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Recovery of Laryngeal Sensation After Superior Laryngeal Nerve Anastomosis

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***Objectives/Hypothesis:* Reliable motor reinnervation has been show in multiple laryngeal transplant studies; however, sensory reinnervation of the larynx after nerve anastomosis has yet to be demonstrated. The role of sensory nerve anastomosis in the transplanted larynx in unknown, but is thought to be necessary to provide airway protection. A canine model was developed to examine the possibility of reformation of sensory pathways in the larynx after nerve section and anastomosis. *Study Design:* Randomized controlled experiment. *Methods:* Ten canines were randomly assigned to two groups. Hydrochloric acid–induced laryngospasm was demonstrated in every dog. All dogs then had their necks explored, and the internal branch of the superior laryngeal nerve was identified and transected bilaterally. Following nerve section all dogs were retested for an acid-induced laryngospasm reflex. The control group had their wounds closed and were then awakened from anesthesia. The study group underwent microscopic anastomosis of their sensory nerves. Following a 6-month period the two groups of dogs were compared for the presence of the laryngospasm reflex. *Results:* No dog in the control group had a response to the acid. All dogs in the study group had some response to the acid, although none of them had return of true laryngospasm. *Conclusion:* We concluded that sensory reinnervation does occur after nerve anastomosis, but the recovery of sensation may be incomplete or altered. *Key Words:* Larynx, sensory, laryngospasm, anastomosis, canine.**

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INTRODUCTION

The development of a canine laryngeal transplant model has progressed significantly in this decade. Modern techniques adopted from microvascular free-flap reconstruction have allowed for successful anastomosis of both the vascular and nervous structures in the transplanted larynx.[1] Immunosuppressive regimens are continuing to be developed in solid-organ transfer, and a variety of agents is currently available.[1,2] Motor anastomosis has been studied extensively within the literature of laryngeal physiology. However, little work has been done with regard to sensory nerve anastomosis or the recovery of sensation after neural anastomosis. The role of sensory nerve anastomosis within the transplanted larynx is unknown.

Sensation within the larynx is provided by receptors supplying two different afferent nerves. The internal branch of the superior laryngeal nerve (Int-SLN) largely supplies the supraglottic and glottic larynx, while the recurrent laryngeal nerve (RLN) supplies the subglottic larynx and proximal trachea. The receptors largely consist of intraepithelial nerve endings and taste buds.[3] These receptors are thought to be responsive to mechanical, chemical, and thermal stimuli.[3] Laryngospasm is defined as a prolonged occlusion of the larynx caused by contraction of the intrinsic laryngeal muscles.[4] In addition, laryngospasm exists as the efferent product of a laryngeal reflex to noxious stimuli.[5] The afferent limb of the reflex is carried from receptors in the supraglottic larynx via the Int-SLN. These impulses feed to the vagal nuclei in the brainstem and then trigger the efferent limb.[6] The result is prolonged laryngeal adductor spasm, and this reflex is thought to be part of the primitive airway protective role of the larynx.[4]

A model of acid-induced laryngospasm model has recently been described in the canine larynx.[6] Briefly, hydrochloric acid (HCl) of pH <2.5 applied to the supraglottic mucosa repeatedly produced a protective laryngospasm response. The response was negated after transection of the Int-SLN, thus demonstrating that this reflex is mediated through the supraglottic mucosal afferents via the Int-SLN.

The development of this acid-induced laryngospasm model has given us an easily identifiable and quantifiable objective response to supraglottic afferent stimulation. Using this model, we designed an experiment to demonstrate whether sensation of the supraglottic larynx could be recovered after Int-SLN severing and anastomosis.

MATERIALS AND METHODS

Ten adult mongrel canine males (Harlan Sprague-Dawley, Indianapolis, IN) were used for this study. All protocols were approved by the University of California Los Angeles Office for Protection of Research Subjects/Chancellor's Animal Research Committee and followed guidelines for humane care as outlined by the National Institutes of Health (NIH publication No. 80–23, revised 1978). With each surgical procedure the animal received an intravenous short-acting induction agent (pentobarbital sodium) titrated to a level of corneal anesthesia followed by oral-endotracheal intubation. Anesthesia was maintained through a closed circuit with an oxygen and halothane mixture. Following establishment of general anesthesia a low tracheotomy was performed, and the oral-endotracheal tube removed so that the larynx would be free for experimentation. The animals were positioned supine on the operating table and had their mandibles retracted with a modified side-biting mouth gag. The larynx was viewed transorally and illuminated with a standard endoscopic light source (Karl Storz, Culver City, CA) secured to the hard palate.

