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Multiple Motor Unit Recordings of Laryngeal Muscles: The Technique of Vector Laryngeal Electromyography

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Objectives: **To display time-series firing rate and recruitment data for multiple, simultaneously active motoneurons activating human laryngeal muscles. These data provide specific information about how laryngeal muscle force is being controlled by the central nervous system at the level of the lower motoneuron.** ***Methods:*** **A quadrifilar needle electrode was used to record multi-channel myoelectric signals from thyroarytenoid muscle of normal subjects during tasks ranging from quiet breathing to a short sentence. Motor unit action potentials of the signal space were identified and tracked throughout task productions using pattern recognition and Precision Decomposition software.** ***Results:*** **We present the first recordings and analyses of multiple motor unit activations in the larynx. The firing times and mean firing rates are plotted for each identified motor unit, which reveal recruitment and decruitment information and the database from which common firing statistics across motor units may be derived.** ***Conclusions:*** **This study provides new information about neuromuscular physiology of the larynx. Specifically, the results reveal the ordered recruitment and firing patterns of multiple motor units and the existence of common drive from the central nervous system. The technique may prove fundamental to understanding various neuromuscular pathologies such as laryngeal spasm and to assist clinical prognosis of laryngeal paresis and the diagnosis of certain neurogenic disorders.**

Key Words: **Electromyography, myoelectric recording, EMG, decomposition, motoneuron, laryngeal physiology.**

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INTRODUCTION

Electromyographic recordings of laryngeal muscles have up until now been either the single-fiber or multiple-fiber type, with the latter being more common and easier to obtain. The type of electromyographic (EMG) signal acquired is highly dependent on the geometry of the recording electrode and its orientation to the muscle fibers.[1] A *single-fiber EMG recording* is accomplished by using an intramuscular detector that is of sufficiently small size so that depolarization potentials of a single fiber dominate the recording field, whereas a larger-sized detector is sensitive to a greater number of fibers and results in the *composite EMG recording*.

In the case of single-fiber recordings, detailed firing activity of one motoneuron is revealed, but information about other motoneuron activity in the neural pool is unknown. Composite EMG recordings, by comparison, represent the spatial–temporal summation of multiple motor unit action potentials (MUAP) in the volume of muscle surrounding the electrode. Thus, in the case of composite recordings, aggregate information about motor pool activity is known, but only sparse details can be obtained concerning the underlying neural drive. When the muscle is active, the superposition of MUAPs that form the signal "hash" is too complex to derive specific information about recruitment and firing rate activity of multiple motoneurons except in isolated instances.[2]

Although EMG studies based on each of these recording types have greatly advanced our understanding of both normal physiology and of neuromuscular diseases affecting laryngeal function, much remains unknown.[3–6] In the case of laryngeal spasm, for example, the work of many researchers has served to characterize the disorder and identify effected neuromotor subsystems.[7–11] However, comparatively little is known about the underlying

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neural mechanism, because detailed observations of neural activation during spasm have not been achieved.

The goal of modern electromyography is to obtain features from EMG signals that relate to one or more aspects of normal or pathologic function of the muscle, nervous system, or both. In the simplest scenario, it may be sufficient to know merely if myoelectric activity exists and therefore crude observations will suffice. However, usually it is required to obtain quantitative features from the EMG signal. Historically, these have been extracted along three domains: *amplitude*, *time*, or *frequency* (or combinations thereof). Extant literature reveals that the great majority of features extracted from laryngeal EMG signals are amplitude-based[5,8,12] with a few being temporal-based.[10,13]

Repetitive firing of an active lower motoneuron creates the action potential train for a nerve axon and its associated motor unit, as illustrated in Figure 1A, B. In turn, arrival of the action potentials at the motor end plates results in depolarization of the muscle fibers of the associated motor units and creation of the resulting MUAP (Fig. 1D). The action potentials created by the motoneurons are all equal in amplitude, varying only in their dynamic activations (i.e., firing rates).[14] Thus, once activated, muscle force is controlled by a frequency-modulated scheme of the central nervous system and not by amplitude modulation.[1]

Sufficient incentive therefore exists to use a frequency-based approach when analyzing laryngeal EMG signals, whether single fiber or composite. Limited success has been reported from scant few investigators who have applied spectral and time-frequency analyses of intralaryngeal myoelectric signals.[15–17] Despite their ability to expose myoelectric phenomena not detected by amplitude or temporal methods, frequency-based analyses do not provide specific information about the controlling paradigm of neuronal action potentials.

