INDUCING VOCAL REGISTER TRANSITION IN AN IN VIVO EVOKED PHONATION CANINE MODEL

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Background and purpose: The nature of vocal registers is still a subject of controversy. The purpose of this study was to demonstrate the induction of timbre transition of vocal register in an in vivo evoked phonation canine model and thereby confirm vocal register transition as a laryngeal event.

Materials: A canine midbrain stimulation evoked phonation model was used in this study. To repeat a low-pitched evoked phonation in the model, the low activity of the thyroarytenoid (TA) muscle and coordinate actions of other intrinsic laryngeal muscles were kept in a consistent condition by stimulating the same midbrain point with the same electric current intensity at the same timing in the respiratory cycle. The cricothyroid (CT) muscle was activated with an electrical current delivered directly to the muscle during the evoked phonation. Under constant subglottal pressure, CT muscle activity was varied while changes in vocal register of the evoked phonation were monitored.

Results: The fundamental frequency (F0) of the evoked phonation increased as the stimulating current to the CT muscle increased. In addition to the increase in F0, data collected from six animals demonstrated that timbre register transition was induced by a stepwise increase of current to the CT muscle. The abrupt escalation of F0 and sudden change in sound quality, which could be verified perceptually, manifested the register transition. Frequency spectrum analysis showed that the sound in the modal register contained abundant harmonics that were different from those of the sound in the falsetto register, which contained fewer harmonics.

Conclusion: The results of this study indicated that intrinsic laryngeal muscles (especially CT and TA muscle interactions) regulate timbre register transition in a canine model.

Vocal register is defined as perceptually nearly identical phonatory quality over a range of consecutive voice frequencies [1, 2]. There is little overlap in fundamental frequency (F0) between adjacent registers [3]. When the voice frequency shifts from the range of one register to another, the voice quality changes abruptly. This phenomenon is termed register transition [2]. In general, there are two register transitions between three registers. The term periodicity transition refers to the change between vocal fry (pulse register) and modal voice (chest register). The voice of vocal fry is characterized by the pulse-like quality and the modal voice is non-pulsed [2]. The timbre transition is the change between modal and falsetto registers. The difference in voice quality between registers can be distinguished both perceptually and acoustically.

Register transitions result mainly from the regulation of intrinsic laryngeal muscle activities [1, 4]. The coordinated action of the cricothyroid (CT) and thyroarytenoid (TA) muscles plays the primary role in the process of F0 control during phonation [5]. The action of the CT elongates the vocal folds, increases the tension, and thins the edge of the vocal folds. By contrast, the action of TA muscles shortens, slackens, and thickens the vocal folds [6]. The laryngeal muscular mechanism involved in the transition between modal and falsetto registers has been studied in human subjects using electromyography (EMG) to monitor the...
intrinsic laryngeal muscle activity [7]. It is well documented that in the light (eg, falsetto) register the TA is much less active than in the heavy (eg, modal) register [1, 2, 4, 7]. Shiotani et al simulated the actions of the CT and TA in excised canine larynges to demonstrate this register transition [4]. In particular, they showed that a mechanically reproduced CT action can induce register transition. However, their study was conducted in the absence of intrinsic laryngeal muscle activity.

The in vivo evoked phonation canine model uses electrical stimulation of the midbrain and provides a suitable paradigm for studying the action of laryngeal muscles during timbre register transition [8, 9]. In this model, midbrain stimulation activates both laryngeal and respiratory muscles producing a phonatory response. The response is repeatable and consistent. Using this model, we investigated the CT and TA muscle interactions involved in timbre transition in order to induce timbre transition by modifying the action of the CT muscle while the midbrain-stimulated coordinated activity of the other laryngeal muscles (including the TA muscle) was maintained at a constant level.

**Methods**

Six healthy 20- to 25-kg hound-like mongrel dogs were anesthetized to the surgical level with pentobarbital (25 mg/kg, injected intravenously), and then each animal was immobilized on a stereotaxic apparatus. A 1-cm-diameter burr hole was bored into the skull in the parietal area at about 10 mm anterior and 5 mm lateral to the ear bar zero, and a coaxial bipolar electrode (Rhodes NE-100, Woodland Hills, CA, USA) was inserted. The electrode was vertically placed into the brain to 20 mm dorsal to ear bar zero with the help of a stereotaxic manipulator.

To expose the larynx and trachea, the animals were placed in the supine position. Bipolar hooked-wire electrodes were inserted into the CT, TA, lateral cricoarytenoid (LCA), and posterior cricoarytenoid (PCA) muscles after identifying the larynx. A pressure sensor (for subglottal pressure measurement) was then inserted in the tracheal lumen and a microphone was set at about 5 cm in front of the mouth for voice recording.