Laryngospasm Model

An acid-induced laryngospasm model was used for evaluation of laryngeal sensation. This method was selected because it has previously been shown to be valid in the canine laryngeal model by an independent laboratory.[6] In addition, this model was chosen because it specifically shows restoration of an airway protective reflex, rather than just sensation. Measurement of sensory evoked potential was considered, but was not used because it is prone to false-positive indication of reinnervation in cases where there is partial but inadequate sensation. As has been shown previously,[6] a light plane of anesthesia is necessary to permit laryngeal reflex monitoring. The plane of anesthesia was maintained so that spontaneous respiration and occasional spontaneous swallowing occurred. This minimizes, although it does not eliminate, uncontrolled variables (pco_2, core body temperature), but was necessary and desirable with regard to established criteria for humane animal experimentation.

After establishment of general anesthesia the larynx was visualized transorally and a concentric electromyography needle (Nicolet, Madison, WI) was placed permucosally within the belly of the right thyroarytenoid (TA) muscle. Spontaneous electromyographic (EMG) activity was recorded with the Viking IV EMG computer (Nicolet, Madison, WI). This pattern was recognized as confirmation of placement within a laryngeal adductor muscle and was used to confirm subsequent placements on the same animal in later experiments. Permucosal needle placement also decreases the risk of electrode misplacement as the thyroid lamina serves as a barrier to recording from the strap muscles. Three milliliters of a pH 1.0 HCl-saline solution was dripped onto the supraglottic larynx via a blunt-tipped, 20-gauge spinal needle, and spontaneous EMG activity was recorded. The needle was not allowed to touch the mucosa directly. The solution was made by mixing 1 N HCl (Sigma Chemical Company, St. Louis, MO) with 0.9% sodium chloride solution for irrigation (Baxter Pharmaceuticals, Deerfield, IL) until a pH of 1.0 was measured with a Corning model 220 pH meter (Corning, New York). The larynx was irrigated with 50 mL of normal saline solution with another blunt-tipped spinal needle after each experimental trial. Spontaneous EMG activity was measured at baseline, after irrigation with the HCl-saline solution, and after irrigation with saline alone. The animals were maintained in a slight Trendelenburg position so that gravity would prevent the irrigation solution from coming into contact with the subglottic or trachea mucosa, to avoid stimulation of the recurrent laryngeal nerve afferents.

Experimental Design

The 10 canines were segregated randomly into two groups of 5; one group was designated the control group, and the other, the study group. All dogs had their spontaneous right-side TA EMG activity measured at baseline, after acid stimulation, and after saline wash. Following this, all dogs had their necks explored through a horizontal skin incision just inferior to the hyoid bone using aseptic technique. The Int-SLN was identified bilaterally as it entered the larynx through the thyrohyoid membrane. The nerve was bilaterally severed, and spontaneous right-side TA EMG activity was measured at baseline, in response to HCl-saline solution, and after plain saline wash. Animals in the control group had a 2-cm portion of the Int-SLN resected; then their wounds were closed in layers. The tracheotomy tube was removed, and the site was closed in layers after the circuit was switched to transoral intubation. Following wound closure, the dogs were taken off the inhalation anesthesia, extubated, and transferred to a special kennel for recovery.

Animals in the study group also had right-side TA spontaneous EMG activity measured at baseline, following stimulation from HCl-saline, and after saline wash. After post-transection measurement of EMG activity, the proximal and distal Int-SLN stumps were anastomosed with four 10–0 nylon epineural sutures using a Zeiss operating microscope. The nerves were sutured without tension. Decannulation and recovery followed wound closure, similar to the control group.

After recovery, the animals were returned to their regular kennels and resumed their usual diet and activity. They were monitored for weight loss, fever, or abnormal activity.

Six months after the initial experiment the animals were returned to the operating theater and had spontaneous right-side TA EMG activity measured at baseline, after stimulation with HCl-saline solution and after saline wash. As was performed previously, a low tracheotomy was made after induction of general anesthesia so that the larynx was clear for experimentation. Following the EMG experiments the neck was opened from hyoid to sternum and the Int-SLNs were explored where they entered the thyrohyoid membrane. After completion of all experiments the animals were humanely sacrificed with a lethal intravenous injection of Eutha-6 solution (Western Medical Supply, Arcadia, CA).

