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Selective recurrent laryngeal nerve stimulation using a penetrating electrode array in the feline model

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Abstract

Objective—Laryngeal muscles (LMs) are controlled by the recurrent laryngeal nerve (RLN), injury of which can result in vocal fold (VF) paralysis (VFP). We aim to introduce a bioelectric approach to selective stimulation of LMs and graded muscle contraction responses.

Study Design—Acute experiments in cats.

Methods—The study included six anesthetized cats. In four cats, a multichannel penetrating microelectrode array (MEA) was placed into an uninjured RLN. For RLN injury experiments, one cat received a standardized hemostat-crush injury, and one cat received a transection-reapproximation injury four-months prior to testing. In each experiment, three LMs (thyroarytenoid, posterior cricoarytenoid, and cricothyroid muscles) were monitored with an electromyographic (EMG) nerve integrity monitoring system. Electrical current pulses were delivered to each stimulating channel individually. Elicited EMG voltage outputs were recorded for each muscle. Direct videolaryngoscopy was performed for visualization of VF movement.

Results—Stimulation through individual channels led to selective activation of restricted nerve populations, resulting in selective contraction of individual LMs. Increasing current levels resulted in rising EMG voltage responses. Typically, activation of individual muscles was successfully achieved via single placement of the MEA by selection of appropriate stimulation channels. VF abduction was predominantly observed on videolaryngoscopy. Nerve histology confirmed injury in cases of RLN crush and transection experiments.

Conclusions—We demonstrated the ability of a penetrating MEA to selectively stimulate restricted fiber populations within the feline RLN and selectively elicit contractions of discrete

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LMs in both acute and injury-model experiments, suggesting a potential role for intraneural MEA implantation in VFP management.

Keywords

recurrent laryngeal nerve; RLN; RLN implant; RLN stimulation; multichannel electrode array; posterior cricoarytenoid; PCA

Introduction

The recurrent laryngeal nerve (RLN) is a critical branch of the vagus nerve that innervates the laryngeal musculature and facilitates voice production, swallowing, and airway protection. Damage to the RLN can result from iatrogenic and traumatic injury, neoplastic mass effect, or an idiopathic process, and lead to vocal fold paralysis (VFP).¹ The incidence of VFP is not well established but has been previously reported to be as high as 42 in 10,000 persons per year.² Unilateral VFP can generate significant patient morbidity and can present with hoarseness, difficulty swallowing, and in some cases, aspiration. Bilateral VFP often presents a more substantial problem, as it can result in shortness of breath and airway obstruction.

Upon initial diagnosis of VFP, a period of observation is typically indicated prior to more definitive treatment, as 14% to 58% of patients will have complete spontaneous recovery.^{3,4} In cases of permanent VFP, there are currently no means of restoring normal physiologic vocal fold function, and although generally effective, current treatment options for VFP have some limitations. For symptomatic unilateral VFP, surgical interventions include injection laryngoplasty, medialization thyroplasty, arytenoid adduction, and laryngeal reinnervation, among others. While medialization thyroplasty has led to well-established and satisfactory vocal outcomes and is increasingly performed⁵⁻⁷, this technique may lack long-term efficacy due to possible vocal fold atrophy and implant migration.⁸⁻¹⁰ In cases of bilateral VFP, surgical interventions to augment the airway, including vocal fold cordotomy and arytenoidectomy, are at times indicated. Reported complications of these procedures include voice impairment and aspiration.¹¹⁻¹³ In certain cases of bilateral VFP, a tracheostomy is required for airway protection due to narrowing at the glottis and consequent inadequate airway opening. While current treatment modalities for both unilateral and bilateral VFP are generally safe and adequate, newer technologies may offer opportunities for further functional and vocal outcome improvement.

