments on vocal folds replica: pioneering setup designed by Muller [2] was either simplified to investigate the peculiarities of the flow in idealized glottal channel (either static [3] or motorized [4]) or rather augmented in order to improve the decoupled control of several geometrical and mechanical parameters (see, e.g.,

Ref. [5]) involved in the self-sustained oscillations. They are usually made of an elastomer envelop containing water [5] or a bioreactor [6], or of a tissue-like hydrogel [7].

Nevertheless, by now, none of these synthetic designs manages to reach to combine observability to realistic anatomical or physiological conditions. Either mammals or human excised larynges provide an interesting trade-off between those requirements, and the last decades witnessed a large variety of experimental setups involving excised larynges (see Garcia and Herbst [8] and Luo *et al* [9] for recent overviews). However, even these setups may be affected by oversimplified controls: as an example, the automated setup designed in Erlangen [10] suffers from an over-constrained mobility of the arytenoids cartilages as the chosen electromechanical actuators inhibits the essential rocking motion of the arytenoids on the cricoid facets.

The aim of this communication is thus to present the last developments of the excised human larynx test bench hosted at the Medecine Faculty in Marseille, with the motorized animation of some of the intrinsic laryngeal muscles (Sec. 2) designed to be physiologically realistic. As an evidence of the possibilities of such experimental devices, we present three studies: first the validation of the *ex vivo* laryngeal articulation (Sec. 3.1), then the control of the fundamental frequency f_o using the tensioning of the crico-thyroid muscle (Sec. 3.2), and finally a quantitative analysis of the contact pressure between vocal folds in presence of a Reinke's edema (Sec. 3.3).

2. DESCRIPTION OF THE ANIMATED TESTBENCH

2.1 Larynx preparation

Human larynges are harvested from fresh cadavers, within 24 to 48 hours after death, in accordance with the French ethical laws. Larynges (including the upper three tracheal rings) are then rapidly frozen in 0.9% saline solution. Before experiment, the larynx is thawed in saline solution and cleaned with removal of the extraneous tissues and supraglottic structures (epiglottis and vestibular folds), within literature standards [11]. Ref. [12] evidences that dehydration of the tissues during experiments seems to

have more impact on phonation characteristics (such as the f_o range) and on tissue damaging than successive freezing/thawing cycles.

The larynx is then secured on the experimental bench, the posterior edges of the thyroid cartilage lying horizontally upon adaptable steel workpieces (with hose ties on the superior horns). The gap (adjustable in order to deal with the interindividual variability in larynx dimensions) between the latter enables the cricoid cartilage and the trachea to move freely during the crico-thyroid tilt.

2.2 Actuation

The intrinsic laryngeal musculature being disabled on excised organs (except for preliminary *ex vivo* perfused larynx under neuromuscular electrical stimulation [13]), it is necessary to set up substitutes enabling the reproduction of the effects of the muscle activation. It was the aim of the ANIMAGLOTTE project (funded by the Mission for Interdisciplinarity CNRS, 2018, coordinated by Th. Legou) not to impose the motions of the cartilages (as done, e.g., in Ref. [10] using needles pricked to the cartilages) but to mimic the forces resulting from muscular activation (see Ref. [1] for a review of the muscles and their orientation). For most of the controls, this means that we focus on the approximation of the origin and the insertion of the essential intrinsic muscles of the larynx. The whole actuation setup is shown on Fig. 1 and is controlled using an Arduino board.

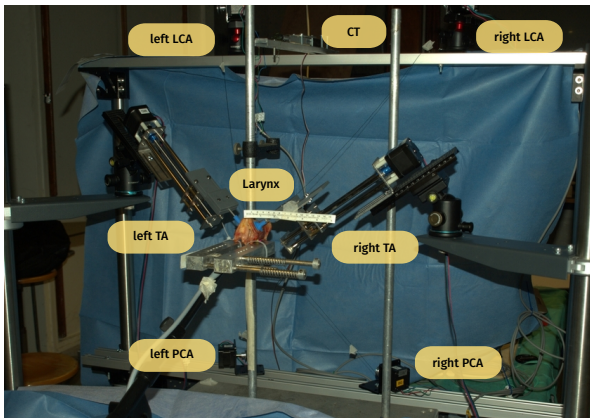


Figure 1. Full experimental setup, showing the positioning of the actuators with respect to the excised larynx.