RESULTS

All dogs demonstrated baseline expiratory activity of the right-side TA on spontaneous electromyography. When the supraglottic mucosa was exposed to the HCl-saline solution, all dogs developed laryngospasm as demonstrated by direct visualization and by continuous hyperactivity on the electromyogram (Figs. 1 and 2). Following the saline wash all dogs had their EMG activity return to baseline levels. After severing of the Int-SLN, no dog had a laryngospasm response to the acid solution; their EMG activity remained at baseline. Also, reanastomosis of the nerves in the study group did not cause a return of the laryngospasm response during the initial surgery (Fig. 2).

During the 6-month waiting period no dog had a significant change in weight. Furthermore, no dog limited its oral intake or developed a cough or other symptoms of aspiration. There were no postoperative fevers or other objective signs of pneumonia or demise. There were two

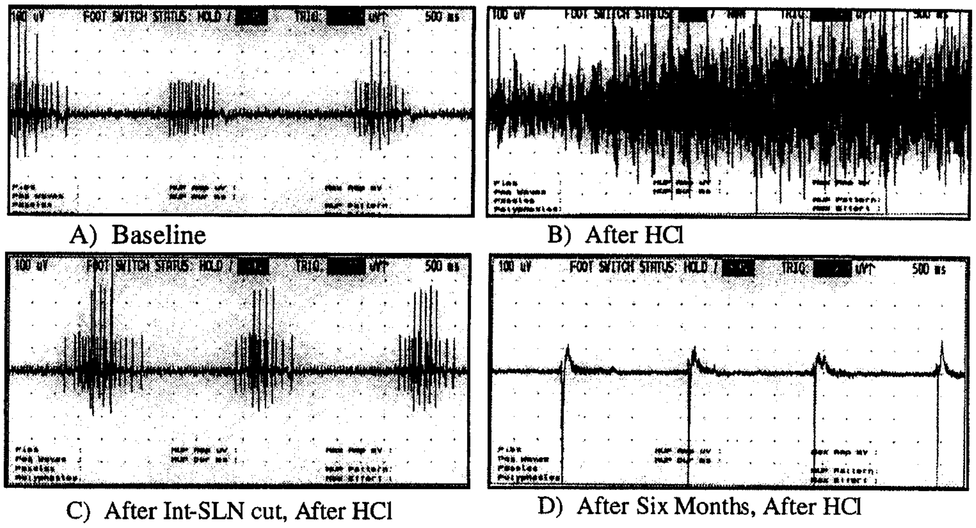

Fig. 1. Examples of control dog spontaneous right thyroarytenoid (TA) electromyography (EMG). **A.** Normal expiratory phasic activity at baseline. **B.** Laryngospasm response to HCl. **C.** After cutting the internal branch of the superior laryngeal nerve (Int-SLN) there is no response to HCl. **D.** Six months later there is still no change in baseline phasic activity to HCl.

small postoperative seromas that regressed without intervention.

After 6 months all dogs in the control group still had no response to the acid solution (Fig. 1). EMG activity was unchanged from baseline to saline wash and to acid stimulation. No laryngospasm was observed. On re-exploration of the neck the stump of the Int-SLN was not found, and the surrounding tissue was mildly fibrotic. No spontaneous reinnervation of the Int-SLN was noted at time of surgery.

All dogs in the study group demonstrated a response to the acid stimulation after 6 months. However, none of them demonstrated a true protective laryngospasm response (Fig. 3). Instead, all dogs demonstrated increased right-sided TA activity while maintaining the typical expiratory phasic cycling. All dogs also had coughing and swallowing in response to the supraglottic acid stimulation. Two dogs demonstrated spasm of the pharyngeal constrictor muscles. These responses were unique to the acid stimulation and did not occur in response to the saline wash. There were no changes in baseline activity noted after saline wash. At re-exploration of the neck all Int-SLNs were intact with preservation of the anastomosis. The epineural sutures could still be seen under magnification.

DISCUSSION

In 1974 Silver and Rosen[7] published their findings with regard to laryngeal sensory reinnervation. At that time, they were performing experiments with laryngeal transplantation, and they asked the question whether an anastomosed Int-SLN could provide airway protection to prevent pneumonia in these animals. They totally denervated the larynges of 10 dogs. An arytenoidectomy was performed in all 10 dogs to provide a glottic airway, and the Int-SLN was anastomosed in 4 dogs. The researchers evaluated the degree of lung consolidation at autopsy in long-term survivors (>5 mo). Three dogs of the reinner-

