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Preliminary Study of Open Quotient in an Ex-Vivo Perfused Human Larynx Model

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Abstract

Importance—Scientific understanding human voice production to date is a product of indirect investigations including animal models, cadaveric tissue study, or computational modeling. Due to its invasive nature, direct experimentation of human voice production has previously not been possible. The feasibility of an *ex-vivo* perfused human phonatory model has recently allowed systematic investigation in virtually living human larynges with parametric laryngeal muscle stimulation.

Objective—In this study, the relationship between adductor muscle group stimulation and the open quotient (OQ) of vocal fold vibration was investigated using an *ex-vivo* perfused human larynx.

Design—Human perfused tissue study.

Setting—Physiology Laboratory.

Participants—Human larynx is recovered from research-consented organ donors within two hours of cardiac death.

Interventions, Main Outcomes and Measures—Perfusion with donated human blood is re-established shortly after cardiac death. Human perfused phonation is achieved by providing subglottal airflow under graded neuromuscular electrical stimulation bilaterally to the intrinsic adductor groups and cricothyroid muscles. The phonation resulting from the graded states of

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Study concept and design: Mendelsohn, Zhang, Berke

Acquisition, analysis, or interpretation of data: Mendelsohn, Zhang, Luegmair, Orestes, Berke

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neuromuscular stimulations are evaluated through high-speed vibratory imaging. OQ is derived through digital kymography and glottal area waveform analysis.

Results—Under constant glottal flow, step-wise increase in adductor muscle group stimulation decreased OQ. Quantitatively, OQ values reached a lower limit of 0.42. Increased stimulation above maximal muscle deformation was unable to affect OQ beyond this lower limit.

Conclusions and Relevance—For the first time in a neuromuscularly activated human larynx, a negative relationship between adductor muscle group stimulation and phonatory OQ was demonstrated. Further experience with the *ex-vivo* perfused human phonatory model will work to systematically define this causal relationship.

INTRODUCTION

The compendium of human voice production understanding is a product of observational associations and direct control laryngeal models. However, such direct systematic control of physical variables had previously been confined to comparative laryngeal models. Specifically, laryngeal modeling has been applied in a number of methods including phonation within: excised human larynges,[1,2] *in-vivo* physiologically active animal larynges,[3,4] and computational modeling.[5,6] Yet these experimental constructs hold limitation towards the successful extrapolation to fully describe human voice production. For instance, creating phonation by mechanically adducting excised cadaveric human larynges does not incorporate the effects of physiologically active thyroarytenoid muscle, which is known to increase the bulk and tension of the vocal fold body. Additionally within the cadaver laryngeal model, many changes to the soft tissue are seen without physiologic blood flow such as epithelial dehydration and loss of tissue elasticity. *In-vivo* animal phonation is limited by anatomic and physiologic variances between the number of proposed mammalian larynges described in the literature and human larynges. Computational phonatory modeling is also limited as it utilizes specific quantitative data from the other described models. By using specific data related to vocal fold compliance or elasticity from limited scientific platforms, the computational models also demonstrate limitations to direct clinical application.

In response to these known shortcomings in human voice production research, a novel phonatory model utilizing *ex-vivo* perfused human larynges was developed by Berke *et al.*[7,8] As described, human larynges are recovered shortly following cardiac death and are re-perfused in order to maintain physiologic viability of the larynges. By applying neuromuscular electrical stimulation, physiologic laryngeal muscle activation was accomplished, and the feasibility has previously been described. In continued preliminary experience with this new phonatory model, a relationship between muscle stimulation level and phonatory open quotient was observed. We therefore set out to describe our preliminary observations regarding this relationship for the first time within physiologically viable human larynges.

METHODS AND MATERIALS

Institutional Board Review (IRB) of the methodology determined that this study was exempt from IRB approval.

Ex-Vivo Method

The general methodology of the *ex-vivo* perfused human phonatory model has previously been described;[7,8] however, improvements in the methodology have been selectively applied during the intervening time period. A focused description of the updated *ex-vivo* methods specific to the presently reported phonatory data will be discussed.