The procedure for evoking phonation in canines by midbrain stimulation was previously reported [8, 9]. To elicit vocalization, sites described previously in the midbrain were electrically stimulated. The location of the ideal stimulation points for evoking stable low-pitched phonation was determined by trial and error (ie, movement of the electrode 1 mm at a time). Stimuli were given in 2- or 3-second trains with 0.2 ms pulses transmitted at a rate of 200 Hz while current levels were maintained at about 0.5 mA. Low-pitched phonation was characterized by the absence of CT muscle activity and relatively low activity in the TA muscle [8–10] (Fig. 1). The F0 of the evoked phonation at this point was less than 150 Hz. Once the stimulation site in the midbrain that could elicit a stable low-pitched phonation was chosen, it was kept in place throughout the whole experiment.

The trachea was opened to insert two low-pressure-cuffed tracheostomy cannulae. The caudal cannula was used for respiration and the rostral cannula was connected to an air source supplying humidified and warmed air to the larynx for phonation.

The bilateral superior laryngeal nerves were identified and resected at the segment between the internal and external branches to preserve the sensory function of the larynx and to prevent the retrograde conduction of electric current from the ongoing CT stimulation to the brain. To induce CT action, a pair of electrodes was inserted into each side of the CT (Fig. 2). The electrical stimuli to the CT muscle were 2-ms pulses of constant current delivered at the rate of 50 Hz during evoked phonation (Fig. 3). The current ranged from 0 to 10.0 mA and could be adjusted in steps of 0.5 mA. Such electrical stimulation will cause tetanic contraction of the CT muscle [10, 11]. With the stepwise increment of the current, the contraction power of the muscle increases, as has been verified in our previous study [10]. The higher the stimulating current, the stronger the contraction power of the CT muscle, which will be reflected in the increase in the F0 of the evoked phonation [10]. At the same time, a stimulus to the midbrain produced a sustained phonation (lasting about 1.5 s) which was accompanied by sustained, coordinated laryngeal muscle action. This evoked pho-
nation was repeated at different levels of electrical stimuli delivered simultaneously to the CT muscle.

A pressure-regulated valve (Fairchild model 10, Winston-Salem, NC, USA) was used to maintain the airflow pressure in the rostral tracheal cannula (measured with a pressure transducer [Micro Switch 143PC03G, Freeport, IL, USA]) and to prevent changes in the F0 of the vocal fold vibration.

While the TA and other intrinsic laryngeal muscle activity remained relatively constant, the pitch of the evoked phonation rose in proportion to the increase in current to the CT muscle. During this process, the point of perceptual timbre transition was recorded. Additionally, all the signals (such as EMG from laryngeal muscles [CT, TA, PCA, LCA], the subglottal pressure, voice, and the stimuli to the midbrain and CT) were read on an 8-channel DAT data recorder (TEAC RT-130D) for further analysis. At the end of the experiment, the animals were euthanized with a large dose of pentobarbital.

Results

The stepwise increase in stimulatory current to the CT muscle was used to demonstrate timbre register transition in six dogs. Throughout the experiment, subglottal pressure was kept relatively constant (within the variation of 0.35 kPa) (Fig. 4). With the delivery of increasing electrical current to the CT muscle, F0 was raised. An abrupt F0 escalation accompanied by register transition was noticed perceptually in all six animals. A current level to the CT muscle intermediate between the current levels inducing these two registers was found to produce an unstable, rough voice in two dogs (5 and 6 in Fig. 4). In both animals, only a single current (4.0 mA in dog 5 and 3.0 mA in dog 6) to the CT muscle was noted to produce the rough voice that occurs before the register transition in the evoked phonation process. Current levels to the CT higher than the threshold for inducing falsetto voice evoked a whining or voiceless phonation.
The voice in modal and falsetto registers could be distinguished both perceptually and by acoustic analysis in the results of all six animals. Voice samples of one of the dogs in these two different registers are shown in Fig. 5 as an example. The F0 prior to register transition was 391 Hz. Frequency spectrum analysis using fast Fourier transformation (FFT) showed abundant harmonics. Harmonics existed even at frequencies higher than 2,500 Hz. The FFT in the adjacent falsetto register with a 508-Hz F0 contained fewer harmonics, and all were below 1,500 Hz.