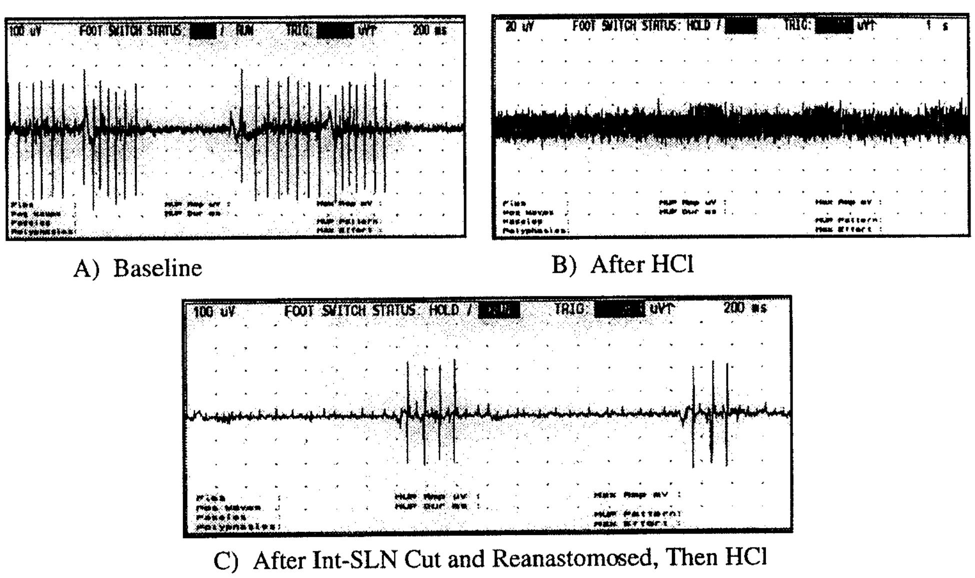

Fig. 2. Examples of study dog right TA spontaneous EMG. **A.** Normal expiratory phasic activity at baseline. **B.** Laryngospasm response to HCl. The EMG gain in this run is one fifth of that in the other runs. **C.** After Int-SLN cut there is no response to HCl.

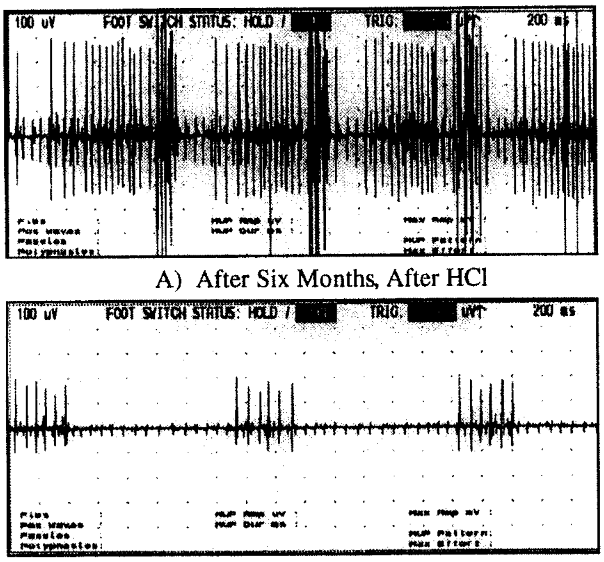

Fig. 3. Examples of study dog right TA spontaneous EMG 6 months after Int-SLN anastomosis. There is an increase in the normal expiratory phasic activity of the right TA after acid **(A)** as compared with saline **(B)**, but the dogs did not respond with a true laryngospasm response.

vated group survived and had 0 to 1+ consolidation of their lungs as measured on a scale of 0 to 4+ , with 4+ representing extensive gross consolidation. The denervated group had four long-term survivors that had lung consolidation ranging from 2 to 4+. The authors thought that sensory reinnervation had occurred and that aspiration was prevented in the reinnervated group.

In the last several years there has been renewed interest in laryngeal transplantation. Berke et al.[1] have outlined four criteria that must be fulfilled before human laryngeal transplantation. While ethical considerations continue to be pondered, successful revascularization and reinnervation of the motor supply have been achieved. Strome et al.[2] have written extensively on the development of an immunosuppressive regimen in the rat model. None of these studies, however, investigated the role of sensory reinnervation of the larynx.

Recent studies by Aviv et al.[8–10] have elucidated in vivo differences in laryngeal and hypopharyngeal sensory discrimination. They developed a technique to deliver a consistent puff of air to the laryngopharynx in normal controls and in stroke patients to identify deficits in sensation of the laryngopharynx. These studies have demonstrated appreciable decreases in laryngopharyngeal sensation in elderly patients and in patients after stroke and have suggested an association of sensory deficit with aspiration pneumonia risk. These findings also allow one to extrapolate that sensory reinnervation of the transplanted larynx would be important to restore airway protection to the patient.