This article reports on a technique that offers the advantages of both single-fiber and composite EMG recordings: the ability to examine the detailed firing characteristics of all individual motor units in the vicinity of the recording electrode and to do so simultaneously. Results were obtained using a quadrifilar needle electrode to record multi-dimensional myoelectric activity and by then applying pattern recognition and the method of Precision Decomposition[2,18] to the vector signal space. From these data, MUAP trains may be obtained that represent individual motor unit action potentials in their correct temporal relations. In short, the method used is able to recover

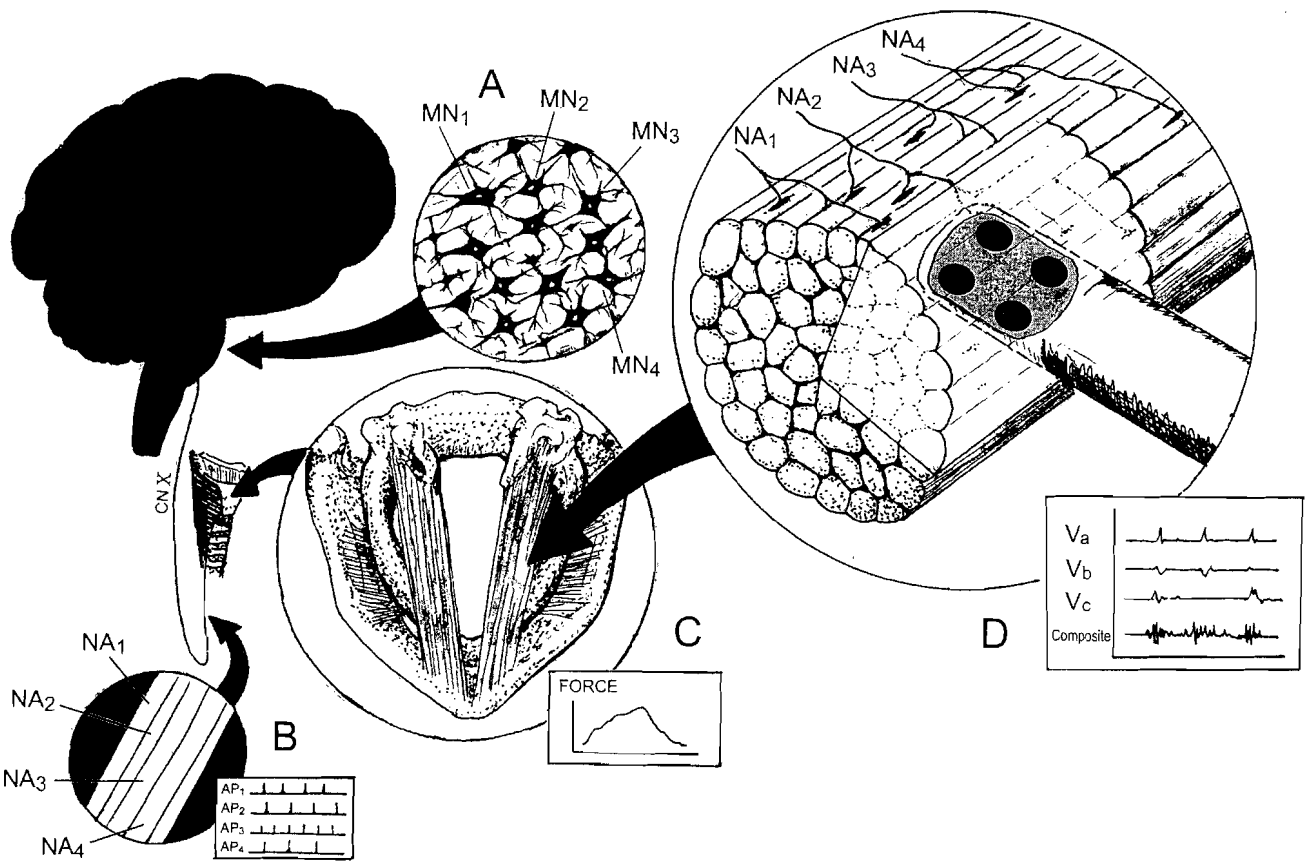

Fig. 1. The morphology of a laryngeal motor unit: In **A**, firings of cranial motoneurons (MN) establish axon potentials (AP) on associated nerve axons (NA) of the recurrent laryngeal nerve (**B**). In **C**, depolarization of the multiple muscle fibers associated with the active motoneurons creates tension (force) of the thyroarytenoid muscle. In **D**, the quadrifilar electrode detects a vector of near-field myoelectric events as well as the conventional EMG signal of composite motor unit action potentials (MUAP).

all useful information contained in the recorded EMG signal.

