Role of motor unit number estimate electromyography in experimental canine laryngeal reinnervation

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Laryngeal electromyography has been used clinically to differentiate neuromuscular pathology from other causes of vocal fold immobility such as arytenoid dislocation, tumor invasion, or cricoarytenoid joint fixation. Electromyography has also been used to predict the prognosis for nerve recovery in laryngeal paralysis. Existing electromyographic techniques either record activity with voluntary motion or study nerve conduction. In this study a new technique, motor unit number estimation, a commercially available quantitative method of electromyographic analysis, is used to study the progress of recovery of vocal fold function after recurrent laryngeal nerve injury. Four dogs underwent transection and immediate reanastomosis of selected branches of the adductor and abductor branches of the recurrent laryngeal nerve on 1 side; the opposite side served as a control. Baseline electromyographic and videolaryngoscopic studies were performed. These measures were then repeated in a longitudinal fashion every 6 weeks after denervation. The motor unit number estimation technique indicated a return of motor unit numbers with time, along with estimates of their size. This was consistent with the expected progress of laryngeal reinnervation. These data and their predictive value for nerve recovery will be discussed. (Otolaryngol Head Neck Surg 1999;121:180-4.)

The treatment of laryngeal paralysis has advanced substantially during the past decade. Therapies include the use of temporary or reversible procedures for early paralysis, when recovery is possible. It would be helpful to predict the chance of recovery in laryngeal paralysis because it would allow earlier definitive treatment. When recovery is unlikely, a more permanent technique such as type I thyroplasty can be performed.

Electromyography (EMG) has been used to follow the status of recovery from paralysis in the larynx and has been shown to give some prognostic information. One EMG method that has not been previously applied to the larynx is motor unit number estimation (MUNE),[1] which was first described in 1971.[2] This application is based on graded increases in electrical stimulation, which evoke incremental responses in the muscle. These incremental responses represent the activation of single motor units. An initial compound muscle action potential is recorded by supramaximal stimulation. The size of the single motor unit is averaged in different ways, but often by a technique of multiple trials at varying levels of submaximal stimulation. The size of the compound muscle action potential is then compared with the average size of the single motor unit, to estimate the total number of motor units within the recorded neuromuscular unit.[1,3,4] The number of motor units changes with aging and various neuromuscular disorders.[5] The activity of the motor units would be expected to decline to 0 at the time of denervation, with recovery after reinnervation.

A commercial software system is now available that uses the MUNE technique. This study was undertaken to compare the timing and findings of the MUNE technique with physiologic, clinically apparent signs of reinnervation. We propose that this method of estimating motor units has potential application in the larynx and may add a more quantitative method of following the progress of laryngeal reinnervation.

METHODS AND MATERIAL

Experimental Design

Four canines were included in this study. Two dogs underwent evaluation of the right and 2 of the left recurrent laryngeal nerve (RLN) and thyroarytenoid (TA) and posterior cricoarytenoid (PCA) muscles. The animals underwent videolaryngoscopy and electromyographic evaluation with surface and/or concentric needle electrodes. The adductor and abductor branches of the RLN on the experimental sides were then dissected out, transected, and reanastomosed. At 6, 12, and 18 weeks after reanastomosis, the animals underwent repeat MUNE and videolaryngoscopy assessment. During the final

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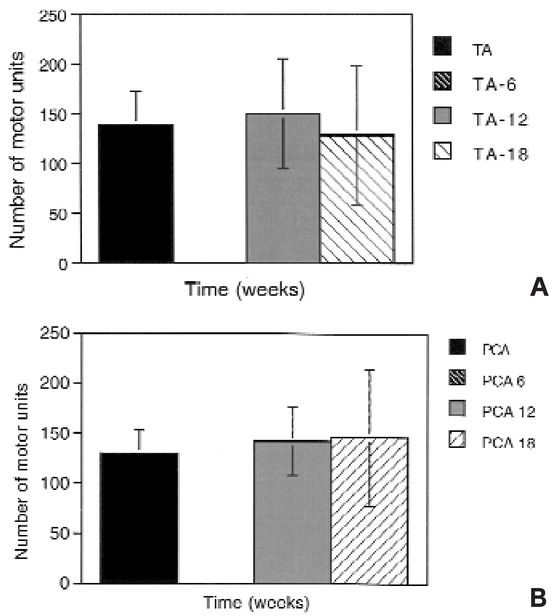

Fig 1. Number of motor units versus time for the TA muscle **(A)** and PCA muscle **(B);** surface electrodes were used.

