Abstract—The present study examined the effects of functional neuromuscular stimulation (FNS) on reinnervation of the posterior cricoarytenoid (PCA) muscle. In each animal, the recurrent laryngeal nerve (RLN) was sectioned and anastomosed, and a patch electrode array implanted for stimulation and recording at selected PCA sites. Following implantation, FNS was applied to two canines for a period of six weeks. Two additional animals served as nonstimulated controls. In each animal, the magnitude and time course of reinnervation was monitored by recording EMG potentials evoked by RLN stimulation. The appropriateness of reconnection was determined by the pattern of spontaneous EMG activity and recovery of vocal fold abduction. Results of this preliminary study indicated that FNS caused an overall repression of reinnervation. However, the major effect was to preferentially inhibit reconnection by foreign nerve fibers, thereby promoting selective reinnervation and preventing synkinesis.

Index Terms—functional neuromuscular stimulation, posterior cricoarytenoid muscle, reinnervation, paralysis, laryngeal pacing, electrotherapy

I. INTRODUCTION

Functional neuromuscular stimulation (FNS) has been proposed as a potential treatment to restore activity to the denervated larynx. FNS also produces a direct beneficial effect on muscle by preserving contractile strength, maintaining muscle mass, and preventing atrophy.1,6,9,11,12 However, its effect on the course of reinnervation remains controversial. Classical studies have indicated that neuromuscular stimulation inhibits sprout formation and the elaboration of extrajunctional receptors following denervation.2,3 Consequently, a deleterious effect on the actual course of muscle reinnervation would be expected. No such impairment has been demonstrated and in fact, there is evidence to suggest that FNS may enhance the onset and extent of reinnervation based on indices of motor recovery.1,4,5,7-10

The purpose of the present investigation was to determine the effects of FNS on reinnervation by assessing the extent and appropriateness of neuromuscular reconnection, and recovery of motor behavior in the canine larynx.

II. MATERIALS & METHODS

This study was limited to an investigation of the paired posterior cricoarytenoid (PCA) muscles, the abductors of the vocal folds. In each of four canines, the left PCA was denervated and reinnervated through recurrent laryngeal nerve (RLN) section and anastomosis. The opposite PCA served as an innervated control. A prefabricated system was implanted in each animal which included a nerve stimulus cuff, a muscle patch containing 36 electrodes, and a skull-mounting receptacle for making external connections.13

The functional status of the PCA muscle and its stage of reinnervation was assessed chronologically in each animal. In each session, monopolar stimuli were delivered at each electrode to establish a map of PCA sites effectively producing abduction. Electromyographic (EMG) recordings were then obtained at each PCA site during RLN stimulation. The amplitude of evoked potentials (EPs) recorded across all these muscle sites was averaged to give a representative index of reinnervation. Spontaneous EMG activity during respiration could also be recorded at each site to characterize the quality or appropriateness of neuromuscular reconnection without synkinesis.

A pacing circuit was encased in a box with an interface plug constructed complementary to the skull receptacle. Two animals (E1 and E2) were electrically paced for a period of 6 weeks using the paradigm: 1 sec pulse train, 2 msec duration pulses, frequency of 30 Hz, and amplitude of 6-8 mA delivered every 7 seconds. Two animals (C1 and C2) served as nonpaced controls. The effects of chronic PCA stimulation were assessed over a period of five months. Data from E1 was excluded from analysis. This animal showed no reinnervation over the entire study because of nerve compression by a dislocated cuff, discovered in the terminal session.

It was anticipated that differences in the level of PCA reinnervation observed among animals might simply reflect variation in the extent of regeneration across the anastomotic site following nerve repair. The total number and diameters of regenerated myelinated axons were measured in each nerve using histomorphometric analysis of electron micrographs (EM).
III. RESULTS

Reinnervation was delayed in the FNS animal.
The onset of PCA reinnervation occurred at four and six weeks in the control animals as evidenced by the appearance of EPs and vocal fold abduction. In contrast, the experimental animal (E2) showed delayed reinnervation with the first indication of evoked EPs and abduction at 10 weeks.

RLN regeneration was greatest in the FNS animal.
Measurements taken from EM cross sections of the RLN showed no difference in the mean or standard deviation of axon diameters between control and experimental animals. However, the nerve from E2 contained twice as many regenerated axons (845) as the nerves from the control animals, C1 and C2 (409 and 446, respectively).

Reinnervation was least in the FNS animal.
Figure 1A shows EP recordings (arrows) taken from the FNS animal and one of the controls (C1) during the terminal session. The reduced amplitude of the EP in the FNS animal suggested that chronic neuromuscular stimulation had repressed PCA reinnervation.