The application of novel treatment options, such as implantable neurostimulation devices, may serve to fill this treatment gap in the management of unilateral and bilateral VFP. Previous studies have demonstrated posterior cricoarytenoid muscle (PCA) neuromuscular stimulation in humans to assist in vocal fold abduction and yield clinically significant improvements.¹⁴⁻¹⁶ More recently, bilateral PCA stimulation in acute and chronic canine studies has demonstrated varying degrees of success.¹⁷⁻¹⁹ Similarly, the use of laryngeal pacing has been shown in recent human studies to be an effective means of PCA stimulation.^{20,21} However, laryngeal adduction through stimulation has not been demonstrated in any model. While in humans, the anterior RLN carries both adductor and abductor motor fibers,

the feline larynx is innervated by branches of the vagus nerve. The external branch of the cranial laryngeal nerve innervates the cricothyroid (CT) muscle, and the anterior branch of the RLN innervating the thyroarytenoid (TA) and PCA muscles.²²

Our group has previously demonstrated the utility of a penetrating multichannel microelectrode array (MEA) to selectively stimulate highly-specific neural fibers within the cochlear nerve and facial nerve.²³⁻²⁵ Due to the limitations in both the current surgical treatment options of VFP and the recent studies using PCA stimulators, we sought to demonstrate the ability of a multichannel MEA implanted in the RLN to selectively elicit contraction of specific laryngeal muscles in a cat. To our knowledge, this is the first animal study to evaluate the efficacy of an intraneural stimulator directly implanted into the RLN to selectively stimulate laryngeal musculature. The goal of this work is to introduce a novel approach that could potentially be used in the surgical management of VFP and circumvent the shortcomings of current surgical interventions, allowing for intraneural RLN stimulation to selectively activate adductor or abductor laryngeal musculature in a graded fashion.

Methods

Electrode array and stimuli

The multichannel intraneural stimulating MEAs (MicroProbes for Life Science, Gaithersburg, MD, USA) have four platinum/iridium-plated electrode sites, or channels, arrayed at 250- μ m intervals spanning a distance of five mm along a single, 241- μ m diameter polyimide tube [Figure 1]. System 3 equipment from Tucker-Davis Technologies (TDT; Alachua, FL, USA) and custom software running in MATLAB (The MathWorks, Natick, MA, USA) were used for stimulus presentation. Electrical stimulus pulses were generated by a channel current source controlled by a 4- or 16-channel digital-to-analog converter (TDT RX8). Stimuli were single charge-balanced biphasic electrical pulses, initially cathodic, 41 or 82 μ s per phase. The illustrated responses were obtained with stimulus charge levels of 26 to 41 nC per phase.

Surgery (Acute Terminal Experiments)

All procedures were performed with the approval of the UC Irvine Institutional Animal Care and Use Committee and according to the National Institutes of Health guidelines. We conducted acute, terminal experiments in four barbiturate-anesthetized cats. A vertical anterior neck incision was made, the strap muscles were lateralized, and the larynx was exposed. A tracheostomy tube insertion was performed. Needle electromyographic (EMG) electrodes were pierced through the thyroid cartilage into the thyroarytenoid (TA) muscle. The larynx was rotated medially and needle EMG electrodes were placed into the PCA muscle. Lastly, needle EMG electrodes were placed into the directly visualized cricothyroid (CT) muscle. Needle EMG electrodes penetrating each individual muscle are demonstrated in Figure 2.

Careful dissection was then performed in the tracheoesophageal groove to expose the RLN. The nerve was stimulated with a Prass monopolar probe of the nerve integrity monitoring system (NIM Response 2.0, Medtronic Inc., Minneapolis, MN, USA) to ensure correct

identification of the RLN via visualization of laryngeal muscle movement and EMG responses. The MEA was manually placed into the RLN, with the goal of inserting all stimulating sites in neural tissue. The frequency at which the RLN was stimulated was typically set at 10 Hz. In one experiment, stimulation frequency was varied from 1 to 100 Hz in 10 Hz intervals while maintaining current levels to evaluate the effect of frequency variation on LM activity. Direct videolaryngoscopy was performed for visualization of vocal fold movement after MEA implantation into the RLN in one cat to evaluate vocal fold motion with varying channels in the MEA, and moreover, in a second cat after MEA implantation to evaluate vocal fold motion with varying stimulation frequencies from a single channel.

