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Exit Jet Particle Velocity in the In Vivo Canine Laryngeal Model With Variable Nerve Stimulation

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Summary: This study extends previous work on exit jet particle velocity in the in vivo canine model of phonation by measuring air particle velocity at multiple locations in the midline of the glottis and across multiple levels of recurrent laryngeal nerve (RLN) and superior laryngeal nerve (SLN) stimulation. In a second experiment, exit jet particle velocity was measured at midline and offmidline positions with constant levels of RLN and SLN stimulation. In this study, peak particle velocity was higher at the anterior commissure than at the posterior commissure in the midline of the glottis, and peak particle velocity was higher at the midline than at offmidline positions. In addition, increasing levels of RLN stimulation resulted in increasing peak particle velocity; however, increasing levels of SLN stimulation failed to produce a uniform effect on peak particle velocity. **Key Words:** Glottis—Vocal cord—Larynx—Aerodynamics—Model—Animal—Canine

The study of the volume velocity through the glottis has been of interest because it is considered the sound source in phonation. However, some recent work has demonstrated asymmetric velocity profiles and turbulence downstream of the glottal constriction.[1-3] Because the glottal volume velocity is the summed or integrated instantaneous velocities through the glottis, measurement of the particle velocity profile can provide important insight into these nonuniformities in glottal airflow.

In 1989, Berke et al first used hotwire anemometry to examine supraglottic airflow using an in vivo canine model of phonation to measure exit jet air particle velocity profiles in the midline of the glottis.[4] Measures were obtained 1 cm above the plane of vocal fold contact, at 5 positions in the midline of the glottis, for 4 levels of input airflow (175-500 ml/s). Nerve stimulation was held constant, and subglottal pressure remained steady at about 30 cm H_2O. Peak particle velocity generally decreased from anterior to posterior positions, and ranged from 19 to 88 m/s. Peak particle velocity also increased with increasing levels of input airflow. Finally, the velocity waveforms were described as characteristically "double peaked." Although this study was limited in its scope, it demonstrated that the canine larynx provides a good model for the study of particle velocity during phonation. Although phonatory input variables such as airflow and laryngeal nerve stimulation were not altered, the use of such an in vivo model allows this type of manipulation.

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Alipour and Scherer used hotwire anemometry to examine particle velocities in excised canine larynges.[3] Measuring at numerous positions on and off the midline of the glottis, they also found the greatest peak velocities near the anterior commissure, and confirmed a velocity profile that varied substantially throughout the glottal cycle. As in the Berke et al study, doublepeaked waveforms were frequently (but not always) observed.[4]

The present study used an in vivo model of phonation to examine spatial patterns of particle velocity in greater detail. It also extended the study of Berke et al by examining how particle velocity changes with varying levels of laryngeal nerve stimulation in the in vivo model.[4] Studies by Sercarz et al and Bielamowicz et al have examined the changes in glottal area, vibration characteristics, and acoustic parameters at different recurrent laryngeal nerve (RLN) stimulation and superior laryngeal nerve (SLN) stimulation levels.[5,6] In these studies, increases in RLN stimulation produced decreases in glottal area, while increases in SLN stimulation produced increases in glottal area. We hoped to examine particle velocity in various states of glottal geometry and vocal fold vibration, resulting from alteration in this stimulation. Further, in this study we sought to replicate in the in vivo model the glottal velocity profiles presented by Alipour and Scherer in an excised canine model.[3]

METHODS

In Vivo Preparation

The in vivo canine model of phonation has been described in detail elsewhere.[7] Briefly, animals were premedicated with acepromazine maleate intramuscularly, followed by an intravenous infusion of sodium thiopental. General endotracheal anesthesia was initiated and the canine was placed in a supine position. General anesthesia was maintained with sodium thiopental to suppress the corneal reflexes.