2.2.1 Crico-thyroid (CT) pars recta

This muscle links the anterior parts of the cricoid and the thyroid cartilages, is innervated by the external branch of the superior laryngeal nerve (contrarily to all other muscles, which are innervated by the recurrent laryngeal nerve) and is generally reported as the primary control for the fundamental frequency. Considering the thyroid as a reference frame, the contraction of the CT muscle induces the tilt of the cricoid that supports the arytenoids, which lengthens, thins and tenses the vocal folds. This is simulated on excised larynges by a pulling system: an inextensible nylon thread is stitched on the anterior arch of the cricoid, runs

along the pars recta and exits through an orifice done into the lamina angle of the thyroid (see Fig. 2), and is attached on the mobile rod of a linear actuator (Firgelli L12-100, range 100 mm). The CT angle related to the tilt and the lengthening of the vocal folds using this pulling system have been reported in Ref. [14], highlighting an intrinsic upper bound for this control corresponding to the situation where the anterior parts of the cricoid and the thyroid cartilages are in contact.

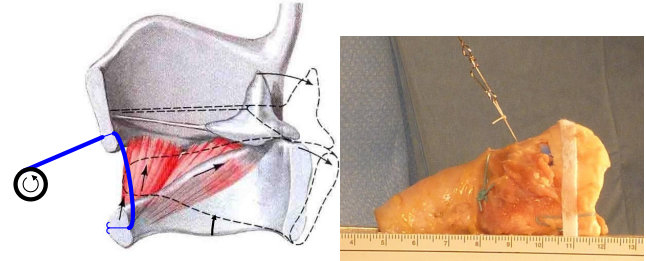


Figure 2. CT actuation: schematics of the pulling system and of the resulting rotation of the cricoid cartilage around the CT joint (left), and realization on an excised larynx (right, from Ref. [15]).

2.2.2 Thyroarytenoid muscle (TA)

These paired muscles run from the angle of the thyroid to the anterolateral surface of the arytenoids, they would shorten the vocal folds. However, being antagonist to the CT, the contraction of the muscular fibers essentially contributes to the thickening of the membranous vocal fold and thus to its medialization. As described in Ref. [14], this effect is reproduced using a type 1 thyroplasty performed by means of Montgomery® implants inserted through bilateral openings done in the thyroid wings (see Fig. 3). The handle of each implant is attached to a linear motion actuator.

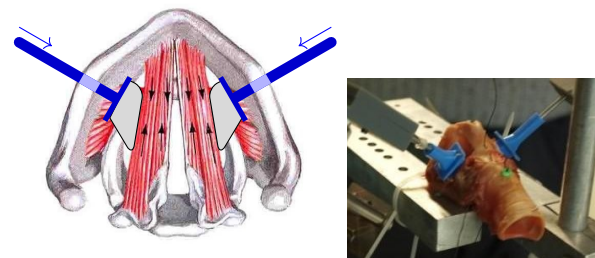


Figure 3. TA actuation: schematics of the medialization system (left, top view), and realization on an excised larynx (right, from Ref. [14]).

2.2.3 Lateral cricoarytenoid muscles (LCA)

These paired muscles run obliquely from the anterolateral surfaces of the cricoid to the lateral tips of the arytenoids. Their activation contributes to the rotation of the arytenoids on their relative cricoid facets, the processes of the vocalis moving inwards which classifies the LCA as adductors muscles.