Each channel was individually stimulated and EMG voltage responses from the three laryngeal muscles were recorded. To vary the neural populations stimulated, the stimulating MEA was removed and repositioned into the distal nerve in varying trajectories and angles along the course of the exposed RLN, and each channel was again stimulated. Each cat underwent three to four insertions of the intraneural MEA followed by escalating current transmission. Consequent EMG responses were recorded. Once the experiment was complete and the animal euthanized, a laryngofissure and laryngectomy were performed to confirm placement of the EMG electrode leads in the TA and PCA muscles [Figure 2]. In all cases, the EMG electrodes demonstrated good placement.

Surgery (Recurrent Laryngeal Nerve Injury)

For survival surgeries to produce a standardized RLN injury, the RLN was identified skeletonized as detailed previously. The nerve was then intentionally damaged by either a nerve crush injury²⁶ consisting of a 30 second one-click crush with a serrated hemostat (n=1) or complete transection with scissors and reapproximation (n=1), to produce a Sunderland 5th degree neurotmesis injury²⁷. For the transection injury model, the nerve endings were aligned but not sutured together. Failure of Prass probe stimulation of the RLN proximal to the site of injury to generate laryngeal muscular contraction and EMG was confirmed. The incision was then closed in layers.

In terminal surgeries four months post-injury, the RLN was again identified, and an MEA was introduced into the RLN either directly adjacent or proximal to the injury site, such that all four channels were in neural tissue. Insertion site and angle was dictated by surgical anatomy and micropositioner-mounted electrode access to the nerve. Each of the MEA channels was individually stimulated, and EMG voltage responses from the three selected laryngeal muscles were recorded by the nerve integrity monitoring system. To vary which neural populations were stimulated, the MEA was withdrawn and reinserted into the nerve in variable trajectories and angles along the course of the exposed RLN trunk, and each channel was again stimulated. Following a lethal dose of barbiturate and transcardial 4% paraformaldehyde fixation, the RLN was harvested for histological examination.

A Pearson's correlation coefficient was measured to determine the strength of correlation between MEA current levels and EMG response. A paired samples t-test was performed in order to determine whether the same muscle had significant differences in activation based on the channel activated. An independent samples t-test was used to determine whether or

not the mean activation was different between the muscles. Statistical analysis was performed using PASW Statistics 18.0 software (SPSS Inc., Chicago, IL). A p-value of < 0.05 was considered statistically significant. The purpose of this analysis was to evaluate for significant increases in EMG response resulting from increasing current delivery, and muscular stimulation selectivity based on channel activation.

Results

Stimulation of an Uninjured Nerve with a Multichannel MEA

Stimulation through each of the four individual channels activated nerve populations selectively, often resulting in EMG activity in individual muscles. Selective activation of one or more distinct muscles was routinely achieved via a single placement of the multichannel MEA by selection of appropriate stimulation channels. Figure 3 (cat 2, position 3) presents the EMG voltages from individual channel stimulation of the RLN in an implant insertion with the greatest degree of muscle selectivity. In this experiment, channels 1, 2, and 3 elicited stronger responses from the TA and CT muscles, while channel 4 selectively stimulated the PCA. Furthermore, increasing levels of stimulation current resulted in increasing EMG voltage responses.

Direct video laryngoscopy revealed primarily vocal fold abduction and rare vocal fold adduction, with stimulation of a single channel, regardless of EMG output data and selectivity. Supplementary video content 1 shows vocal fold abduction in cat 4 after implantation of the left RLN with a 16-channel MEA. Supplementary video content 2 shows representative vocal fold abduction with interspersed and subtle vocal fold adduction when stimulating a different channel in the same animal. Still images of vocal fold abduction and adduction with RLN stimulation are shown in Figure 4.

Analyzing our data from the implant insertion in cat 2, position 3, we found a statistically significant positive correlation between current level and EMG voltage recorded in three different muscles following MEA implantation in the uninjured nerve ($p < 0.001$). When comparing EMG response of the TA and PCA muscle in an individual channel in the uninjured setting, the TA had significantly elevated EMG output when channel 1 was stimulated compared to channel 4 ($p < 0.001$), while the PCA muscle had a significantly increased response when channel 4 was activated compared to channel 1 ($p = 0.039$). When comparing TA and PCA EMG responses within the same channel, again in the uninjured setting, there was a statistically significant difference with the TA showing higher EMG responses than other muscles within channel 1 ($p < 0.001$) and PCA showing higher EMG responses than other muscles within channel 4 ($p < 0.001$).