Consents for the recovery of human larynges from transplant donors are obtained from the donor patients' families by the patient care coordinators of *One Legacy*, the Southern California organ transplant distribution center. Following the recovery of clinically transplantable organs, the larynx, esophagus, trachea, strap muscles, thyroid, carotid arteries, vagus nerves and branches, and the internal jugular veins are excised *en bloc*. The carotid arteries are then irrigated with Wisconsin perfusate solution, placed in 4°C sterile storage, and transported by car to the laboratory.

In the laboratory, the recurrent and superior laryngeal nerves are identified and the arterial branches not related to laryngeal perfusion are tied off. The common carotid arteries are cannulated by 12-French cannulae (DLP Pediatric One-Piece Arterial Cannulae, Medtronic, Minneapolis, MN). Venous outflow was via gravity drainage into the RM3 collection system (RM3 Renal Preservation System, Walters Medical Systems, Rochester, MN). Type O-commercially obtained human whole blood is infused with Ringers Lactate to a hematocrit between 25–40% is used to re-establish blood flow to the larynx. A *RM3 perfusion pump* provides blood flow in a pulsatile manner resulting in arterial blood systolic and diastolic pressure.

Dialysate solution (PrismasateBgk 4-2.5, Gambro Renal Products, Stockholm, Sweden) is utilized in-line with a pediatric dialysis filter to remove the severe hyperkalemia resulting from the previously infused Wisconsin solution and to maintain electrolyte homeostasis. During the experiments, organ pH, oxygen pressure, and serum electrolytes are monitored by blood gas and are adjusted as needed. The RM3 strength is adjusted to provide a systolic pressure ranging between 60–80mm Hg with a minimally acceptable pulse pressure of 10mm Hg, at a pump rate of 60 pulses/min.

Following approximately 1 hour of re-application of blood flow, the organ is assessed for neuromuscular contractility. Initially, the recurrent laryngeal nerves are activated with constant current stimulators (Model WPI 301-T, World Precision Instruments, Sarasota, FL) and the larynx is observed for muscular activation. If muscular contraction is not achieved with nerve stimulation, muscular contraction is produced by direct muscle stimulation. Hook-wire bipolar electrodes[9] are inserted directly into laryngeal muscles. The cricothyroid (CT) and adductor muscle groups are electrically stimulated by two constant current stimulators (Model WPI 301-T, World Precision Instruments, Sarasota, FL) set at 60Hz. The electrodes are placed directly through the thyroid cartilage into the thyroarytenoid muscles.

The CT muscles needles are placed under direct vision of the anterior/superior laryngeal surface. The CT muscles are stimulated during phonatory trials based on the group's prior work in canine phonatory experimentation. The quality and strength of produced phonation is improved with constant high level of CT activation. Within the present description, the level of CT activation is not changed throughout the experiments and the effect of CT activation is not evaluated. This qualitative improvement is likely due to the slight adductory action of the CT muscle, though specific investigation will be required to elucidate the phonatory effects of the human CT muscle.

For initial stimulation observations, current ranged from 0.5 to 10 milliamps in order to identify the range which observable muscular deformation was produced. Following confirmation of appropriate levels of neuromuscular stimulation, warmed, 37 degree Celsius, 100% humidified air is flowed rostrally through the larynx at 350 cc/second, utilizing an endotracheal tube with an inflated cuff. (*Note: Apart from physiologic neuromuscular activation induced laryngeal adduction, no methods of physical approximation of the vocal process were used*) With a constant rate of subglottic airflow, muscular activation is produced in a steadily increasing ramp in a voltage-dependent manner ranging from 2V (volts) to 10V. This ramp was repeated a minimum of three times within the presently reported phonatory trial. Additionally, our findings have been corroborated by continuous experience with the *ex-vivo* model.

Phonatory Data Extraction

Vocal fold vibration was recorded using a high-speed camera (Vision Research Phantom v210) at 3000 fps and a resolution of 512 × 512 pixel. Spatial-temporal plots of vocal fold vibration (or kymograms) were first extracted from the high speed recordings. To generate the kymogram, a medial-lateral line was extracted from each frame of the recordings. These image lines were then stacked consecutively to form a kymogram.[10] For our analysis, a line near to the medial point of the glottal midline was chosen. The glottal area waveform (GAW) was also extracted from the recordings using a region growing algorithm,[11] from which the open-quotient (the fraction of the one cycle the glottis remains open) was calculated for each oscillation period and averaged for each recording.