Neurosensory free flaps have been extensively described in the reconstructive literature.[11–15] The sensation results are quite good, especially in transfers to the hand. Sensory flaps transplanted into the oral cavity and oropharynx have also been successful, but the role of sensory anastomosis in this area has been questioned.[16] All neurosensory tissue transfers contain cutaneous tissue, and no neurosensory *mucosal* free flaps exist. The role of mucosal sensory reinnervation in oropharyngeal and laryngeal reconstruction is unknown.

In the present study we attempted to demonstrate laryngeal sensory return after Int-SLN anastomosis. The control group had no return of laryngeal protective function. It is known that subglottic sensory afferents are mediated through the RLN.[4,17] In addition, microneural anastomoses between branches of the RLN and the SLN have been demonstrated on histological study.[18] In developing the present study, we were concerned that spontaneous reinnervation by the subglottic afferents would occur after Int-SLN denervation. The control group was included so that this phenomenon could be demonstrated if, indeed, it occurred. Because no control dog had return of a response to the noxious stimulus, we believe that no reinnervation took place from the RLN afferents.

Although all study group dogs had a response to the noxious stimulus, none had return of a true protective laryngospasm response. We hypothesize that reinnervation of the Int-SLN afferents may be incomplete or inappropriate. Reports have characterized the Int-SLN afferents as a heterogeneous population of nerve fibers and receptors.[3,19] Neuropeptides are distributed between calcitonin gene–related peptide, substance P, and leu-enkephalin.[19] Afferent nerve endings are responsible for the detection of a variety of sensations, including mechanical stimulation, thermal stimulation, pain stimulation, and a multitude of chemical sensations.[3] Furthermore, the majority of these nerve fiber endings are monomodal.[3] Sensory nerves do not become polymodal until they reach the level of the nucleus tractus solitarius, thereby implying convergence of sensation at this level. Reinnervation proximal to the nucleus tractus solitarius may result in misalignment. For example, a mechanoreceptor fiber may become directed to a chemoreceptor cell body. In addition, the absolute number of functional nerve fibers is likely to be reduced after Int-SLN section and anastomosis. This disordered and incomplete reinnervation may be responsible for the blunting of the laryngospasm response seen in our study. In addition, a longer recovery period may have allowed for more complete reinnervation. General anesthesia itself may have interfered with the measurement of the motor response; however, this is unlikely, since the reflex was universally recorded in all animals before nerve section.

It is well known that reinnervation of cutaneous sensation may provide the patient with dysesthesia, as well as blunted proprioception and two-point discrimination.[11] Brown et al.[15] commented that after neurosensory transfer of digits, training and relearning tasks play an important role in redevelopment of sensory pathways. Perhaps, with time, our animals would "relearn" the laryngeal protective response.

Even though all of our animals had a period after the first procedure without documented laryngopharyngeal sensation, none had any appreciable symptoms or signs of aspiration. In the sensory reinnervation study by Silver and Rosen,[7] approximately half of their dogs did demonstrate lung consolidation as a result of aspiration; however, these dogs also had total motor denervation and an arytenoidectomy to provide a large airway that allowed decannulation of their tracheotomy tubes. These additional procedures by themselves allow for an increased incidence of aspiration via the loss of mechanical airway protection. Although Loughlin et al. [6] demonstrated loss of a protective reflex on severing the Int-SLN, the level of chronic spontaneous aspiration in the sensory denervated dog is unknown. The horizontal position of the larynx in the dog results in a gravity vector perpendicular to its lumen as opposed to the parallel vector of gravity to laryngeal lumen in the human. In addition, the epiglottis abuts the soft palate in the dog but remains unopposed at rest in the human. These anatomical considerations may provide purely mechanical methods to prevent aspiration independent of sensation in the dog, and the lack of these may set up the human for aspiration.

CONCLUSION

Acid-induced laryngospasm is a suitable model for demonstration of an intact Int-SLN afferent pathway. After Int-SLN denervation there is no return of a protective laryngospasm reflex to a noxious stimulus at 6 months. After Int-SLN denervation, anastomosis of the Int-SLN allows for successful sensory reinnervation. Sensory reinnervation occurs in an incomplete or disordered fashion. In the canine there is no apparent negative outcome (i.e., pneumonia) as a result of laryngeal sensory denervation.

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