Role of the Internal Superior Laryngeal Nerve in the Motor Responses of Vocal Cords and the Related Voice Acoustic Changes

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Received: 28 April 2015
Revised: 01 July 2015
Accepted: 02 August 2015

Abstract

Background: Repeated efforts by researchers to impose voice changes by laryngeal surface electrical stimulation (SES) have come to no avail. This present pre-experimental study employed a novel method for SES application so as to evoke the motor potential of the internal superior laryngeal nerve (ISLN) and create voice changes.

Methods: Thirty-two normal individuals (22 females and 10 males) participated in this study. The subjects were selected from the students of Iran University of Medical Sciences in 2014. Two monopolar active electrodes were placed on the thyrohyoid space at the location of the ISLN entrance to the larynx and 1 dispersive electrode was positioned on the back of the neck. A current with special programmed parameters was applied to stimulate the ISLN via the active electrodes and simultaneously the resultant acoustic changes were evaluated. All the means of the acoustic parameters during SES and rest periods were compared using the paired t-test.

Results: The findings indicated significant changes (P=0.00) in most of the acoustic parameters during SES presentation compared to them at rest. The mean of fundamental frequency standard deviation (SD F0) at rest was 1.54 (SD=0.55) versus 4.15 (SD=3.00) for the SES period. The other investigated parameters comprised fundamental frequency (F0), minimum F0, jitter, shimmer, harmonic-to-noise ratio (HNR), mean intensity, and minimum intensity.

Conclusion: These findings demonstrated significant changes in most of the important acoustic features, suggesting that the stimulation of the ISLN via SES could induce motor changes in the vocal folds. The clinical applicability of the method utilized in the current study in patients with vocal fold paralysis requires further research.

What’s Known

- Neuroanatomic studies have indicated axons descending from the internal superior laryngeal nerve to the internal laryngeal muscles.

What’s New

- Previous attempts to impose significant changes on acoustic parameters through surface electrical stimulation (SES) on the larynx have all failed.
- We are the first to significantly change acoustic parameters (e.g., shimmer, jitter, harmonic-to-noise ratio, and intensity of voice) by applying SES on the vocal folds.

Introduction

The scientific application of surface electrical stimulation (SES) to treat dysphagia and especially voice disorders is new.1 LaGorio2 used VitalStim to treat swallowing disorder in a single individual and reported that, in conjunction with improvement in dysphagia
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Symptoms, the voice functions improved. Voice is one of the most important instruments for communication.³ Voice has some acoustic parameters that play an important role in its characterization as normal or abnormal, including fundamental frequency (F0), intensity, shimmer, jitter, and harmonic-to-noise ratio (HNR).³⁷ Accordingly, it is essential that new methods to treat voice impairments be devised.

SES studies were commenced on the subject of dysphagia and its resultant voice changes.² Nonetheless, the unknown details about the procedure and its effectiveness prompted researchers to investigate SES effects on normal subjects’ voice. The existing literature, however, suffers from a dearth of published data on this topic. In addition to SES studies, Kempster⁸ applied implanted electrical stimulation in normal individuals’ internal laryngeal muscles, which resulted in increased F0. Although implanted electrical stimulation evokes laryngeal muscles more specifically, it is invasive and not widely applicable. Some researchers were, therefore, prompted to draw upon SES.⁹⁻¹³

Previous studies have demonstrated that SES is ineffective in causing changes in acoustic voice parameters and also in closing the vocal folds.⁹⁻¹¹,¹³⁻¹⁵ Flower¹⁵ found that SES resulted in statistically significant unfavorable effects on voice parameters in experimental participants.

According to the published studies on SES in normal individuals, generally SES is not effective in inducing changes in voice parameters. This finding may be attributed to the application of SES protocols, which are designed for dysphagia therapy and are not useful for voice and larynx specifically. Consequently, this method cannot induce a remarkable effect on voice in normal subjects, although we should not discount SES as an important agent.

A possible method to impose changes on the internal laryngeal muscles and create subsequent acoustic changes may be the use of the potential capability of the internal superior laryngeal nerve (ISLN) to move the internal laryngeal muscles. Contrary to earlier studies that knew only a sensory role for the ISLN, some neuroanatomical studies in the last 3 decades have found that the ISLN in addition to its sensory role also contributes to the movements of the internal laryngeal muscles by its distributed motor branches to those muscles.¹⁶⁻¹⁹ However, in awake humans, the literature contains only 2 studies that stimulated the ISLN with needle electrodes inserted around the ISLN and induced motor reactions, which were monitored via flexible laryngeal endoscopy and also electromyographic recordings. The results indicated that ISLN stimulation caused movement and electromyographic responses in the internal laryngeal muscles.²⁰⁻²¹ Although the findings asserted the motor role of the ISLN,²⁰⁻²¹ the stimulus instrument used (i.e., needle electrodes) are invasive and not applicable at clinics for repeated efforts.