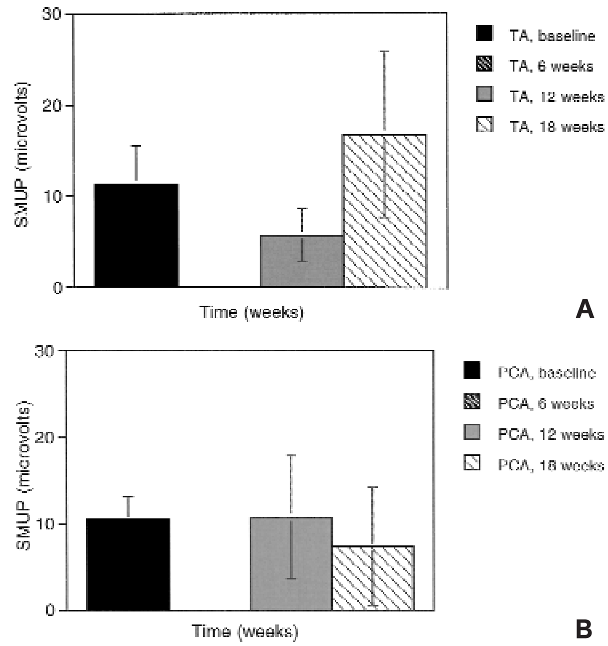

Fig 2. SMUP in microvolts versus time is indicated for the TA muscle **(A)** and PCA muscle **(B);** surface electrodes were used.

experiment, the animals also underwent evoked phonation trials with videostroboscopy and acoustic analysis.

Surgical Technique

This study was performed in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 USC et seq). The animal use protocol was approved the institutional animal care and use committee of the University of California, Los Angeles.

Each dog was anesthetized with intravenous pentobarbital. The dog was then placed supine on an operating table, and direct laryngoscopy was performed to confirm normal laryngeal anatomy. The dog was then intubated with 7.0 endotracheal tubes (ETTs) under direct visualization. The animal was kept under general anesthesia with inhalation of halothane in the range of 1% to 1.5%, with 100% O_2 at 4 L/minute. Once the dog was under adequate anesthesia, he was shaved, prepared, and draped in a sterile fashion.

An approximately 6-cm midline vertical incision was made just above the sternal notch. The strap muscles were separated along the median raphe and retracted laterally. The cervical trachea was exposed, and a low tracheostomy incision made 2 or 3 rings above the sternal notch. A 7.0 ETT was placed through the tracheostomy, the balloon was inflated, and the ventilator was connected to this tube. The balloon on the first ETT was deflated, and the tube was removed. The RLN was identified on 1 side. Once the nerve was isolated, 2 silver-disk electrodes were placed over the RLN, in sequence, approximately 1 cm apart. Overlying strap muscles were used as necessary to stabilize the position of the electrodes by suturing the muscles together over the electrode.

Attention was turned to the oropharynx and larynx. The dog's mouth was opened with a mouth guard, and the epiglottis was suspended by placing a suture attached to a washer through the epiglottis and the neck skin. Video data were collected by positioning the endoscope a fixed distance from the vocal cords while the following tasks were performed: tube occlusion stimulating respiratory dyspnea, pharyngeal stimulation, and nerve stimulation. A centimeter ruler was videotaped at the level of the glottis to use for image calibration for subsequent analysis.

EMG

A silver-disk electrode was placed along 1 true vocal cord to record the TA muscle. The ground electrode was tucked into the vallecula. The MUNE program was then run. The recording electrode was repositioned over the PCA muscle, and the protocol was repeated. Additional trials were completed with use of concentric bipolar electrodes placed directly into the experimental muscles.