Following Refs. [12, 16], sutures are done on the muscular processes of the arytenoids, the threads then course anteriorly, medially and inferiorly, passing below the thyroid wings to preserve the mean orientation towards the muscular origins on the anterolateral surfaces of the cricoid (see Fig. 4). Each thread is then attached to the wheel of a stepper motor 17HM15-0904S.

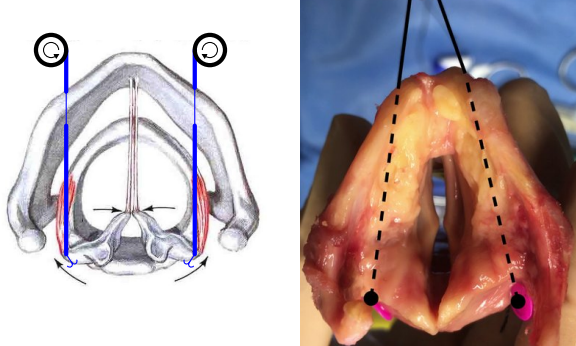


Figure 4. LCA actuation: schematics of the pulling system and of the resulting rotation of the arytenoids cartilages around the CA facets (left, top view), and realization on an excised larynx (right, threads as black lines - dashed when within tissues).

2.2.4 Posterior cricoarytenoid muscles (PCA)

These paired muscles attach to the posterior face of the cricoid and the muscular process of each arytenoid. Their activation contribute to rotate the latter (thus abducting the vocal folds) and to pull them posteriorly, acting then as direct antagonists to the LCA muscles. As for LCA actuation, threads are sewed to reproduce physiological force directions and are attached to wheels of servo (same as for LCA actuation, see Fig. 5).

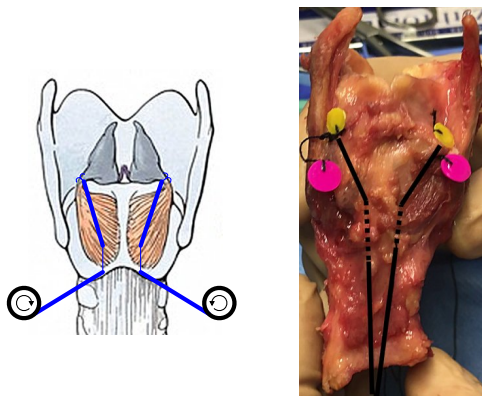


Figure 5. PCA actuation: schematics of the pulling system (left, rear view), and realization on an excised larynx (right).

2.3 Aerodynamics and Sensors

For experiments implying aerodynamics (needed for phonation), airflow may be supplied from a turbine (Werei Rietschle, Fürstenfeldbruck, Germany) through

an electrovalve (Burkert 2875, now replacing the previous quarter-turn manual valve, see Ref. [17]), a flowmeter (TSI4043, full scale 200 L min^{-1}), a heater/humidifier (Dräger, Aquador, Dräger) and an intubation tube (Rüsch Elit, inner diameter 6.5 mm, with balloon cuff fitted in the tracheal rings), respectively. Finally, the subglottal aerodynamical pressure is measured by inserting a venous catheter (23-gauge) through the crico-thyroid membrane. The catheter is then connected to the piezo-resistive differential pressure sensor provided by the EVA workstation (SQLab, Aix-en-Provence, France).

Concerning acoustics, a microphone (AKG C520) may be placed 30 cm away from the larynx to record the radiated acoustic pressure. Additionally, a probe microphone (GRAS 40SC) can be inserted within the trachea along the balloon cuff in order to measure the acoustic component of the subglottal pressure. Vibrations of the vocal folds may also be monitored by electroglottographic electrodes placed on the anterior aspect of the thyroid laminae and connected to the Laryngograph microProcessor (Wallington, United Kingdom), and/or by a high-speed camera (Fastcam SA3, Photron, external trigger, with a micro-lens Nikon AF MicroNikkor 105 mm, f/2.8D, and a light source STORZ XENON NOVA 175) and recording up to 5000fps.