Accordingly, in this pre-experimental study, we employed SES as a noninvasive, cost-effective, and repeatable stimulus modality, to evoke the ISLN in awake subjects and then recorded the reaction of their internal laryngeal muscles in terms of the resultant acoustic changes. To our knowledge, the present study is the first research to apply SES in order to evoke vocal cords through the ISLN in awake normal humans. The aim of this study, as part of a larger research project, was to investigate the probability of utilizing the motor potentials of the ISLN on the vocal cords via SES, which can be used in future studies as an electrotherapy modality to treat patients with recurrent laryngeal damage.

Materials and Methods

The current study was performed on the students of School of Rehabilitation Sciences, Iran University of Medical Sciences in 2014. Twenty-two female and 10 male individuals (n=32) between 20 and 33 years old (mean age=23.87) participated in this study. All the subjects were selected according to the accessible sampling method, although participation was on a completely voluntary basis. Because of the lack of a previous similar study, the sample size was defined according to the data gathered from our study’s 10 pilot subjects. According to the applied formula to calculate the sample size presented below, the sufficient number was 18 persons:

\[ n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2}{d^2} \]

First, all the subjects completed a consent form approved by the Ethics Committee of Iran University of Medical Sciences. All the study participants were nonsmokers and reported a negative history of neck and head surgery and neurological, respiratory, serious cardiac, psychiatric, swallowing, hearing loss, voice, and speech disorders. There were also no systemic diseases such as diabetes. According to the subjects’ negative self-reported voice complaints and the perceptual judgment of a speech-language pathology expert, who was experienced in voice disorder evaluation and treatment, all of the participants’ voice was diagnosed normal at the time of test. In addition, during the study, any subject who could not tolerate a gradual rise...
in SES intensity was excluded from the study. Likewise, inability to prolong the/a/vowel at least for 10 seconds without any changes in voice acoustic parameters led to the exclusion of the subject from the study.

Procedure
SES was presented to the subjects via 2 self-adhesive circular active monopolar silver electrodes, 0.7 cm in diameter (F-55). They were placed on the thyrohyoid membrane between the hyoid bone and the thyroid cartilage at the entrance location of the ISLN into the larynx on both sides. Additionally, one 6×5 cm rectangular farther dispersive electrode was used on the back of the neck. The electrical current was generated by a commercially available electrotherapy system (ELPHA II 3000, Danmeter A/S, Denmark). The system was set to produce a 3-second current with 5-second rests between each 2 stimuli. The current parameters were programmed on a 200-microsecond duration of pulses and a frequency of 100 Hz. Amplitude was started from 0 mA and increased gradually according to the participant’s tolerance. Before electrode placement, the skin of all the subjects was cleaned with alcohol. Additionally, in some males, the extra hair on the site of electrode placement was shaved.

Controlling volume conduction (diffusing the current to the contiguous tissues, especially the vocal cords and the laryngeal recurrent nerve) was a challenge. Thus, after the termination of ISLN stimulation via electrodes, the 2 active electrodes were once again placed on the thyroid lamina (the nearest place to the vocal cords and the recurrent nerve) and the previous current was repeated precisely. The objective was to define the amount of the direct conduction of the current to the vocal cords and the recurrent nerve and to exclude evoked ISLN effects.

Voice Recordings
As the intensity of the stimulant reached up to 10% of the subjects’ ultimate tolerance, they were asked to prolong the/a/vowel for 13 seconds: 5 seconds without stimulation (rest period), 3 seconds with stimulation (SES period), and finally another 5 seconds’ rest period. The second rest period was meant to coordinate the time of phonation with the rest periods of the electrical stimulation. Hence, only 2 seconds of phonation from the mid-point of the first rest period and the SES period were compared with each other. The subjects were free to practice during stimulation and were instructed to prolong a comfortable and consistent natural vowel. Voice recordings were obtained using a condenser microphone (Rode NT6, Australia), connected to a preamplifier (M-Audio, Avid technology, Inc.). The distance of mouth to microphone was 20 centimeters. The collected voice samples were analyzed using Praat (version 5.3.13; http://www.fon.hum.uva.nl/praat/download_win.html). The acoustic parameters digitized comprised F0, standard deviation of F0 (SD F0), shimmer, jitter, voice break, HNR, and intensity of voice.