Reinnervation

These dogs then underwent transection of the anterior and posterior branches of the RLN on the side of study, with immediate microvascular reanastomosis. Four sutures of 9-0

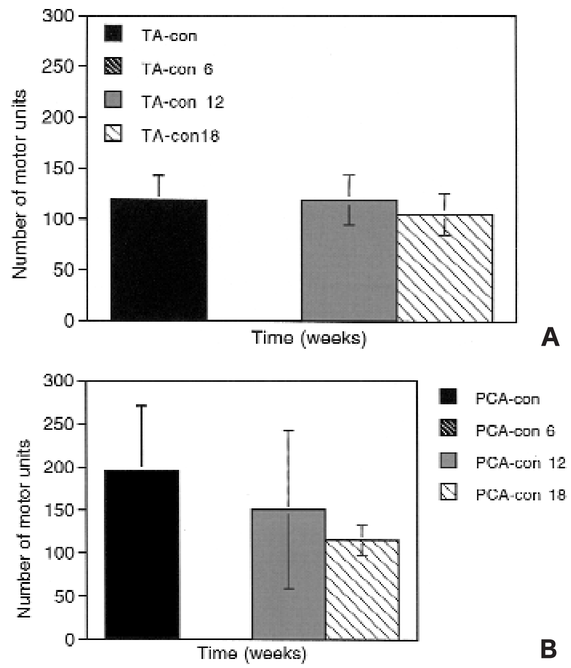

Fig 3. Number of motor units versus time for the TA muscle **(A)** and PCA muscle **(B);** concentric needle electrodes were used.

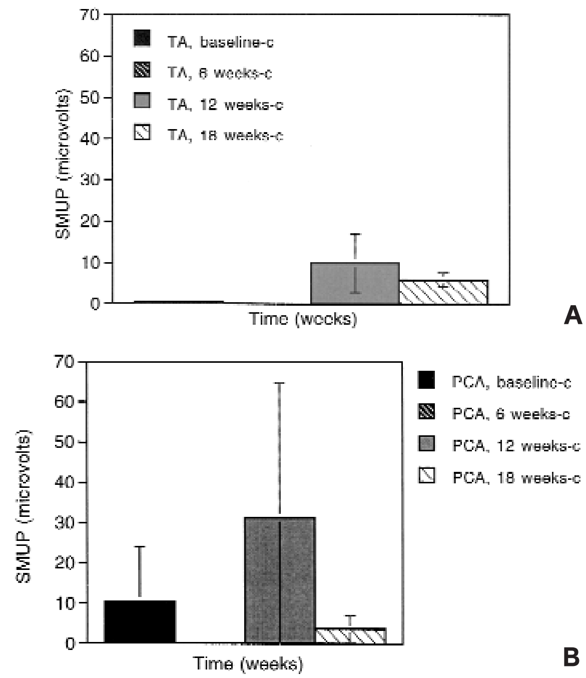

Fig 4. SMUP in microvolts versus time is indicated for the TA muscle **(A)** and PCA muscle **(B);** concentric needle electrodes were used.

nylon were used for each nerve branch. The wounds were closed, and the dogs recovered from anesthesia.

Interval Evaluation

The dogs were brought back at intervals of approximately 6, 12, and 18 weeks, at which time electrodes were positioned as described and the MUNE protocol was run. Video data were collected with repeat calibration. At the final experiment, videostroboscopy data were also collected with electrically induced phonation of the animal. The anterior and posterior branches of the RLN on the experimental side were dissected out and placed in neural fixative. The TA and PCA muscles were isolated and fast frozen.

Equipment

Silver-disk surface electrodes (Grass Instrument Co, Quincy, MA) were used both for stimulating and as receiving electrodes. Concentric bipolar needle electrodes (26 gauge, with a recording area of 0.068 mm^2; Nicolet Biomedical, Madison, WI) were also used as receiving electrodes in separate trials to compare surface to needle recording techniques. Nicolet Viking IV software (Nicolet Biomedical) was used to do the MUNE protocol, as described in the manual. For video-laryngoscopy, a 0-degree Storz endoscope was placed through the mouth and above the vocal cords. A video camera was used to record images on a Sony U-matic videocassette recorder (model VO-9850; Sony, Tokyo, Japan). Analysis was performed with ImagePro for Windows 3.1 software (Media Cybernetics, Silver Spring, MD) on a Zeos 486 personal computer, with a FlashPoint ISA (version 2.0; Integral Technologies, Indianapolis, IN) color frame grabber. Measurements were made of vocal cord excursion under the given conditions, and the relative excursion of each side was compared against the midline.