Finally, a piezo-resistive transducer (Model 105, Precision Measurement Company, Ann Arbor; full scale 207 kPa, 2.67 mm diameter and 0.508 mm thickness) can be positioned between the vocal folds to provide access to the contact pressure during the closed glottis episode.

3. SOME EXPERIMENTAL STUDIES

3.1 Ex vivo laryngeal articulation

We first present experiments testing the effectiveness of the actuation described in Sec. 2.2. As a first step, the ranges of laryngeal motions (described in terms of vocal folds angle, antero-posterior diameter, inter-arytenoid and mid-vocal folds distance) have been estimated from clinical *in vivo* fibroscopic videos ($N = 28$ healthy subjects performing breathing, M1 and M2 phonation; 24 females and 4 males) in order to provide normalized reference data.

Then the designed actuation has been tested on two excised larynx (2 female). As a first step, individual actuations (one muscle simulated at a time) were performed and recorded (see Fig. 6). It is visible that the medialization of the membranous vocal folds by the Montgomery implants, the adduction of the vocal processes (with a net hourglass shape), the abduction by the simulation of CAP are effective. The CT actuation is less noticeable on the picture but has already been validated in Ref. [14]. As a last comment, it is worth noticing that individual actuations were performed here with no effort applied on the other muscles. Those are expected to stabilize the tissues, i.e., to reduce the magnitude of the displacements obtained in this individual actuation experiment and to reduce the hysteresis visible for some of the muscles (left PCA, right TA and right LCA).

Coactivations of the muscles have also been tested, in a

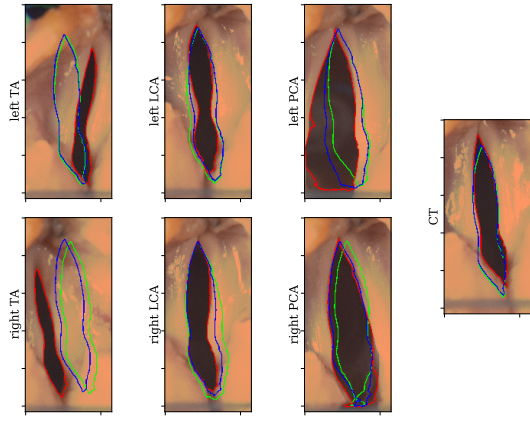


Figure 6. Effect on the glottis of each individual actuation. Position of the glottis at rest (blue: before activation, green: after activation) and during the activation (background image and red contour).

scenario mimicking sequentially breathing, prephonatory posturing, M3 phonation, and successive transitions to M2 and M1 phonations. All these data are still to be compared to the *in vivo* reference data, to the magnitude of the vocal process displacement reported in Ref. [18] and to the correspondence between laryngeal EMG data and the image-based estimation of vocal folds angle discussed in Ref. [19].

3.2 Control of the fundamental frequency

Another experiment focused on the control of the fundamental frequency. Air supply line being connected to the excised larynx prepared as in Ref. [14] (i.e., the LCA and PCA actuations described in Sec. 2.2 being replaced by the double adduction technique). Traction and release cycles were performed on the CT actuation leading to changes in the fundamental frequency of the radiated acoustic pressure as displayed on Fig. 7. On this recording, the control is coarse-grained ($\pm 1mm$ steps) and not all commands seem to induce a step in f_o . This may be due to a failure in the motor control (the linear actuator does not respond to

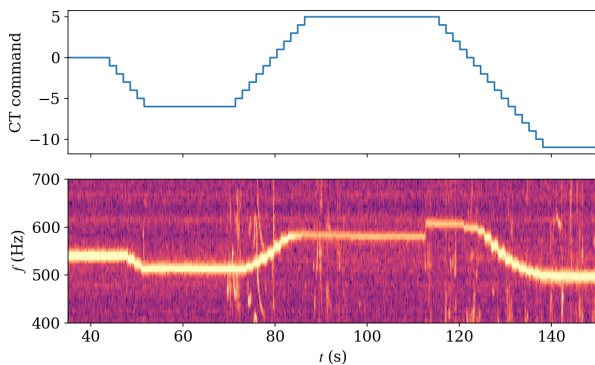


Figure 7. CT actuation commands (u.a., top) and spectrogram of the radiated acoustic pressure (bottom). Effect of the tension of the CT thread on the phonation fundamental frequency.

the electrical input, which seems a common issue on such devices) or either a saturation effect related to the cricoid and the thyroid being in contact on their anterior edges.