Statistical Analysis
The digitized acoustic parameters of rest and SES periods were analyzed using SPSS (version 17) and were compared in terms of the induced changes across the 2 conditions of phonation. The paired t-test was utilized to compare the means of the acoustic parameters, namely mean F0, minimum F0, maximum F0, SD F0, jitter, shimmer, HNR, mean intensity, maximum intensity, and minimum intensity at rest and SES periods.

Results
Figure 1 demonstrates mean F0, maximum (max) F0, and minimum (min) F0 for the rest and SES periods related to ISLN electrode placement. The paired sample t-test showed no statistically significant effects of SES on mean (m) F0. As the figure indicates, mean F0 for rest was 198.35 (SD=49.93) while it was 193.97 (SD=50.15) for the SES period, which did not constitute significant statistical difference (t=1.03; P=0.31). Also, the means of max F0 during rest (m=202.28, SD=50.78) and SES (m=203.93, SD=52.64) were not significantly different (t=0.35; P=0.72). However, the mean of min F0 for the rest time was 194.54 (SD=49.19) versus 182.62 (SD=53.39) for SES, duration which the difference between the 2 means was significant (t=2.61; P=0.01).

The SD F0 means of the rest and SES durations related to electrode placement on the ISLN are shown in figure 2. The rest SD F0 mean was 1.54 (SD=0.55) versus 4.15 (SD=3.00) for SES. The paired t-test indicated significant differences between the SD F0 means (t=4.95; P<0.001).

Table 1 demonstrates jitter, shimmer, HNR, mean intensity, maximum intensity, and minimum intensity results related to electrode placement on the ISLN. As the table presents, the means of rest compared to those of the SES period for both jitter and shimmer increased: The changes in the means were 0.09 and 0.9, respectively, which were statistically significant (jitter: P<0.001 and shimmer: P<0.001). HNR mean during rest versus SES decreased by 2.21 dB, with the change being significant according to
the paired t-test (P<0.001). However, there were only 2 participants who demonstrated phonation break during the SES phase.

The means of the 3 token values of intensity decreased from rest to the SES phase. Mean intensity changed 1.48, minimum intensity 3.99, and maximum intensity 0.38. The paired t-test indicated that the changes in both mean intensity and minimum intensity were statistically significant (P<0.001), whereas the changes in maximum intensity were not statistically significant (t=0.92; P=0.36).

Table 2 demonstrates the acoustic changes created by SES when active electrodes were placed on both sides of the thyroid lamina. As the table shows, the means of all the investigated acoustic parameters did not differ significantly between the rest and SES periods.

Discussion

The present study examined the possibility of imposing changes on the vocal cords via SES on ISLN topical location on skin. The results showed that an evoked ISLN did not give rise to a significant change in mean F0. Given that the contraction of the cricothyroid muscle heightens mean F0, it can be concluded that there were no descending branches or anastomoses from the ISLN to the cricothyroid muscle, which is in agreement with the results of previous anatomical studies. However, as the stimulation did not evoke the external SLN, which innervates the cricothyroid muscle, the stimulated location on skin was correct. It is not possible to exclude the external SLN from stimulation as Ludlow et al. asserted in their study even after using needle electrodes around the ISLN. Nevertheless, in our study, as SES did not lead to an increase in mean F0, SES effects on the external SLN were too little to be taken into account. The same result was observed for maximum F0, which again confirms the above conclusions. This result is in accordance with the previous studies that indicated insignificant effects of SES on mean F0. However, those studies reported increased F0 after SES, which may be due to their larger electrodes and consequent decreased current density distributed to the external laryngeal muscles. SES resulted in decreased minimum F0 compared to the rest state. This may have been due to the effects of SES on the thyroarytenoid muscle via the stimulation of the ISLN, lowering the frequency of voice. SD F0 was increased by SES. By taking into consideration that SD F0 reflects frequency variability for a large time segment, it can be concluded that SES plays an important role in imposing variability on muscles and thus contributes to muscular tension stability. Our findings do not chime in with the results of the previous studies performed on the effects of SES on voice parameters inasmuch as in none of those investigations SD F0 and minimum F0 changed significantly after SES.

Perturbation, including jitter and shimmer, is another acoustic factor which increased during the SES phase. The irregularity of the vibratory behavior of the vocal cords is referred to as perturbation. As a result, SES led to higher irregularity during the vibration of the vocal cords. This finding is not in accordance with the previous studies insofar as they did not show significant differences in their subjects’ perturbation of voice. Therefore, it is impossible to create perturbation changes via SES directly throughout the thyroid cartilage and into the vocal cords. Presumably, it is because of the lack of stimulus penetration due to incorrect electrode placement and electrical stimulation parameters. Nonetheless, our findings demonstrated that it
was possible through the stimulation of the vocal cords directly via the ISLN.