A midline cervical incision from the hyoid bone to the sternal notch was performed and the trachea was exposed. A low tracheostomy was performed at the level of the sternal notch and cannulated with an endotracheal tube for ventilation. A second tracheostomy was performed superiorly, and a cuffed endotracheal tube was passed rostrally with the tip positioned 10 cm below the vocal folds. Airflow was controlled by a valve at the laboratory wall outlet and measured with a flowmeter (Gilmont Instruments, model F1500; Great Neck, NY) prior to airflow humidification. The flowmeter had been calibrated for air using gas equations provided by the manufacturer. The Gilmont flowmeter allowed macroscopic control of airflow. Airflow was humidified and heated by bubbling through a 5cm depth of heated water such that the air was 37°C when measured at the glottis, and then passed through the rostral endotracheal tube to drive phonation. Subglottal pressure (SGP) was measured with a cathetertipped pressure transducer (Millar Instruments, model SPC30; Houston, Tex) passed rostrally through the superior tracheostomy and placed 2 cm below the glottis.

One centimeter segments of the RLN and SLN were isolated bilaterally, and Harvard bipolar electrodes (South Natick, Mass) were applied. Separate constant current nerve stimulators (Model S2LH; WR Medical Electronics Co, St. Paul, Minn) provided stimulation to the recurrent and superior laryngeal nerves bilaterally. Nerves were stimulated at 70 Hz, with a current between 0.5 and 2.0 mA (RLN) or between 0.5 and 1.2 mA (SLN) with a 1.5ms pulse duration. The exact stimulation amperage for low and high RLN and SLN stimulation levels in experiment 1 will be detailed in the "Methods: Experiment 1" section below. For experiment 2, constant RLN stimulation was maintained at 1.0 mA and SLN stimulation at 0.75 mA.

A custom-made photoglottography (PGG) sensor was placed over the cricothyroid membrane. A xenon light source was secured in the oral cavity to provide transglottal illumination for excitation of the PGG sensor. Electroglottography (EGG) sensors were placed on either side of the thyroid cartilage, while the ground electrode was secured to the sternocleidomastoid muscle. The complete experimental setup is illustrated in Fig. 1.

Constant-Temperature Anemometry

Exit jet particle velocity was measured with a Dantec 56C01 (Mahwah, NJ) constant temperature anemometer (CTA). Consistent with previous studies, the hotwire anemometer probe tip (Dantec, type

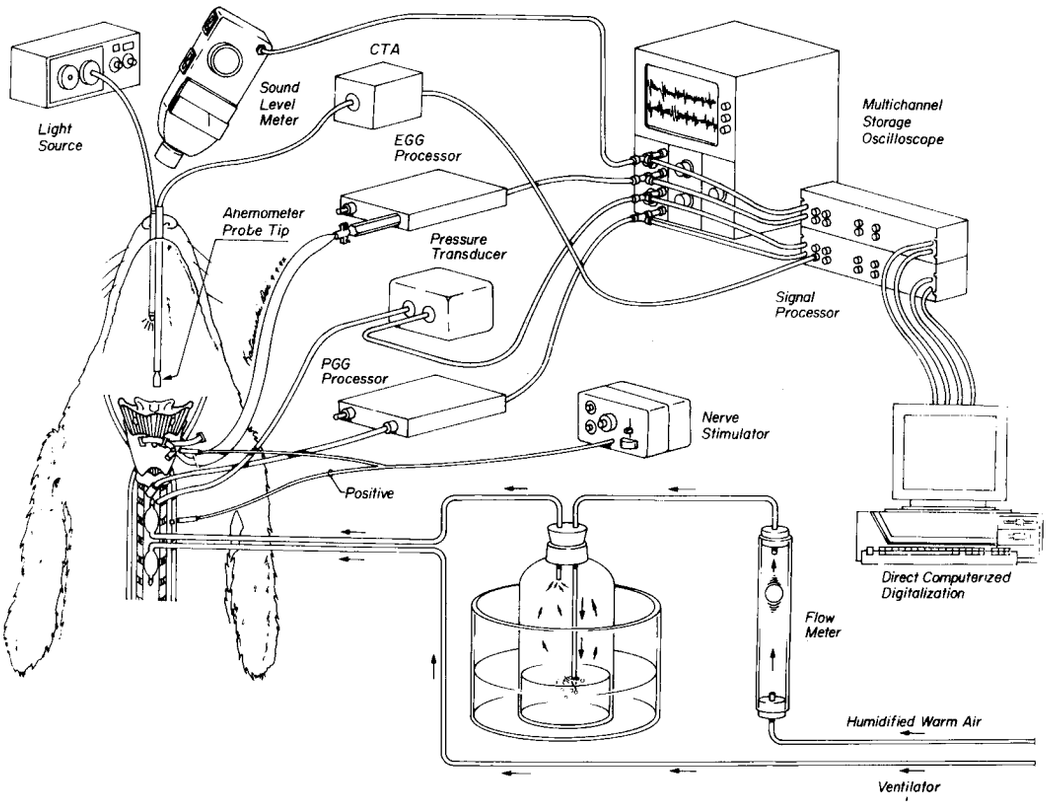

FIG. 1. In vivo canine model setup for collection of EGG, PGG, SGP, and anemometer data.

