Evaluation of Functional Recovery of Recurrent Laryngeal Nerve Using Transoral Laryngeal Bipolar Electromyography: A Rat Model

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Objectives: We developed a standardized method of minimally invasive transoral laryngeal (ToL) bipolar electromyography (EMG) for evaluation of recurrent laryngeal nerve (RLN) recovery after a controlled crush injury in a rat model.

Methods: Ten 200- to 250-g Sprague-Dawley rats underwent a controlled crush injury to the left RLN performed with 60 seconds of use of a calibrated aneurysm clamp with a closing force of 0.61 N. Serial ToL bipolar EMG was performed on adductor muscles and the posterior cricoarytenoid muscle during spontaneous vocal fold motion under anesthesia. Each animal underwent ToL EMG immediately after surgery and 1, 3, and 6 weeks after surgery.

Results: The EMG signals showed normal motor unit potentials and recruitment patterns 3 weeks after crush injury. Endoscopic evaluation of vocal fold mobility yielded consistently normal findings 6 weeks after crush injury.

Conclusions: We have developed a standardized method of crush injury to the rat RLN model and a minimally invasive transoral bipolar spontaneous EMG technique to serially evaluate and follow nerve injury and recovery in rats. This model is intended to simulate intraoperative RLN injury, to elucidate the electrophysiological events that occur during nerve recovery, and to form the basis for studying agents to enhance such recovery.

Key Words: animal model, crush injury, rat, recurrent laryngeal nerve injury, transoral electromyography.

INTRODUCTION

The recurrent laryngeal nerve (RLN) can be injured during surgery from thermal damage, stretching, cutting, compression, or vascular compromise. Most iatrogenic injuries of the RLN are not recognized at the time of injury. The recovery from RLN insult is unpredictable at best. Therefore, in order to study the pathobiology of RLN injury and induce functional recovery, an experimental animal model is necessary. Rats have been utilized as popular experimental animal models for nerve injury studies because of their low cost, availability, and ease of handling. Studies using the rat larynx as an experimental model are uncommon. However, the rat larynx model has been used as a feasible method of studying RLN injury and recovery.

Because of the variability of inducing injury to the RLN with reproducible results, the need for an experimental model is paramount. Several animal models have been developed to evaluate crush injury to peripheral nerves. The crush injury is commonly performed with jeweler’s forceps or a serrated hemostat. Many authors refer to a “standardized” crush injury without mentioning the chosen standards. Such a standard should include the number, intensity, and duration of crushes. Aneurysm clips have been used as a reliable “standard” method to perform crush injury to peripheral nerves.

Laryngeal electromyography (EMG) in experimental animals has required invasive surgical procedures to gain access to the laryngeal muscles. We have developed a minimally invasive endoscopic method to perform transoral bipolar EMG in a rat after a standardized crush injury to the RLN. Transoral laryngeal (ToL) EMG has enabled us to perform spontaneous EMG recordings that allow the evaluation of the electrophysiological changes that correspond to acute RLN injury. The technique per-

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Presented as a poster at the meeting of the American Laryngological Association, San Diego, California, April 26-27, 2007. Awarded second-place prize.

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MATERIALS AND METHODS

Experimental Animals. Ten female Sprague-Dawley rats weighing 200 to 250 g were used to validate the endoscopic transoral EMG and perform crush injuries. Project approval was obtained by the New York Medical College Animal Research Committee, and all animals were maintained in a facility approved by the National Academy of Science and the National Society for Medical Research. Humane care was provided for all animals, and all institutional and national guidelines were observed.