Statistical Analysis

Statistical analysis was performed with 2-way analysis of variance (ANOVA), muscle by time, with repeated measures on muscle at each time interval. Experimental muscles were compared within and between time. In addition, a 3-way ANOVA, type of electrode by muscle by time, with repeated measures was performed. Electrodes were compared within and between time and within and between muscle. Data were analyzed with BDMP statistical software (BDMP Statistical Software, Los Angeles, CA).

RESULTS

MUNE

The results of the MUNE assessment with surface electrodes are shown in Fig 1. The averaged results show a baseline number of motor units of 139 ± 34.12 for the TA muscle and 130.5 ± 22.75 for the PCA muscle. At the second assessment, there were no signals, and therefore no motor units were recorded. By 12 weeks, the number of motor units estimated was 150 ± 55.27 for the TA and 142.75 ± 33.90 for the PCA. At 18

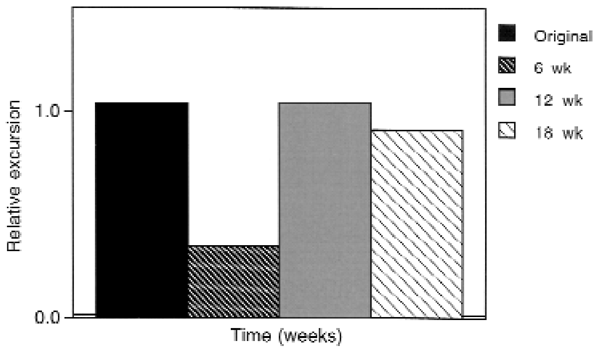

Fig 5. Relative excursion of the vocal fold versus time is indicated. A value of 1 indicates that the excursion on the experimental side is equal to that of the control side.

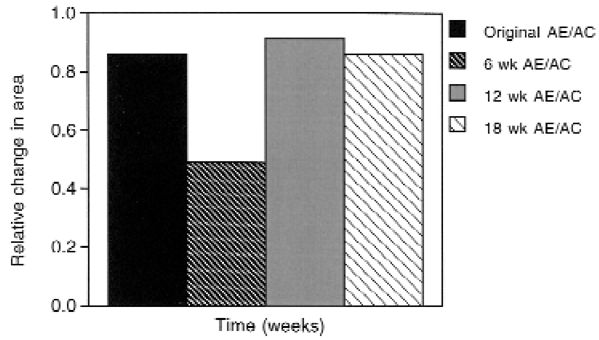

Fig 6. Relative change in the area of the glottal opening was measured on digitized video images and plotted versus time. The area measured is an indication of glottal dilation during respiratory effort.

weeks the TA and PCA demonstrated 128.4 ± 71.30 and 146.4 ± 68.23 motor units, respectively. These numbers analyzed for each animal, muscle by time, showed no statistically significant difference.

The averaged sizes of the single motor unit potentials (SMUPs) for the surface electrodes at the baseline study were 11.33 ± 4.16 µV for the TA and 10.5 ± 2.65 µV for the PCA (Fig 2). Again, there was no recordable signal at 6 weeks. At 12 weeks the single motor units were estimated to be 5.75 ± 2.9 µV for the TA and 10.8 ± 7.1 µV for the PCA. The final assessment yielded single motor units for the TA and PCA of 16.8 ± 9.12 µV and 7.4 ± 6.9 µV, respectively. There was a statistically significant difference between muscles across time, analyzed for each animal ($P < 0.05$, $F_{1,3} = 11.5$).

Concentric needle trials show a baseline of 119 ± 24 motor units for the TA and 196.5 ± 74.3 for the PCA muscle. There were no recordable signals at 6 weeks, and at 12 weeks there were 119 ± 24.6 and 150.5 ± 91.7 motor units for the TA and PCA muscles, respectively (Fig 3). At 18 weeks there were 105 ± 20.6 motor units for the TA and 114.8 ± 18.2 for the PCA. These results analyzed for each animal also were not statistically different.