55R01) was placed 1 cm superior to the glottis, thus providing a measure of exit jet particle velocity rather than glottal particle velocity.[3,4,8] Glottal particle velocity cannot be measured directly, because placement of the probe tip at the glottal level would prevent normal vibration of the vocal folds and would damage the probe tip. The probe had an active length of 1.25 mm and was quartz coated for use in a humid environment. The number of trials recorded for each experimental condition was limited in both experiment 1 and experiment 2. The in vivo canine model of phonation is a harsh environment for the CTA probe. The canine model continues to secrete pharyngeal mucus throughout the experiment, requiring removal of the CTA probe after each trial for cleaning of protein debris from the CTA wire in an ultrasonic bath. Accumulation of debris during a trial results in a loss of high frequency data. Trials recorded without high frequency data were eliminated in this study. The total number of trials was limited by the number of CTA probes available for each experiment (usually 2), since the CTA wire would eventually break after repeated cleanings.

The probe was held in position by a micromanipulator mounted on a tripod at the head of the operating table. The micromanipulator controlled movement in the x, y, and z planes. The micromanipulator was aligned such that the x-y plane was parallel to the superficial surface of the vocal cords. Once the micromanipulator was positioned, direct measurement of the CTA probe 1 cm above the vocal folds was confirmed in all x-y positions. The CTA probe was calibrated with a rotameter, which was independently calibrated against a mercury Utube manometer ($r^2 > 0.995$ for all probes used) to determine the dynamic head of saturated air.[8,9] The rotameter had a 3mm diameter opening with a converging throat to avoid flow separation and to provide a uniform velocity across the opening. The gas was humidified and heated for calibration through the rotameter, so that the temperature of the gas was 37°C when measured at the outlet of the rotameter.

For both experiments, anemometry, SGP, EGG, and PGG signals were lowpass filtered at 3 kHz and simultaneously digitized for 2.8 seconds at 10,000 samples per second, with 12bit resolution. SGP and PGG signals were verified on an oscilloscope (Tektronix, model 5116; Beaverton, Ore) before recording. The digitized anemometry signals were converted from volts to meters per second for each trial using a third order polynomial calibration curve, which provided excellent fit to the original data (r^2 = .995- .997).

F_0 was calculated from the PGG waveforms as the average of 10 consecutive cycles for each trial. Peak and minimum exit jet velocities were handmarked from the anemometer waveforms for the same 10 cycles. Data were not phase averaged.[3]

Experiment 1

Experiment 1 examined the effects of SLN and RLN stimulation on exit jet particle velocity. Because mean glottal area decreases with increasing RLN stimulation and increases with increasing SLN stimulation, we hypothesized that peak particle velocity would increase with increasing RLN stimulation and decrease with increasing SLN stimulation.[10]

Three animals were studied in this experiment. Phonatory trials were produced with a constant airflow of 318 mL/s. The CTA probe was positioned 1 cm above the glottis in the midline at the midvibratory portion of the vocal cord (grid x-0, y-3, as described below). Levels of low and high RLN stimulation and zero, low, and high SLN stimulation were selected to maintain target levels of SGP, as shown in Table 1. A minimum of 1 trial at each stimulation condition was recorded for each animal. As noted in the table, SLN stimulation altered the subglottic pressure only as a function of the RLN stimulation level. This finding can be explained by the reciprocal relationship of RLN and SLN stimulation levels and glottic area noted in our previous studies.[5,6] For the low SLN stimulation condition, current was set at 0.7 mA; current for the high SLN stimulation condition was set at 1.5 mA. For the low RLN stimulation condition, current varied from 0.5 to 0.7 mA as necessary to maintain a constant SGP of 30 cm H_2O. In the high RLN stimulation condition, current was varied from 0.7 to 0.8 mA to maintain SGP at 65 cm H_2O. While these are extremely high subglottic pressures for humans, this level is easily obtained in the in vivo canine model and most likely underlies the vocalization demands of this species. Canine vocalizations of 100 dB are common.