RESULTS

Following the confirmation of perceptually normal phonation in *ex-vivo* human larynx, stimulation ramps were applied symmetrically to the adductor muscle groups under constant cricothyroid muscle stimulation. Figure 1 displays the vibratory images seen with high-speed laryngoscopy, which highlights the OQ variation between the lowest (2V) and highest (10V) levels of adductor muscle stimulation. With high levels of adductor group stimulation, the glottis closed longer. Figure 2 shows the kymographs for each of the recorded adductor muscle stimulation levels that display quantitatively the OQ variances. Additionally, Figure 3 displays the GAWs that were extracted to compliment the demonstrated kymographs as kymography is limited to the selected horizontal line of analysis whereas GAW integrates the whole glottal aperture. Figure 4 graphs the calculated OQ by level of adductor stimulation suggesting a direct relationship. OQ decreased with increased stimulation levels

from 2V (OQ=1) to 5V (OQ=0.68) to 8V (OQ=0.42), and plateaued at 10V (OQ=0.42). OQ did not change with stimulation level larger than 8V suggesting that following maximal adductor neuromuscular activation, no change in OQ can be produced through adductor muscle stimulation. The phonatory principles reported here were repeated multiple times to ensure the reliability and repeatability of the model.

DISCUSSION

In this study, human voice production within a physiologically active human larynx was investigated. For this report, the authors have chosen to describe the vibratory characteristic of open quotient (OQ). The observable relationship suggested that OQ was directly decreased with increased adductor muscle group stimulation.

The origins of OQ descriptions began with Timke, defining OQ as the time ratio of a single vibratory cycle spent with the glottis in an open configuration over the duration of the entire vibratory cycle. Timke was amongst the first to identify OQ variability with vocal modulation specifically voice intensity and pitch.[12] Yet while changes in OQ have been related to changes in voice quality,[13,14] these and other reported relationships between OQ and voice measures are only loosely associated, and the control of OQ has also been poorly described. However, our control of the vibratory cycle during phonation is thought to be a critical aspect in normal and abnormal voice production.

For instance, several studies have examined the relationship between OQ and vocal intensity without a consensus conclusion regarding a causal relationship.[12,15–17] In the canine in-vivo model, while increased medial tension of the phonating vocal folds produced increased vocal intensity,[18] a follow-up clinical study could not show a causal relationship between increased vocal intensity and OQ.[16] To postulate the source of this discrepancy, it has been suggested that the global laryngeal aerodynamics and vocal tract mechanics can supersede the relationships seen between OQ and vocal parameters seen in experimental data.[19] Otherwise stated, human voice production with the numerous postural and behavior complexities can obscure the physiologic mechanisms for OQ control.

The components which directly modulate OQ are thought to be directly associated with the viscoeleastic properties of the vibrating folds.[20] With prior evidence that adductor group stimulation increases vocal fold tension of the body layer,[21] the utilization of physiologically viable larynges is critical towards the study of phonatory OQ. With the use of the *ex-vivo* perfused larynx, the data presented here suggests that an increase in adductor group stimulation does in fact lead to direct modulation of the OQ. Further systematic variations in the *ex-vivo* model, we can have a greater understanding of the source mechanisms of OQ modulation and thereby voice quality.[22] Future studies will be targeted to further define normal phonatory mechanisms, such as vocal fold tension during phonation, as well as pathologic states of voicing, such as unilateral vocal fold paresis with asymmetric tension.