With concentric needle electrodes, the baseline SMUPs were 0.5 ± 0.7 µV for the TA and 10.5 ± 13.4 µV for the PCA. No units were recorded at 6 weeks. At 12 weeks, the SMUPs were 10 ± 7.2 µV for the TA and 31.3 ± 33.4 µV for the PCA (Fig 4). At 18 weeks the motor units were 6 ± 1.7 µV for the TA and 3.6 ± 3.4 µV for the PCA.

Excursion/area

Comparisons were made of the movement of the experimental versus the control sides with maximal abduction under conditions of obstructive respiratory dyspnea. The results demonstrate an equal excursion at the initial assessment, before surgical transection of the RLN (Fig 5). At 6 weeks the experimental excursion represented 34.5% of the control side. By 12 weeks the excursion reached an equal level versus control and maintained this level at 18 weeks.

The change in area of the experimental versus control sides under the same conditions showed a change in area on the experimental side of approximately 86% of control at initial study (Fig 6). This dropped to 49% at 6 weeks and increased to 91% and 86% at 12 and 18 weeks, respectively.

DISCUSSION

The current preliminary study examined the use of an automated, commercially available software system that provided estimates of motor units. If the MUNE proves to correlate with functional recovery, it could allow the laryngologist to predict the outcome in acute laryngeal paralysis.

The estimate was consistent for each muscle and across animals. The total number of motor units for the TA and PCA were in the ranges of 119 to 130 and 130 to 196, respectively. This correlates well with histologic findings: estimates of the number of axons found in distal branches (adductory and abductory branches) of the RLNs in canines from a previous study in this laboratory[6] give a range from 143 to 370 for each branch. Previous reports in the literature have given the number of myelinated axons in normal RLNs to be around 403 to 421 in the cat,[7] with 237 to 713 axons found in reinnervated or reanastomosed nerves, using a branch of the phrenic nerve to the distal RLN stump. The number of axons in the RLN would be expected to be greater than that estimated in evaluating the branches of the nerve to only 2 laryngeal muscles.

The recovery of the number of motor units in this study correlated with the functional recovery of vocal cord mobility as demonstrated by video assessment of excursion. The timing of neural recovery, both by EMG and functionally, occurred within the 6- to 12-week window. The larynx was not studied often enough to compare the precise onset of EMG and functional recovery, but we can say that it occurs between 6 and 12 weeks with a distal anastomosis. Further study should better resolve the timing of recovery in laryngeal reinnervation. In a planned follow-up study, MUNE will be used to compare RLN function after a permanent nerve injury (transection) and a crush injury (to allow complete nerve recovery). It is possible that MUNE is an all-or-none phenomenon and that no stepwise increase in the number of motor units can be verified after laryngeal reinnervation.

The size of the single motor units was different between muscles, and this difference was parallel across time. For the TA, the motor units recorded at 12 weeks were about half the size of original units, consistent with early reconnections of nerve to muscle fibers before terminal sprouts of nerves have had time to reconnect to their full quota of fibers. At 18 weeks the sizes of the units were back up to and slightly higher than baseline, suggesting essentially complete reinnervation and reshaping of motor units by this time. For the PCA muscle, both the size and number of motor units were back to baseline at 12 weeks, suggesting faster reinnervation for this muscle. Comparison of the different types of electrodes demonstrated no statistically significant difference between the estimates. The consistency with different electrodes is important to allow clinical application of this technique. Placement of surface electrodes would require general anesthetic, whereas use of concentric needle electrodes might be possible in a clinic setting. Before human applications of this technique, methods of nerve stimulation in awake patients will need refinement. Although the neuroanatomy of the larynx is similar in canines and human beings, the relative number of motor units has not been compared directly. Therefore some caution in applying these results to human beings is reasonable.

In conclusion, the MUNE technique correlates with functional recovery after laryngeal denervation with reanastomosis. Further study is necessary to determine whether this technique is capable of documenting a graded increase in the number of motor units with the recovery of laryngeal function after paralysis.

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