Surgical Procedures. The animals were sedated with isoflurane and then anesthetized with an intramuscular injection of a mixture of ketamine hydrochloride and xylazine hydrochloride (1:1). A final dosage of 45 mg of ketamine hydrochloride per 100 g body weight and 0.9 mg per 100 g body weight of xylazine hydrochloride plus 1 mg/kg acepromazine maleate produced sufficient anesthesia. The animals were held in a stereotactic apparatus, a vertical midline neck incision was performed, and the strap muscles were separated. The left RLN was carefully identified in the tracheoesophageal groove with a dissecting microscope. The nerve was crushed for exactly 60 seconds at the level of the seventh tracheal ring with a commercially available Sugita calibrated aneurysm clamp with 0.61 N closing force (Mizuho Ikakogyo, Tokyo, Japan). The strap muscles and overlying fascia were closed with 4-0 chromic suture material, and the skin was reapproximated with 5-0 nylon sutures.

Transoral Laryngeal EMG. All animals underwent ToL EMG during spontaneous, unevoked respiratory cycles under anesthesia. The animals were placed supine on a modified stereotactic operating table with a 15° incline in a modified Faraday cage room. The tongue was retracted with a 3-0 silk suture placed midline in the anterior third of the tongue and suspended. A moistened conductive strap that was placed around the operator's forearm and a clip that was placed on the rat with conductive gel served as ground electrodes. A 0° Storz operating endoscope with a newly developed epiglottis elevator was transorally inserted to visualize the endolarynx (Fig 1). The elevator was fashioned from the tip of an Endoscope endoscope sheath (Medtronic Xomed, Jacksonville, Florida). With insertion of the tip of the elevator into the vallecula, excellent visualization of the glottis was obtained. Vocal fold movements were graded on a continuous scale of 0 to 4 before performing each ToL EMG. A score of 0 was given for an immobile vocal fold, and a score of 4 was given for full and vigorous adductor and abductor movement. The right vocal fold movement was used as a subjective control to define normal vocal fold motion. The scoring was performed by 2 separate observers. The mean score of the 2 observers was used as a final score.

The ToL EMG recordings from the adductor muscles and the posterior cricoarytenoid muscle were performed with a 45-mm-long, 25-gauge bipolar needle electrode. Because of difficulty in obtaining consistent signals with the bipolar needle, we successfully changed the electrode to a 45-mm-long, 25-gauge quadrifilar needle electrode11 modified to record bipolar signals. Filter settings of 20 Hz to 10 kHz and a sweep speed of 10 ms per division were used. Four animals were used to identify the best electrode and standardize the insertion points that consistently elicited the strongest signals (Fig 2). Transoral laryngeal EMG was performed immediately after nerve crush and at 1, 3, and 6 weeks after the operation.

Recordings from the posterior cricoarytenoid
muscle were obtained from the rat larynx during spontaneous, unevoked phasic respiratory cycles. Adductor muscle recordings were obtained during moments of spontaneous adduction due to coughing or swallowing.

RESULTS

All experimental animals had no vocal fold movement (grade of 0/4) immediately after RLN crush injury. At week 3, all vocal folds had a moderate recovery and were graded 3/4. Normal vocal fold motion (4/4) was observed in all animals 6 weeks after crush injury (Fig 3).

The EMG signals recorded from the right side were used as a control for each time point (Fig 4). Immediately after crush injury, complete EMG silence was noted in all animals when ToL EMG was performed. On day 7, the EMG recordings had polyphasic motor unit action potentials with an intermittently normal recruitment pattern. Three weeks after crush injury, the EMG signals had normal motor unit action potentials and a normal recruitment pat-

Fig 2. A) EMG electrode insertion points for measuring consistent strong signals from posterior cricoarytenoid (lower stars) and thyroarytenoid (upper stars) muscles of rat larynx. B) Artist's rendition.

Fig 3. Normal vocal fold movement visualized with modified 0° endoscope with epiglottis retractor. A) Vocal folds completely abducted. B) During adduction.
tern despite abnormal vocal fold movement (graded 3/4). At 6 weeks after crush injury, all animals had EMG signals and vocal fold movement similar to those of the control side (Fig 5).