As the *ex-vivo* human phonation model continues to be refined, the current shortcomings of this model can be improved. Planned refinements include a quieter blood pump system to allow for high fidelity acoustic recordings to accurately investigate the vocal changes which

are seen with vibratory alterations. The model will also include electromyographic analysis as well as subglottic pressure recordings during *ex-vivo* phonation. However, a continued challenge for this model is the lack of statistical analysis which appears to be a significant challenge for this model due in large part to the inherent organ-to-organ variability. Factors which lead to this inter-organ variability include the variances in clinical data (including: *gender, age, body height, medical comorbidities, cause of death, length of intubation prior to organ recovery, knowledge of pre-recovery laryngeal function*) and methodological data (including: *intraoperative medication administration including neuromuscular blockade, time from aortic cross-clamp to laryngeal preservate-solution infusion, transport time from recovery hospital to laboratory, inability to measure consistent re-establishment of whole organ microcirculation perfusion*). As the available donor pool is limited, strict control of these variables cannot be selected for and statistical variance would preclude accurate analysis. However, as the experience with the phonation model increases, phonation from variable-similar organs may be combined to allow for reliable statistical analysis.

CONCLUSION

Utilizing human larynx *ex-vivo* perfused phonation, the relationship between adductor muscle group stimulation and glottal open quotient was investigated. Observational study suggests that increased adductor group neuromuscular stimulation may modulate open quotient. Specifically, increasing adductor stimulation resulted in decreasing open quotient ratio. Future work utilizing the *ex-vivo* perfused phonatory model will concentrate on direct study of human voice production.

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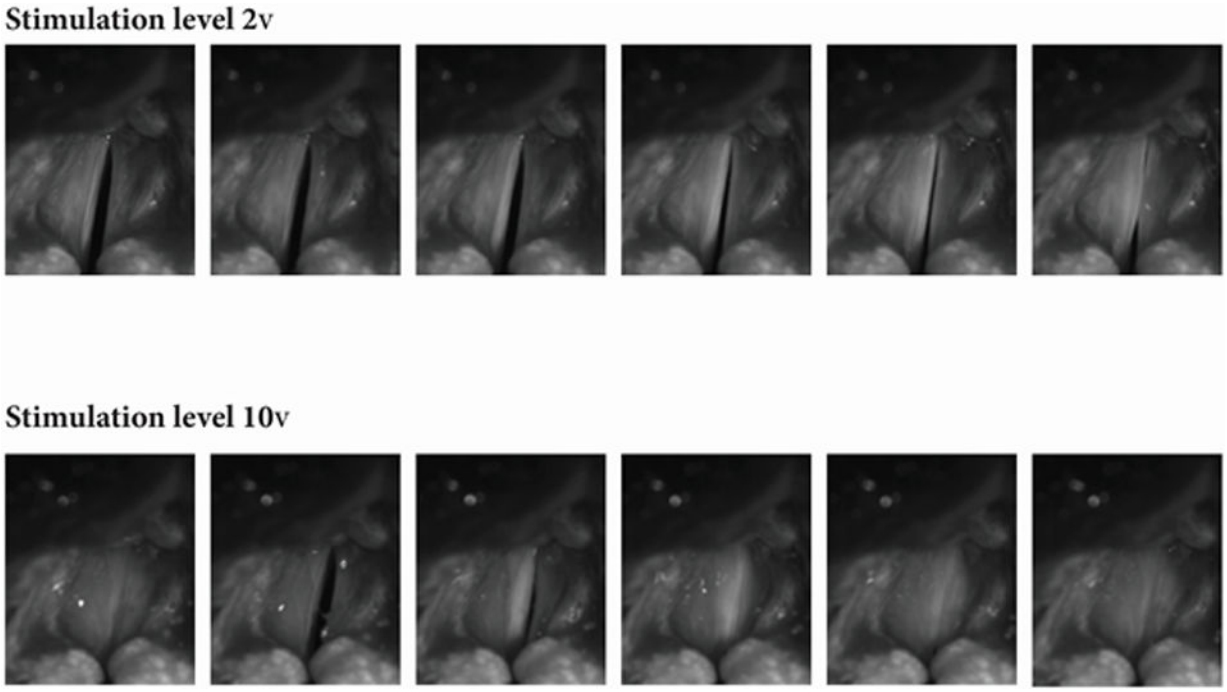

Figure 1. Still images of a human *ex-vivo* perfused phonatory cycle

Demonstrated are selected images from high-speed video capturing a full vibratory cycle at adductor stimulation of 2V (*above*) and 10V (*below*). Seen is the increased time with open glottal configuration during lower adductor stimulation.