METHODS

EMG Electrodes

Figure 2A shows a schematic of the specially manufactured quadrifilar needle electrode used in this study (25 ga, length 45 mm). The distinguishing feature of the electrode is the side-port detector array located 3 mm from the cannula terminus, consisting of four 50-μm diameter platinum selective surfaces spaced 200 μm in a square pattern. These four detectors are routed to a pin connector at the needle head that likewise provides communication to the inner and outer cannula for a total of six detector surfaces for the electrode. The quadrifilar needle electrode and its characteristics are further detailed by De Luca and Adam.[18]

The four side-port detectors are connected to three differential amplifiers in a fashion typified by the schematic in Figure 2B, providing the vector EMG signal array V_a, V_b, and V_c. A fourth amplifier connects between the outer cannula and one of the side-port surfaces to detect myoelectric activity over a broader field than that of the microdetector array, thereby providing a composite EMG signal, V_d, in the fashion of a conventional needle electrode. Isolation buffer amplifiers (not shown) are located in a lightweight, microencapsulated base that serves as the female connector at the needle head, thus reducing noise artifact in the downstream signal path.

The detector arrangement shown in Figure 2 provides a miniature multi-dimensional array that produces a vector "image" of myoelectric field activity, E, in the volume neighborhood of the electrode side-port. Examples of these myoelectric images are shown in Figure 2C. The myoelectric event noted at time T_1 in Figure 2 represents the tertiary template of myoelectric activity detected by the electrode–amplifier combination, which forms a *signature* for a motor unit firing. The myoelectric event noted at time T_2 results in a different signature, and therefore will be identified by the processing system as being associated with a different motor unit in the laryngeal muscle. The geometry and size of the detector array and its position relative to the anatomic arrangement of the interspaced muscle fibers (Fig. 1D) assure that the myoelectric event of each motor unit firing will have a unique signature.

By contrast, the signatures obtained from a bipolar electrode, either needle or hooked-wire, often fail to provide sufficient distinguishing quality so that motor unit identification can occur. This is illustrated by examining only the V_a component in Figure 2C, as if V_b and V_c were unavailable. From the perspective of the bipolar detector surfaces associated with V_a, the two myoelectric events at times T_1 and T_2 appear to arise from the same motor unit. The additional information provided by the other perspectives, V_b and V_c, indicate that the events are actually associated with two different motor units. This example illustrates why "software-only" EMG decomposition techniques using bipolar electrodes are less accurate than the approach taken here. The