**Discussion**

The nature of vocal registers has been a subject of controversy for decades and the register transition is thought of as a laryngeal event [1]. In this present study, timbre register transition was induced in all six animals. The TA and other intrinsic laryngeal muscle activities remained relatively constant in our experiments. The F0 of the evoked phonation rose in proportion to the increase in current to the CT muscle. The stepwise increase in the stimulating current to the CT muscle induced an abrupt F0 escalation of the evoked phonation and was accompanied by timbre register transition. In most previous reports, vocal registers were defined as perceptually distinct vocal quality regions, each one of which consists of a series of voice frequencies [1, 2]. The F0s of adjacent registers have little overlap [3]. Abrupt changes in vocal quality may occur, when the F0 shifts from the region of one register to another [1, 2]. The transition between modal and falsetto register, the timbre register transition, is primarily regulated by the actions of the CT and TA muscles, though other intrinsic laryngeal muscles are also involved [7]. The results of previous EMG studies of intrinsic laryngeal muscles in human subjects showed that TA muscle activity is greater in modal register than in falsetto register. On the other hand, CT muscle activity is greater in falsetto register [12]. Other evidence shows that a delicate balance between the CT and TA muscle activities regulates the timbre register transition [4, 5, 7, 12]. The results in our study also revealed the interaction of the CT and TA muscle regulating the timbre register transition, thus demonstrating the timbre register transition to be a laryngeal event.

Shiotani et al successfully demonstrated the register transition in excised canine larynges [4]. In their model, CT muscle action could be simulated mechanically by CT approximation; however, no active TA muscle tension existed. We used a relatively physiologic (in vivo) canine model of phonation evoked by midbrain electrical stimulation to study voice production mechanisms in the larynx [8, 9]. Delivery of a stimulus to a suitable point in the midbrain induces a sequential coordinate action of respiratory and laryngeal muscles and results in evoked phonation. When the midbrain is stimulated at the same site with the same electrical current intensity and at the same time in the respiratory cycle, the evoked phonation and laryngeal muscle action pattern are consistent and can be repeated more than 50 times. Owing to these unique characteristics, the action of individual laryngeal muscles (eg, the CT in this experiment) can be modified and studied by resecting its innervated nerve and directly stimulating the muscle [9]. In a low-pitched phonation evoked by midbrain stimulation, there is relatively little elicited activity in the TA muscle and almost no elicited activity in the CT muscle [9]. Under this condition, direct stimulation of the CT muscle

**Fig. 5.** Voice samples from Dog 3 before and after register transition. The upper tracing is the acoustic signal and the lower panel is the frequency spectrum. A) The modal voice at 391 Hz showed abundant harmonics even in the area with a frequency higher than 2,500 Hz. B) The falsetto voice at 508 Hz contained fewer harmonics and all were in the area below 1,500 Hz.
results in CT approximation and raises the F0 of evoked phonation [10]. To a certain extent, when the current to the CT muscle is greater, the muscle contraction and the tension of the vocal folds increase. Under constant subglottal pressure, a higher tension resulted in a higher frequency of vocal fold vibration [10]. When the evoked phonation is under low TA activity, the stepwise increase in CT muscle activity gradually raised the F0 of vocal fold vibration [10]. In addition, as CT muscle contraction increased, the edge of the vocal folds became thinner. If the action of the CT muscle dominates that of the TA muscle, the bilateral folds will be adducted to a position at the posterior cricoarytenoid ligament insertion and the glottal configuration changes abruptly [12]. The sudden glottal configuration change might play an important role in the register transition in our experiments.

Titze demonstrated that the sudden change in perceptual and acoustic quality of timbre register transition results from the change in the glottal waveform character [2]. The spectral slope of glottal flow waveform in the modal register is less than 12 dB per octave. As a result, more high-frequency harmonics appear in the spectral analysis of the modal voice. In the experiments of the present study using a canine model, the differences in the FFT of the modal and falsetto voices clearly supported this finding (Fig. 5).

In this present study, a certain degree of CT muscle action causing a rough voice during the register transition was demonstrated in two dogs. In the process of register transition, vocal folds wobbled for a short period before readjusting to the configuration of the falsetto register. This phenomenon (falsetto break or vocal break) has been reported in human subjects [13, 14] and in excised canine laryngeal experiments [4]. In this present experiment, the increase in CT muscle action was stepwise but not continuous. The narrow frequency range of falsetto break may explain why the irregular vibration of vocal folds during register transition was found in only two of the six dogs.

In summary, using midbrain electrical stimulation to evoke phonation in a canine model, we demonstrated how register transition between the modal and falsetto voices occurs. During low-pitched evoked phonation (while TA muscle activity is relatively low), the stepwise increase in CT muscle action could induce timbre register transition. This experiment revealed that intrinsic laryngeal muscles (especially the interaction between CT and TA muscles) regulated the timbre register transition.

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References