Another interesting situation is depicted in Fig. 8 where a bifurcation from the M1 laryngeal mechanism to the M2 one occurs about $t = 28s$. The opposite situation when releasing the tension at $t = 43s$.

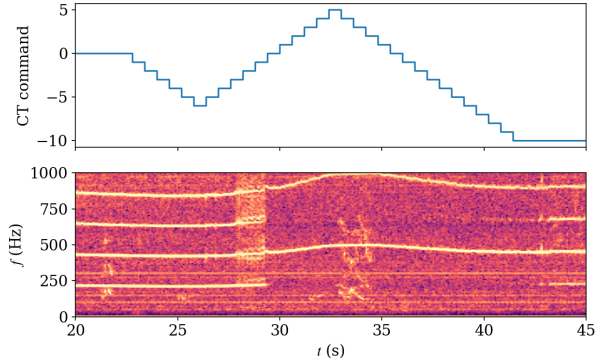


Figure 8. Same quantities as in Fig. 7 for a different larynx and different controls on the subglottal pressure and on the CT actuation.

3.3 Contact pressure on a larynx with Reinke's edema

As a last example, we refer to Ref. [20] by some of the current authors. It is the first comparison of the contact pressure between vocal folds for two excised human larynges, one of them being pathological with a bilateral loose swelling of the vocal folds diagnosed as bilateral grade 1 Reinke's edema. Both larynges were tested on the testbench (with controls and sensors depicted in Fig. 9) with the airflow controlled in a kind of *Messa di Voce* singing exercise (progressively increased, maintained, then decreased), scanning the subglottal pressure range for human phonation.

While most of the usual descriptors (onset and offset subglottal pressures; sound level; fundamental frequency; RMS values for EGG; and Harmonics-to-noise ratio) are comparable and consistent with classical knowledge on patients suffering from Reinke's edema, the main observation is the extremely high level of contact pressure during stable phonation (up to 296 kPa) in the pathological larynx, compared to the healthy larynx (no more than 34 kPa). This suggests that the phonotrauma may contribute to maintain

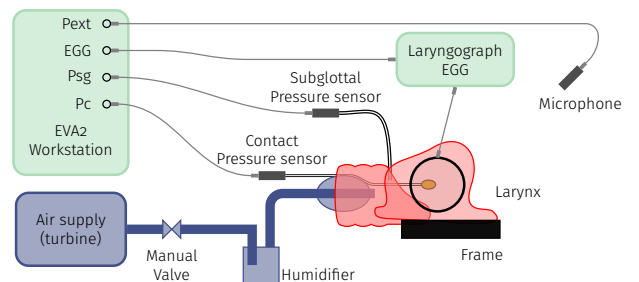


Figure 9. Setup for the estimation of the contact pressure on healthy and pathological larynges.

the lesion after its appearance.

4. CONCLUSION

The excised human larynx test bench designed and hosted at the Medecine Faculty in Marseille has shown to be a valuable tool to investigate phonation and its pathologies. To the best of our knowledge, this is the first implementation of this full set of actuators and sensors on excised larynx testbeds that offers, in addition to a laryngeal flow control, a physiologically realistic motorized animation of the intrinsic laryngeal musculature with less constrained motions of the cartilages and the soft tissues than other setups prescribing idealized translations and rotations.

Further work is still needed to provide a calibration procedure (in order to automatically determine the bounds and reference state of each actuator) and to map high-level controls to low-level actuation commands.

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