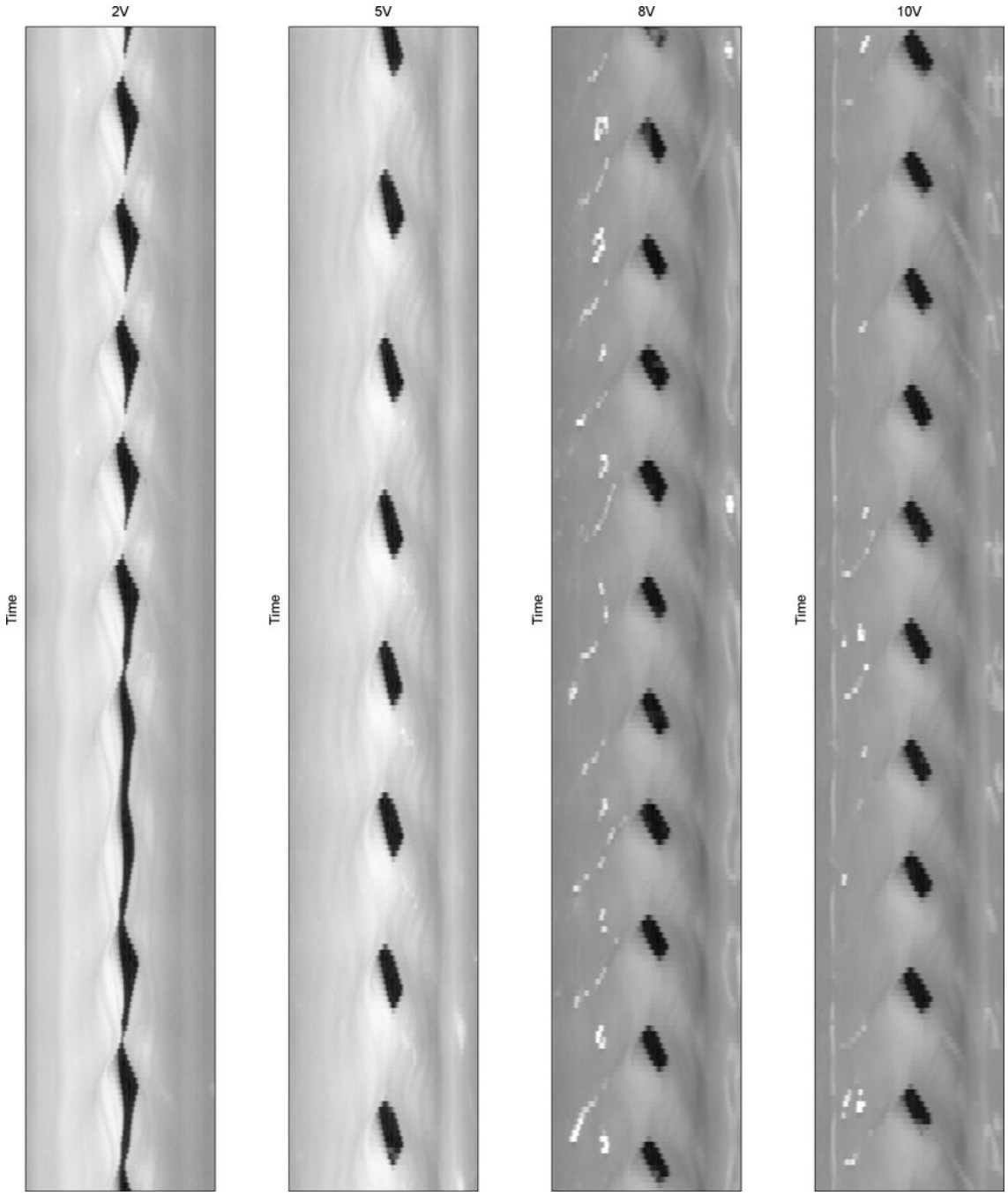

Figure 2. Digital high-speed kymography by adductor stimulation level of human *ex-vivo* perfused phonation

The relationship between increasing adductor stimulation and decreasing open quotient is seen by the diminishing open glottal component of the kymograms.