TABLE 1. *Research Design for Experiment 1**

SLN Stimulation	RLN Stimulation	Subglottic Pressure (cm H_2O)
Off	Low	30
Off	High	65
Low	Low	30
Low	High	65
High	Low	30
High	High	65

*Levels of SLN and RLN stimulation were set to obtain the target subglottic pressure.

Experiment 2

Experiment 2 examined the particle velocity profile of the glottal exit jet during phonation in a plane 1 cm above the plane of vocal fold contact. Previous studies indicate that peak particle velocity decreases from anterior to posterior.[3,4] However, Berke et al[4] measured particle velocity only in the midline of the glottis, and Alipour and Scherer used an excised larynx model of phonation to evaluate both midline and offmidline positions.[3] The present study extends these findings by measuring velocity in vivo across a 2 dimensional grid. We hypothesized that peak particle velocities would be greatest near the anterior commissure and the midline of the glottis, compared to posterior and offmidline measurements.

Differences in particle velocities across the plane of the glottis were studied in a single animal. The CTA probe was moved across a 5 × 4mm x (medial to lateral) by y (anterior to posterior) grid by a micromanipulator mounted on a tripod at the head of the operating table. Grid positions were 1 mm apart. $y = 1$ was located at the anterior commissure, and $y = 4$ was located at the posterior commissure. Thus the $x = 0$, $y = 4$ position was located over the most closed portion of the glottis. Given the anatomical restrictions of the in vivo model, only a single measurement position was used for $y = 1$ and $y = 4$. Five measures were obtained for $y = 2$ ($x = -2, -1, 0, 1, 2$) and 3 for $y = 3$ ($x = -1, 0, 1$). Two trials were

recorded at each grid location. Phonation for all trials was obtained with a constant level of airflow (388 mL/s), SLN stimulation (0.75 mA), and RLN stimulation (1.0 mA). F_0 was handmarked from the EGG signal, and the peak and minimum particle velocities were obtained for each trial by handmarking of the velocity waveforms using CSpeech software.

RESULTS

Experiment 1

The mean values for each of the SLN and RLN conditions are listed in Table 2. Significant main effects of RLN stimulation ($F_{1,414} = 652.80$, $P < .01$) and SLN stimulation ($F_{2,414} = 314.29$, $P < .01$) on peak velocity were observed, along with a significant interaction ($F_{2,414} = 377.58$, $P < .01$). Scheffe post hoc comparisons indicated that when SLN stimulation was present, particle velocity increased significantly with increasing RLN stimulation for every level of SLN stimulation; however, no effect of RLN stimulation was observed in the absence of SLN stimulation. The combination of low SLN stimulation and high RLN stimulation produced the highest particle velocities; the lowest velocities were observed when RLN stimulation was low and SLN stimulation was present at either level.

Experiment 2

Mean values for peak velocities at different locations within the glottis are shown in Table 3 and Fig. 2. Complete glottal closure was achieved during all

TABLE 2. *Peak Particle Velocity (m/s) by RLN and SLN Stimulation Condition for Experiment 1 Listed as Mean Value (Standard Deviation) and n = Number of Data Points*

RLN Stimulation	SLN Stimulation		
	None	Low	High
Low	45 (3.3) n = 30	27 (2.9) n = 90	26 (3.8) n = 91
High	55 (6.2) n = 90	191 (65.1) n = 59	52 (8.4) n = 60

TABLE 3. *Particle Velocity Profiles for the x-y Grid in Experiment 2**

X Coordinate	Y Coordinate	Mean F_0, Hz	Mean Peak Velocity (in m/s)	Mean Minimum (in m/s)
0	1	277	222	133
−2	2	280	18	7
−1	2	295	46	6
0	2	290	50	6
1	2	291	43	6
2	2	301	17	6
−1	3	235	13	5
0	3	294	41	18
1	3	239	28	13
0	4	252	8	6

*$Y = 1$ corresponds to the anterior commissure and $X = 0$ corresponds to the midline.