Stimulation of an Uninjured Nerve with Varying Frequency

In the experiments with which the frequency of stimulation was varied, there was no evidence of laryngeal muscle selectivity on EMG. Despite this lack of selectivity on EMG, there were changes in vocal fold movement visualized on direct videolaryngoscopy. Supplementary video content 3 demonstrates the apparent vocal fold abduction with RLN

stimulation at a frequency of 10 Hz in cat 3. Supplementary video content 4 illustrates a dissimilar laryngeal muscle contraction pattern with RLN stimulation of 100 Hz in cat 3.

Stimulation Post-RLN Injury with a Multichannel MEA

Following RLN injury, MEA implantation resulted in an attenuated degree of selective stimulation of individual muscles, with the PCA consistently contracting more robustly than the TA or CT in both the transection and crush injury settings (Figure 5). The amplitudes of the EMG responses were also diminished following RLN injury, with maximal EMG outputs reaching ~600 μ V (in comparison to ~6000 μ V in the uninjured nerve). Following a RLN transection injury, the PCA was activated to a statistically significantly greater degree than both the CT and TA regardless of which channel was activated ($p < 0.01$; Figure 5A-B). Following a RLN crush injury, the PCA was activated to a statistically significantly greater degree than both the CT and TA when channel 2 was activated, but only statistically significantly greater than the CT (not the TA) when channel 4 was activated ($p < 0.01$; Figure 5C-D). On histological examination, crushed and transected RLN nerve fibers show atypical axons, fibrosis within the neural bundle, vacuolization, and neuroma formation under hematoxylin and eosin (H&E) and trichrome staining [Figure 6], confirming the presence of degenerative and regenerative processes in the experiments.

Discussion

This study establishes the ability of a multichannel MEA implanted within a feline RLN to selectively stimulate neural populations and allow for contraction of distinct laryngeal muscles with a graded muscle response. The overall robustness of the response did decrease following recovery from transection-reapproximation or crush injuries, as expected given the histological findings, but the ability to stimulate the PCA with a graded response was still present after injury. Despite decreases in maximal EMG responses and decreases in selectivity, these results suggest that MEA implantation can continue to selectively elicit contractions in laryngeal muscles following a neurotmetic nerve injury.

As previously discussed, current treatment options (e.g. medialization thyroplasty, injection laryngoplasty, arytenoid adduction) for VFP are limited. An alternative technique, laryngeal reinnervation, was introduced many decades ago to prevent the possible long-term muscle atrophy associated with static medialization options. Reinnervation procedures can restore adductor tone. Reinnervation techniques include primary end-to-end anastomosis, ANSA to RLN anastomosis, and primary interposition grafts. Case series and a multicenter randomized clinical trial have shown broadly positive outcomes with reinnervation procedures.²⁸⁻³¹ However, current reinnervation options do present limitations, with overall outcomes being only marginally better than static medialization techniques.⁹ Bilateral VFP has even further limited treatment options. Reinnervation can be performed in a selective fashion, in which abduction is targeted by reinnervation of the PCA, or in an unselective fashion, where muscle tone is obtained without regained function.³² While these reinnervation options have demonstrated success with the possibility of decannulation³³, there is possible room for improvement with the selectivity of reinnervation in cases of bilateral VFP.

Due to this gap in the definitive interventions for bilateral VFP, previous studies have evaluated the utility of neuromuscular stimulation of the PCA to evoke vocal fold abduction. The utility of laryngeal pacing stimulated by inspiration has been examined in both animals and humans.^{14-17,20,21,34-43} Most notably, a multicenter clinical trial with an implantable PCA stimulation device (Itrel II) was conducted.¹⁴⁻¹⁶ In five of six patients, unilateral laryngeal stimulation via 1-2 second trains of pulses paced with inspiration improved airflow without affecting voice or swallowing, and three of six patients were successfully decannulated. This device was limited by its design, which only allowed for unilateral implantation. More recent studies have evaluated a newer generation device (Genesis XP) in bilateral PCA stimulation in acute and chronic canine studies with success in restoring bilateral motion in a paralyzed larynx.¹⁷⁻¹⁹ These PCA stimulators are limited as they can only lead to vocal fold abduction in the setting of paralysis and do not allow for laryngeal adduction.