Greater aperiodicity of vibration may show up as greater noise in the spectrum and lesser HNR. In the present study, HNR decreased during the SES period. As a result, heightening of aperiodicity can be concluded consequent to electrical stimulation on the ISLN. Gorham-Rowan et al. reported similar results regarding the change direction of jitter, shimmer, and HNR after SES, but the changes were not statistically significant. It may be attributed to the differences in methodology between the study by Gorham-Rowan et al. and the present study.

In spite of these reported aperiodicity and instability in the participants’ voice during the SES period, SES was able to cause only voice break in 2 subjects. Ludlow CL et al. stimulated the ISLN by inserting needle electrodes around the ISLN for normal awake participants and reported voice break during phonation in 1 of their 3 subjects. The authors attributed it to increased vocal fold adduction, which disrupted vibration. Similarly in our research, voice breaks occurred in 2 subjects but the other 30 participants did not show any voice break during phonation at SES time. This suggests that SES, unlike needle electrode stimulation, is not strong enough to interrupt the phonation process completely.

In addition to frequency and perturbation, the results showed that while mean and minimum intensity decreased with SES, maximum intensity decreased but it was not statistically significant. As the average intensity is a criterion to present the strength of the vibratory behavior of the vocal cords, the effects of SES in terms of reducing vocal intensity may be due to glottal closure interruption consequent to SES presentation. This result was in contrast to the results of a study by Flower and et al., who did not report significant changes in voice intensity after SES in healthy individuals.

There are some reasons for reporting nonsignificant changes in acoustic parameters after using SES in the previous studies. The most important reason relates to the electrical stimulation parameters applied in those studies compared to our own. The previous investigations applied an electrical current with a low frequency (80 Hz) and long duration pulses (700 microseconds) compared to our study with a high pulse frequency (100 Hz) and short duration pulses (200 microseconds). It is possible to increase the intensity of pulses with short durations and high frequencies well above the motor threshold without stimulating pain endings. Stimulation of the deeply placed nerves (such as the ISLN) will, therefore, be feasible. Accordingly, the current method applied in the present research had more power to penetrate deeply and reach the ISLN.
The other reasons include different methods in electrode placement, different stimulated organs (internal laryngeal muscles versus ISLN in our study), different sizes of electrodes, and dissimilar time of recording voice (after or during SES presentation, as was the case in the present study).

As the results of electrode placement on the thyroid lamina indicated, SES did not bring about significant changes in any of the acoustic parameters. This finding confirms the notion that by dispossessing and excluding the ISLN from the SES-affected area and also by delivering the current at the nearest place to the vocal cords and the recurrent nerve, SES cannot stimulate the vocal cords or the recurrent nerve. It also shows that all the findings on delivering SES with electrodes placed on the thyrohyoid hint at the stimulation of the ISLN and not the direct conduction of the current to the vocal cords or the recurrent nerve. The results of electrode placement on the thyroid lamina are in agreement with those reported by the previous studies whose method of electrode placement was aimed at stimulating the vocal cords directly.9-11,13 This confirms the importance of correct electrode placement when seeking to impact voice through SES.

Although the findings on perturbation and intensity suggested exacerbation of voice during SES presentation, they indicated the effectiveness of SES in impacting the internal laryngeal muscles from the ISLN as a gate for motor responses in the vocal cords. The results of electrode placement on the thyroid lamina indicated the impossibility of stimulating the recurrent laryngeal nerve by SES. Therefore, the clinical application of this finding would see SES utilized to evoke the motor potential of the ISLN in order to treat patients suffering from recurrent laryngeal nerve damage.

The current study has some limitations, first and foremost among which were problems in fixing electrodes on a defined location, not least when the subjects sweated and the test had to be repeated after cleaning the skin. In addition, some subjects feared the first sensation of tingling caused by SES and were, thus, excluded from the study.

Conclusion

The results of the present study demonstrated the significant impact of SES on voice changes in healthy participants. To the best of our knowledge, ours is the first study of its kind to report significant voice changes in consequence of SES application. The reason for the dissimilarities between our results and those of the previous studies may be attributed to the methodology of stimulation and the time of recording voice. Our results indicated that the application of this method via the ISLN at least in healthy people could induce motor pulses in the vocal cords and cause voice changes. The importance of this finding is that it hints at the possibility of SES application in patients who need to have the motor neurons of their vocal folds stimulated when there is no accessibility owing to damage to the recurrent nerve (e.g., patients with vocal paralysis). Confirmation of the applicability of this method, however, requires further research.

Acknowledgment

We appreciate all the participants who assisted us in performing this study.

Conflict of Interest: None declared.

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