DISCUSSION

Traditionally, rat laryngeal EMG in experimental animals has been performed via a midline cervical incision to gain access to the adductor muscles or the posterior cricoarytenoid muscle. Transoral endoscopic laryngeal EMG of the human larynx was initially performed by Thumfart et al using a bipolar hooked-wire electrode or needle electrodes. Inagi et al developed a specialized laryngoscope and operating platform in an attempt to perform selected transoral EMG recordings from a rat larynx and measure vocal fold movements. In the present study, we developed and standardized a minimally invasive method to perform transoral EMG by use of an operating endoscope fitted with an epiglottis retractor to expose the endolarynx. The current mode has several advantages compared to previous attempts in performing transoral EMG on the rat larynx. It is rapid, economical, minimally invasive, and easily reproducible, although there is a steep learning curve, especially if the investigator has not worked with an operating endoscope. Moreover, each procedure can be recorded with a camera attached to the endoscope for blinded evaluation and comparison of vocal fold movement as nerve recovery continues.

Compression of nerve fibers can cause various clinical symptoms depending on the cause, magnitude, and duration of the compression trauma. It is often difficult to analyze the results obtained by investigators on nerve compression injuries, owing to differences in the method of pressure application and inconsistent pressure levels with different recovery periods. This inconsistency creates confusion in replication of the data, and the method does not lend itself to testing various neuroprotective agents. In the present study, we overcame these problems by using a specially designed and commercially available device. Aneurysm clips have been used to produce quantitative crush injury to the rat sciatic and optic nerves. The advantage of utilizing aneurysm clips to perform crush injury is the ability to apply a specific force over a limited area for a defined duration.

Controlled crush injury to peripheral nerves can produce neurapraxia or axonotmesis, depending on the force used. Chen et al showed that application of a crush force of up to 1 N to the rat sciatic nerve caused neurapraxia only. The rat RLN is significantly smaller in caliber than the sciatic nerve. The lack of consistent fibrillation potential in our EMG recordings supports neurapraxia with partial axonal injury when a standardized force of 0.61 N is applied to the rat RLN.

This report concentrates on the techniques we developed for achieving controlled nerve injury and transoral EMG recordings in this animal model, which is a necessary requirement for applying this model to controlled RLN injury studies. Our next report will describe quantitative EMG findings ob-
tained after crush injury in a much larger population, with the goal of substantiating this animal model for comparing crush-related RLN injuries that occur in humans.

In this study, our purpose was to reliably demonstrate the ability to obtain EMG signals in an animal model that are associated with a controlled crush injury. The definition of functional recovery was based on endoscopic observations of vocal fold movement obtained after crush injury in a much larger population, although quantitative EMG measurements were not used to show neuroaxonal recovery, we have highlighted some features observed in our data, such as polyphasic potentials, that are consistent with axonal recovery in humans.

Although animal models may not duplicate all types of neural injuries seen in humans, the model enables investigators to follow the electrophysiological changes that occur after specific injuries to the RLN. Moreover, in future studies, the effects of targeted therapy for laryngeal reinnervation after controlled injuries to the RLN can be followed from the acute to the chronic phase by using this model.17,18 It is important to note that the degree of nerve injury is dependent on several factors, one of which is the force applied during the crush. If the injury to the nerve is not predictable, the results cannot be studied to draw a meaningful conclusion. Our model allows for consistency in the injury, as well as the predictability of recovery of function.

**CONCLUSIONS**

We have developed and validated a standardized, minimally invasive endoscopic ToLEMG technique to perform serial recordings from the rat larynx to evaluate RLN injury and recovery. A quantifiable crush injury to the RLN was performed with a commercially available Sugita aneurysm clip with a calibrated closing force of 0.61 N causing neurapraxic injury. We were able to successfully follow the recovery from this RLN injury with serial EMG recordings.

The above-described ToLEMG animal model is a rapid, economical, noninvasive, and easily reproducible method of obtaining serial EMG recordings from the rat larynx and is well suited to studies consisting of a large number of animals and testing neuroprotective agents for their potential to prevent peripheral neuropathies.

**REFERENCES**

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