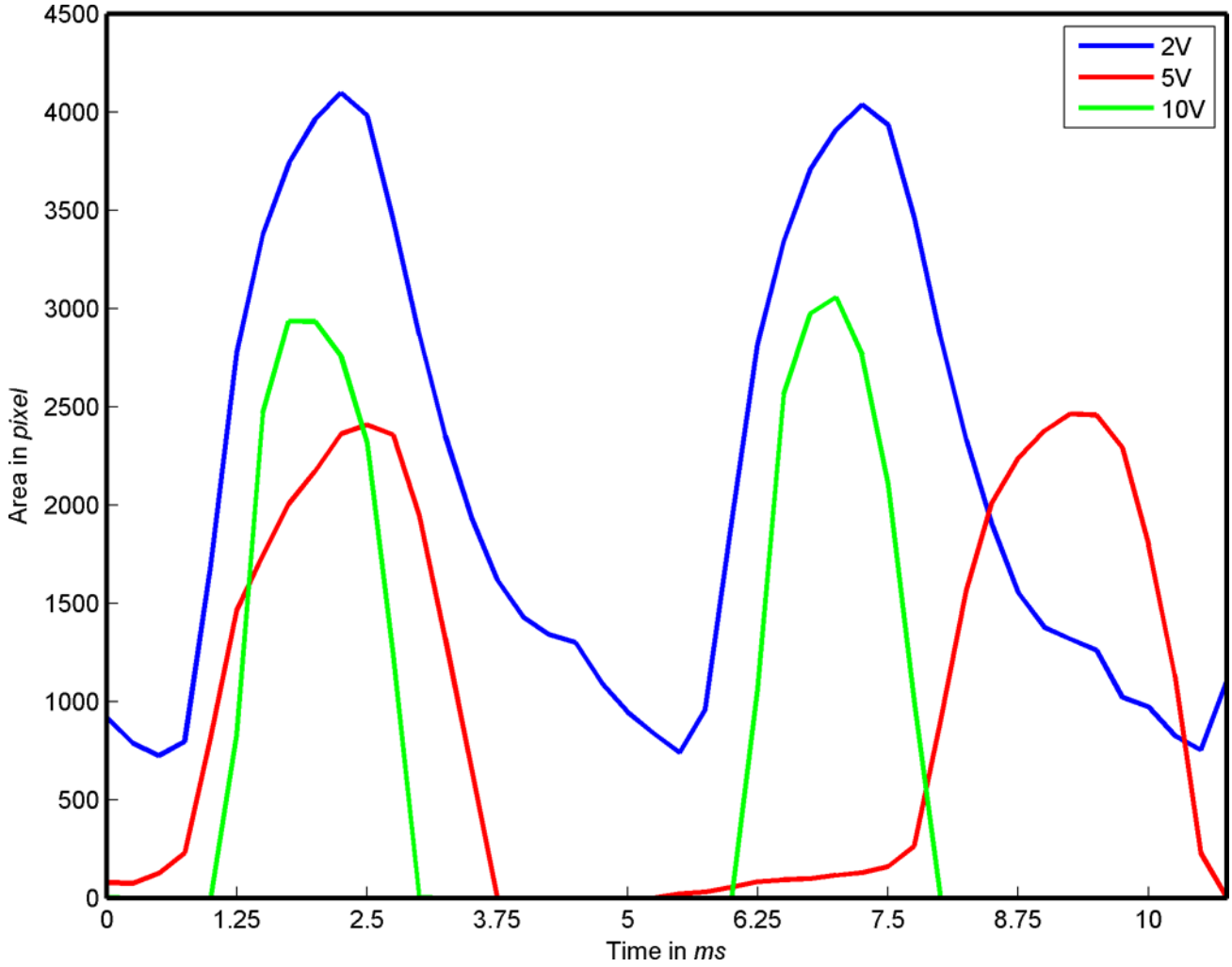

Figure 3. Glottal area waveforms (GAW) by level of adductor stimulation during human *ex-vivo* perfused phonation

Maximum GAW amplitude levels equal 4476 (2V), 2800 (5V), 2506 (10V). The 8V stimulation level was not plotted as it demonstrated very similar waveform characteristics and equivalent OQ to the 10V state.