phonation tasks except at the most posterior aspect of the glottis (associated with the posterior cricoid cartilage cleft found in canines). In this animal, the $y = 0$ coordinate was noted to be in the midline, $y = 1$ corresponded to the midportion of the true vocal fold, and $y = 2$ corresponded to the medial portion of the ventricular fold. As noted in previous studies, we again identified a doublepeaked nature of the CTA waveform (Fig. 3).[1,4,8] While the doublepeaked waveform is common, this feature is not uniformly present at any particular locations within the x-y grid and was not consistent from cycle to cycle. F_0 was significantly ($P < .01$) but minimally correlated with peak velocity ($r = .13$). F_0 was used as a covariate in all analyses. Analysis of covariance (ANCOVA) was used to examine changes in particle velocities as the probe was moved from anterior to posterior in the midline and from medial to lateral at positions $y = 2$ and $y = 3$.

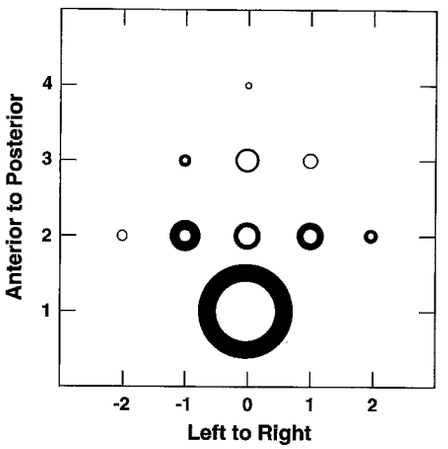

FIG. 2. Plot of peak particle velocity by x and y coordinates. The size of the circle is proportional to particle velocity.

For $x = 0$, a 1way ANCOVA (dependent variable= peak velocity; independent variable = y; covariate = F_0) showed significant differences in peak particle velocity as the probe position was moved in the midline from the anterior commissure to the posterior commissure ($F_{3,205} = 7388.13$, $P < .01$). Scheffe post hoc comparisons showed significant differences between all probe positions, except that position $y = 2$ did not differ significantly from probe position $y = 3$. The data for positions $y = 2$ and $y = 3$ were collapsed for the remainder of the statistical analyses.

For $y = 2, 3$ (across x in the center of the glottis), a 1way ANCOVA again revealed significant effects of probe placement in the x dimension on peak particle velocity ($F_{4,474} = 358.39$, $P < .01$). Scheffe post hoc comparisons indicated that peak velocity differed significantly for all pairs of probe positions, except that position 0 did not differ from position 1.

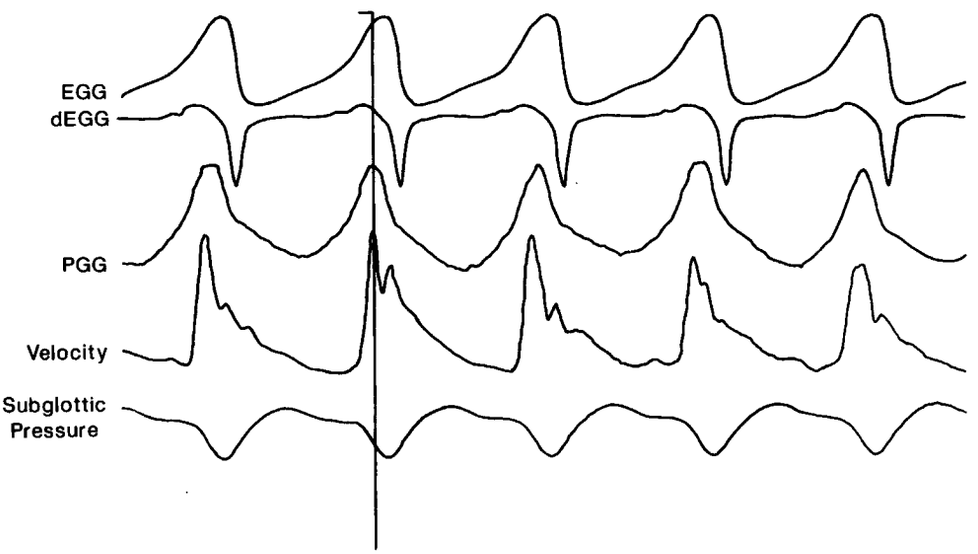

FIG. 3. Example of the simultaneous EGG, PGG, particle velocity, and SGP waveforms recorded in the experimental design. Note the doublepeaked velocity waveform.