The application of intraneural multichannel MEAs to the RLN can provide a dynamic treatment option for bilateral VFP. Analysis of our data demonstrated that laryngeal muscles can be selectively activated and produce differential levels of contraction in both the acute and, to a lesser degree, injury models. Specifically, the PCA muscle demonstrated distinct selectivity, at times being the only muscle stimulated in a single channel, which could be most beneficial in cases of bilateral VFP. Additionally, MEA implantation in the RLN allows for graded muscular contraction, allowing for enhanced and optimized control of laryngeal muscle response. Theoretically, a programmable device coupled to an intraneural multichannel MEA could be surgically and securely inserted into a permanently damaged RLN in cases of VFP. Intraoperative confirmation using electromyographic nerve monitoring guidance could verify activation selectivity of laryngeal adductor and laryngeal abductor muscles using different channels in a single implant, as previously used in hypoglossal nerve stimulation.⁴⁴ The use of an intraneural stimulator can prevent the possible risk of long-term atrophy and stiffness that can occur with deinnervation, and that is not addressed with medialization thyroplasty and injection laryngoplasty. Furthermore, direct neural stimulation has been shown to mitigate muscle atrophy and improve muscle rehabilitation to a greater degree than direct muscle stimulation.⁴⁵⁻⁴⁸

Importantly, the potential clinical applicability of this proposed approach to VFP treatment is yet to be determined. In our feline experiments, certain implant placements demonstrated more laryngeal muscle selectivity than others, and we would expect a similar clinical result. Gacek et al⁴⁹⁻⁵¹ previously demonstrated in a series of feline experiments that there is a diffuse arrangement of adductor and abductor nerve fibers in the vagus nerve and these collections of fibers separate prior to entering the larynx. We subjectively noted improved selectivity with more distal implant placement, possibly due to described separation of adductor and abductor fibers, although this observation will need to be further investigated and confirmed in our future studies. Natural variability in the somatotopic distribution of the RLN fibers innervating specific muscles, the limitations of the electrode composition and array design, and the variability in the surgical placement of the array can impact the selectivity of muscle contraction. With higher stimulation current levels, there is a greater risk of stimulating nearly all of the fibers in the small diameter nerve and thereby limit selectivity, and theoretically improvements in the array design can improve selectivity.

Additionally, poor regeneration of neural fibers after injury has previously been described.⁵² In certain scenarios of poor regeneration or misdirected re-innervation, we suspect that RLN injury may alter the somatotopic distribution of the nerve fibers, reduce selectivity, and decrease overall EMG response following current delivery. Lastly, further elucidation of chronic implantation parameters and the effects of head and neck movement on RLN implants must be more fully addressed prior to contemplating translational and clinical applications of this RLN implant system.

It must furthermore be acknowledged that the feline model is not identical to the human larynx. While the variations in RLN anatomy and innervation in the human larynx is well defined⁵³, few studies have demonstrated feline RLN anatomy and innervation patterns. In our study, we found that our intraneural implant stimulated the CT muscle. While there is a remote possibility that this may be due to unintended stimulation of the external branch of the cranial laryngeal nerve, we believe that this is most likely due to direct CT stimulation by the RLN, which has been previously described in humans.⁵⁴⁻⁵⁷ We also found that, under direct videolaryngoscopy, the clinical response was more consistently vocal fold abduction in the feline model, despite the laryngeal muscle EMG selectivity more often favoring TA activation. This may be due to the underlying differences in the feline anatomy compared to human anatomy, where vocal fold abduction may be the primary laryngeal response to RLN stimulation. Additionally, while previous studies have demonstrated laryngeal muscle selectivity with varying frequencies using transcutaneous and transesophageal RLN stimulation,^{58,59} we were unable to replicate this relationship using intraneural stimulation at frequencies ranging from 10-100 Hz. These studies were able to demonstrate abduction at frequencies less than 30 Hz and adduction above 40 Hz. We did not demonstrate clear selectivity with changing frequencies. However, we did find that the vocal fold movement varied with escalating stimulation frequencies, as seen on Supplementary Video 3 and 4, which we could not detect in our EMG data. Further studies on stimulation frequency variation may provide additional insight on RLN stimulation selectivity.