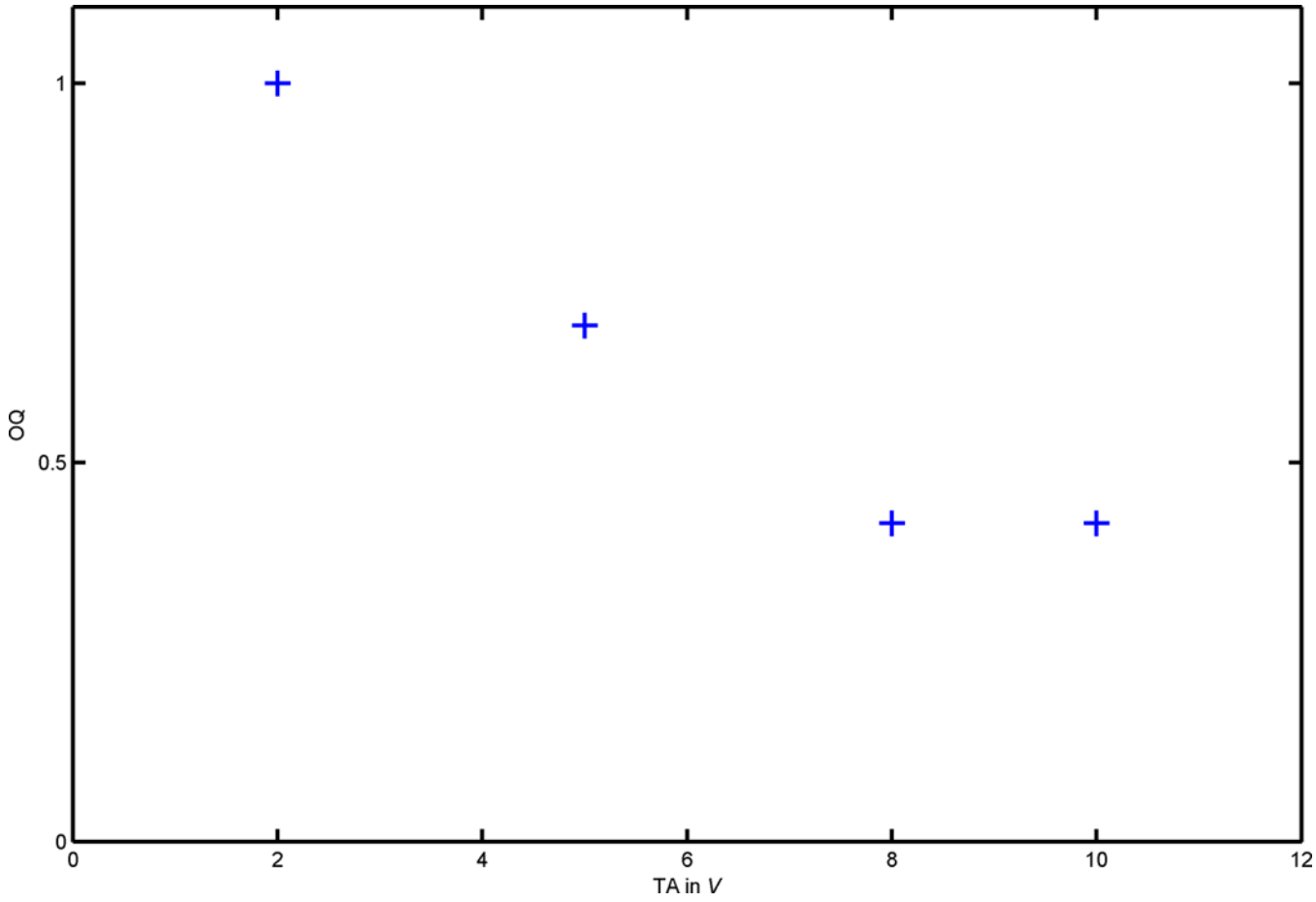

Figure 4. Plot graph of Open Quotient (*OQ*) by level of adductor muscle stimulation (*TA in V*) during human *ex-vivo* perfused phonation

An inverse relationship between the plotted variables is observed with a relative plateau at stimulation above 8V.