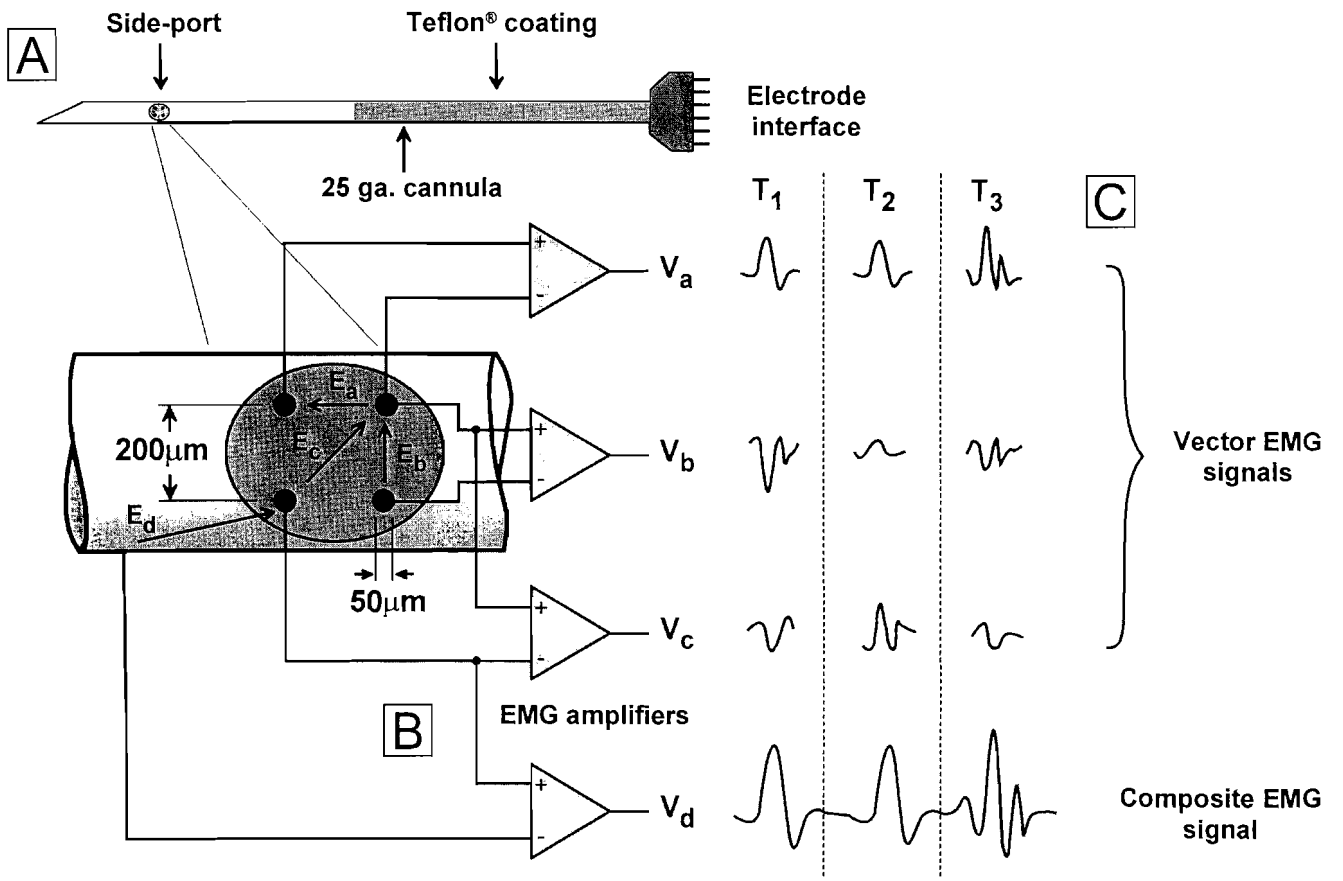

Fig. 2. The quadrifilar needle electrode (**A**) and amplifier configuration (**B**) used in the study. In **C**, the recorded EMG signal array detected during three representative myoelectric events, the first two identified as firings of different motor units and the third identified as a superposition of the two motor units.

technique described in this article, *vector laryngeal EMG* (VEMG), requires both specialized hardware and software components.

Specially prepared wire electrodes were also used in the study. Quadripolar wire electrodes were constructed by forming four strands of 50 μm nylon-coated nickel–chromium wire (Stablohm 800) into a parallelogram arrangement held in place by a small amount of suture glue. A perpendicular scalpel cut was made across the wire cluster to expose the metal, leaving approximately 2 mm of glue at the detector end, which was then formed into a 90° hook. Wire insulation was not further removed from the electrode tip so as to maximize detector sensitivity; only the ends were exposed. Electrode wires were threaded into the cannula of a 25-ga hypodermic needle which was then used to make the percutaneous muscle insertions in a conventional fashion.[4,5] In sum, the same advantages and disadvantages relevant to wire versus needle electrodes[1,5] hold true for these quadripolar types. Because of their facility at minor repositioning, we favor needle electrodes for laryngeal muscles at this juncture.

Instrumentation. The array of EMG signals were amplified using bioinstrumentation amplifiers (Burr-Brown) and filtered using digital filters (Signal Processing Solutions) programmed with a 128-order finite impulse response having exceptional passband and stopband characteristics.[19] Vector EMG signals were band-pass-filtered from 500 Hz to 10 kHz to localize MUAP events and the composite EMG signal band-pass-filtered from 16 Hz to 1 kHz to reduce movement artifact and provide antialiasing. A saline-moistened conductive strap placed around the forearm and a silver ear clip with conductive gel served as reference electrodes. The voice acoustic signal was recorded using an electret unidirectional microphone (Optimus) positioned 30 cm in front and to the side of the subject and low-pass-filtered at 10 kHz. All signals were digitized at a rate of 25 kilosamples per second by a 12-bit A/D board (Data Translation DT2821G) and stored directly to magnetic disk media of a personal computer operating Alamed Voice+ software.