Conclusion

We have established in the animal model the ability of an intraneural multichannel MEA to selectively stimulate restricted fiber populations within the RLN and selectively elicit contractions in specific laryngeal muscles. These results hold true following RLN injury. Despite the need to further refine the selectivity in injury models, these results may suggest a potential role for RLN implanted multichannel penetrating MEAs in vocal fold reanimation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Photograph of a MicroProbes 4-channel stimulating microelectrode array (MEA). (B) Microscopic picture of the shank and distal flexible output table with a metric ruler size reference. (C) High-magnification microscopic photograph of the distal end of the penetrating shank; the 4 electrode sites, or channels, can be seen. Superficial or proximal electrodes are those furthest from the tip of the MEA (to the left in this picture), while deep or distal channels are those closest to the MEA tip (to the right). (D) Microscopic picture of the female Omnetics connector and ground reference wire.

Figure 2.
Images demonstrating the microelectrode array piercing the RLN (A) and needle EMG electrodes within the CT muscle (B). After the larynx was dissected, needle EMG electrode placement was confirmed within the TA muscle (C) and the PCA muscle (D).

Figure 3.

(A-D) Graphic representation of escalating stimulation current levels of a single channel on the x-axis, with correspondingly increasing EMG voltage responses on the y-axis. Also demonstrated are the distinct voltage responses of different muscles to graded stimulation of individual channels. (A-B) Channels 1 and 2 demonstrate selective stimulation of adductor laryngeal muscles (TA and CT) [cat 2, position 3]. (C-D) Channel 4 demonstrates selective stimulation of abductor laryngeal muscles (PCA) [cat 2, position 3].

Figure 4.

Vocal fold images from direct videolaryngoscopy in cat 4 after implantation of the left RLN with a 16-channel microelectrode array. The vocal folds are outlined in black to better demonstrate airway expansion and narrowing with vocal fold abduction and adduction respectively. (A) Vocal folds at baseline. (B) Left vocal fold abduction. (C) Vocal folds at baseline. (D) Left vocal fold adduction.

Figure 5.

(A-D) Graphic representation of escalating stimulation current levels of a single channel on the x-axis, with correspondingly increasing EMG voltage responses on the y-axis. Also demonstrated are the distinct voltage responses of different muscles to graded stimulation of individual channels following RLN injury. (A-B) Following a transection-reapproximation injury to the nerve, channels 1-4 stimulate the PCA preferentially in a dose-responsive manner, with increasing current resulting in higher EMG voltage responses [Cat 3, position 3] (phase duration: 41µs; pulse duration: 400µs). (C-D) Following a crush injury to the nerve, channels 1-4 stimulate the PCA preferentially in a dose-responsive manner, with increasing current resulting in higher EMG voltage responses [Cat 4, position 2] (phase duration: 41µs; pulse duration: 400µs).

Figure 6.

(A) Light microscopy image of a normal recurrent laryngeal nerve (RLN) (Hematoxylin and eosin [H&E]) stain, 20× magnification) showing normal axons (star) and no vacuolization. Insert shows light microscopy image of a normal RLN (trichrome stain, 20× magnification) showing normal axons (star), no fibrosis, and no vacuolization. (B) Light microscopy image of a RLN that underwent crush injury, (H&E stain, 20× magnification) showing vacuolization (arrows; insert), atypical axonal fibers with varying thickness (star). (C) Light microscopy image of a crushed RLN (trichrome stain, 20× magnification) showing normal axons (star) adjacent to atypical axons and fibrosis (arrow). (D) Light microscopy image of a RLN that underwent transection and reapproximation injury (H&E stain, 20× magnification) showing an inflammatory reaction (arrow) and neuroma formation.