Evoked EMG activity was sampled at multiple sites along the rostral-caudal axis of each muscle. In figures 1E-G, the EPs recorded were plotted against electrode position to produce a chronological series of response curves for each animal. The gradual upward shift of the curve over time reflected an overall increase in the level of reinnervation. For a given test session, the EPs could be averaged across muscle sites to compensate for differences in recording conditions and give a numerical index of reinnervation. The final index of reinnervation in C1 and C2 (2.448 +/- .762 mV and 2.937 +/- .884 mV, respectively) was much larger than that of E2 (.601 +/- .242 mV). Post hoc analysis in the form of a Dunn test confirmed that reinnervation in E2 was significantly less than reinnervation in either C1 (p=.0056) or C2 (p=.0013).

Synkinetic EMG activity was present in nonFNS animals but absent in the FNS animal.
In figures 1B-D, spontaneous EMG activity from the normally innervated (upper trace) and reinnervated (lower trace) PCA obtained from E2 and C1 during the terminal session can be compared. In deep plane of anesthesia (1B), characteristic inspiratory bursts were recorded from all muscles. In moderate plane of anesthesia (1C), the control animal began to show evidence of expiratory activity from the reinnervated PCA with some repression of inspiratory unit firing. No expiratory units were detected on the control side. In the FNS animal, there was no evidence of expiratory unit activity in either muscle at this level of anesthesia. In light plane of anesthesia (1D), the control animal showed EMG activity characteristic of complete laryngeal synkinesis. Motor units in the reinnervated PCA fired throughout the expiratory phase but no activity was detected during inspiration. In the experimental animal, recordings from both PCAs still showed modulation of activity with inspiration only. In summary, a synkinetic pattern of EMG activity emerged in lighter planes of anesthesia in the control animal, while reinnervation activity in the experimental animal always mirrored that on the normal side, irrespective of the plane of anesthesia.

Synkinetic vocal fold motion was present in the nonFNS animals but absent in the FNS animal.
Initially, C1 and C2 were distinguished from the experimental animal in that they exhibited near-normal inspiratory abduction in deep anesthesia. However, by four weeks post reinnervation onset, the magnitude of abduction began to wane in the control animals apparently due to aberrant reinnervation. Synkinesis in vocal fold motion became more pronounced with time in the control animals such that abduction had disappeared by the terminal session. In contrast, abduction continued to increase in the experimental animal throughout the duration of the study.

In general, inspiratory vocal fold abduction increases as the plane of anesthesia is lightened. In reference to figures 1B-D, there was no vocal fold movement associated with the EMG activity shown for the control animal at any plane of anesthesia. The lack of abduction in deep anesthesia was attributed to antagonistic inspiratory activity of misreinnervated adductor muscles since expiratory units had not yet been recruited. In lighter planes of anesthesia, aberrant expiratory activity in the PCA, as well as inappropriate co-contraction of adductor and abductors, could have accounted for synkinesis. In contrast, E2 showed abduction at all planes of anesthesia, which ranged from 17-52% of normal vocal fold excursion. Although the muscle was paretic, vocal fold movement always paralleled that on the innervated side and contributed to glottal opening.

IV. DISCUSSION

The results of this preliminary study suggest that chronic neuromuscular stimulation of a denervated muscle interferes with neuromuscular reconnection. Although nerve regeneration was twice as great in the experimental animal, the final level of reinnervation was only 1/4 that of the controls. At face value, this observation would seem to discourage clinical use of FNS. However, these findings also suggest that the interference by FNS is selective, and preferentially opposes reinnervation by foreign nerve fibers. The aberrant pattern of EMG activity and loss of vocal fold abduction in the control animals could be attributed to misdirected reinnervation of muscle fibers. In contrast, the appropriateness of EMG activity and progressively increasing abductions in the chronically stimulated animal supports the hypothesis that FNS exerts a selective influence on reconnection. Further studies are in progress to test this hypothesis in a statistically significant number of animals.

V. REFERENCES


FIGURE LEGEND

FIGURE 1: EMG recordings from a control (nonFNS) animal and the experimental (FNS) animal. (A) Evoked Potentials. (B-D) Spontaneous EMG activity from right (upper trace) and left (lower trace) PCA recorded at sequentially lighter planes of anesthesia (top to bottom). An isolated motor unit can be identified by spike activity that is constant in amplitude. In deep plane of anesthesia (B), isolated inspiratory motor units were recorded from the reinnervated PCA of both C1 and E2. In figure C, activity of each inspiratory unit was still present, but a large expiratory unit was also recruited in the nonFNS animal. In figure D, the expiratory unit showed enhanced firing as the inspiratory unit was derecruited. Chronological series of EP response curves: (E) C1, (F) C2, and (G) E2. Sessions were conducted at approximately the same time point for each animal, and were represented by a common symbol. The high reproducibility of the EP at a given electrode site was indicated by small open circles in graphs E and F. Each array was also stable over the entire course of investigation. The contour of a curve, reflecting differences in the recording conditions at electrode sites, was retained in subsequent sessions for the same animal. In E2 (G), values were greater than zero at every electrode site beginning at 73 days.