DISCUSSION

In experiment 1, peak particle velocity increased with increasing RLN stimulation, as hypothesized, presumably because increasing RLN stimulation produces a decrease in glottal area.[10] Since SGP also increases with decreasing glottal area in a constant-flow model, the current results are consistent with Fant's hypothesis that air particle velocity increases with higher transglottic pressures.[11] Fant also postulated that glottal air particle velocity is controlled primarily by SGP. However, recent research in a constant pressure model suggests that subglottic pressure may be controlled by laryngeal muscle contraction.[12] More research will be required to evaluate the relationships of pulmonary driving pressure, SGP, glottal closure, and glottal peak particle velocity.

Bielamowicz et al also found that glottal area increased significantly with increasing SLN stimulation.[10] We hypothesized that these changes resulted from lengthening of the glottis while medial compression remained unchanged. However, the present study found that at a low RLN stimulation level, SLN stimulation had no significant effect on peak particle velocity. At high RLN stimulation, low SLN stimulation resulted in higher peak particle velocity than either the no SLN or high SLN trials. Based on the results of the above mentioned study, we hypothesized a decrease in peak particle velocity with an increase in SLN stimulation. Simultaneous measures of glottal area versus time and CTA waveforms are required to further investigate this relationship. However, simultaneous acquisition of CTA and glottal area measures is difficult due to the restricted supraglottic space in the in vivo model of phonation. These measures may be more easily obtained in an excised canine laryngeal model, where instrumentation space above the glottis is less constrained.

Results of experiment 2 are generally consistent with the findings of Berke et al and Alipour and Scherer.[3,4] Peak particle velocity decreased from anterior to posterior positions in the current study and the studies by Berke et al and Alipour and Scherer, and typical double peaked velocity waveforms were frequently seen. The extremely high levels of peak velocity at the anterior commissure reported here are not consistent with the findings of previous studies. These values are almost an order of magnitude greater than previous findings. One possible explanation for this finding is probe placement at a more anterior location than in previous studies, corresponding to a more narrow portion of the glottal exit jet. Another explanation involves differences between the in vivo model and the excised canine model of phonation. Prior studies have identified significantly higher levels of SGP and decibel output from the in vivo canine model compared to the human system. SGP levels and decibel output are often an order of magnitude higher than those recorded in humans. Vocal tract inertance is absent in the excised model but present in the in vivo model of phonation. Also, the SGPs of the in vivo model are characteristically 3 to 6 times greater than those found in the excised model. This may be partially explained by the method of vocal fold adduction used in each model. Vocal fold adduction is effected by sutures through the vocal processes of the arytenoids in the excised model, while adduction is achieved by RLN stimulation in the in vivo model.

In the current study, a uniform continuum from high to low peak particle velocities was observed in the midline, as noted in the previous study by Berke et al.[4] Values obtained in the most posterior position were noted to occur at the most closed portion of the glottis, where little flow would be expected. In canines, the posterior glottis does not close completely and may be responsible for the DC offset noted in the current study. The lack of closure is due to a posterior glottal cleft found in the canine cricoid cartilage that is not seen in humans.

In the present study, peak particle velocity is significantly higher in midline than in offmidline positions. However, the study by Alipour and Scherer found a relatively uniform peak velocity profile in the midline compared to offmidline measures.[3] In that study, the data were averaged across the trials and were not subjected to statistical analysis. Fundamental differences between the excised and in vivo models of phonation may be responsible for differences in the offmidline CTA findings in these 2 studies. One may hypothesize that a lower peak velocity in offmidline compared to midline measures is due to turbulent interactions with the walls of the phonatory tract in the in vivo model of phonation.

CONCLUSION

In this study, we evaluated the exit jet particle velocity using varying levels of RLN and SLN stimulation in an in vivo canine model of phonation. Higher levels of RLN stimulation resulted in greater maximum peak velocities. Changing levels of SLN stimulation failed to produce consistent effects on peak particle velocity measures. In addition, we evaluated the exit jet particle velocity profile at multiple locations across a grid located 1 cm superior to the glottis. We identified a continuum of particle velocities in the midline, with the greatest values at the anterior commissure and the lowest values at the posterior commissure. Also, we found a continuum of particle velocities from midline to lateral positions, with the highest values at midline.

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