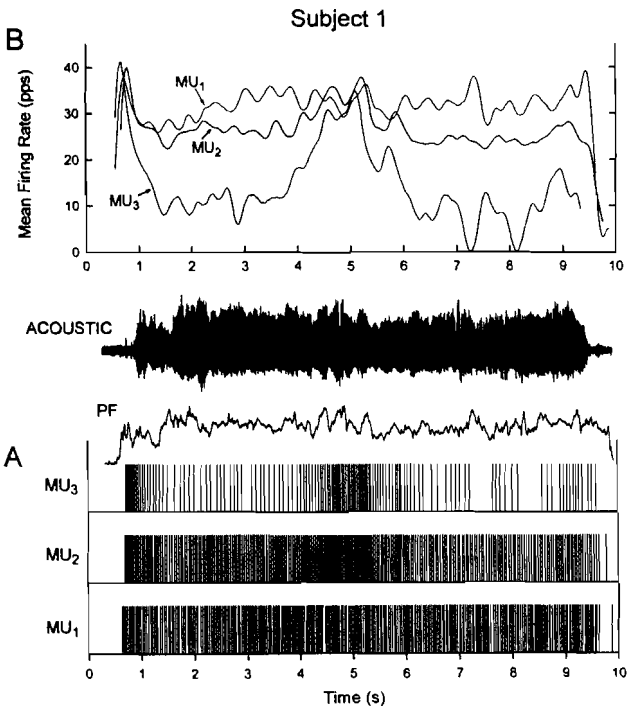

Fig. 3. In **A**, the firing bar plot of three active motor units (MU) of thyroarytenoid muscle during a high-pitched /i/ task for subject no. 1 accompanied by the pseudo-force (PF) and acoustic signals. In **B**, the mean firing rate (MFR) plot.

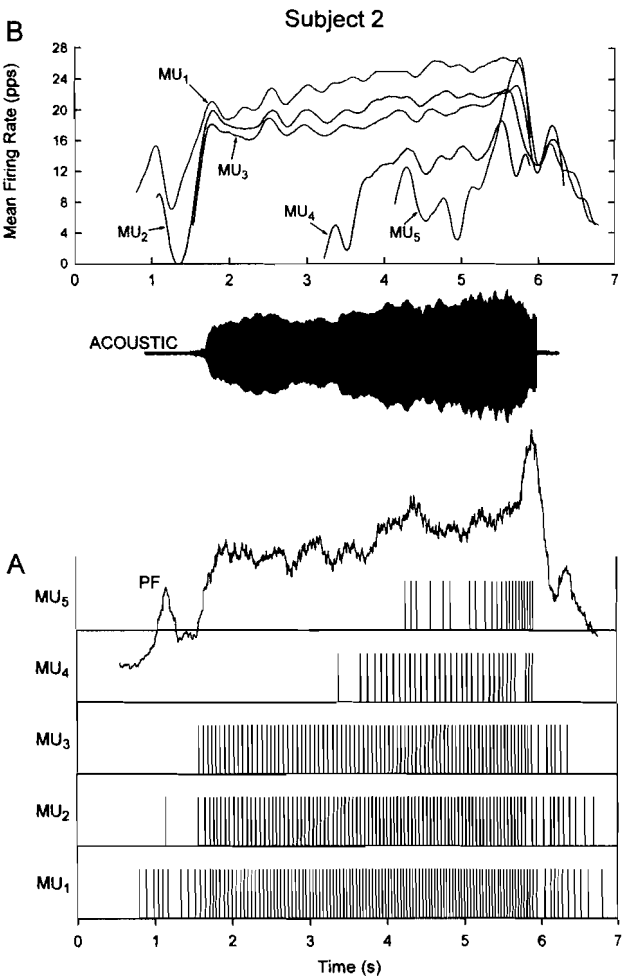

Fig. 4. In **A**, the firing bar plot of five active motor units (MU) of thyroarytenoid muscle during a high-pitched glissando /i/ task for subject no. 2 accompanied by the pseudo-force (PF) and acoustic signals. In **B**, the mean firing rate (MFR) plot.

Muscles, subjects, and tasks. Informed consent was obtained from subjects in compliance with the Institutional Review Board of New York Medical College (NYMC). Electrodes were gas sterilized according to hospital operating room procedures. Electrodes were placed into the midportion of the left or right thyroarytenoid and cricothyroid muscles of awake subjects using percutaneous insertions. No sedation or anesthetic agent was administered. Recordings were made with subjects seated in an ORL examination chair located inside a Faraday cage at the Voice Center at NYMC. The subject database for VEMG has included 14 subjects to date, including spasmodic dysphonic and laryngeal paretic patients as well as normal control subjects. Total data recording time was approximately 20 minutes per subject, during which subjects performed a variety of tasks ranging from vegetative to linguistic. We report here on thyroarytenoid muscle of two normal subjects: a 25-year-old man (subject no. 1) performing high-pitch voiced /i/and a 27-year-old woman (subject no. 2) performing high-pitch voiced glissando /i/. Both subjects were nonsmokers, free of respiratory and speech problems, and did not have a history of head trauma or neurologic problems.

Signal processing. Processing of EMG and acoustic signals was performed at the Voice Center at NYMC and at the

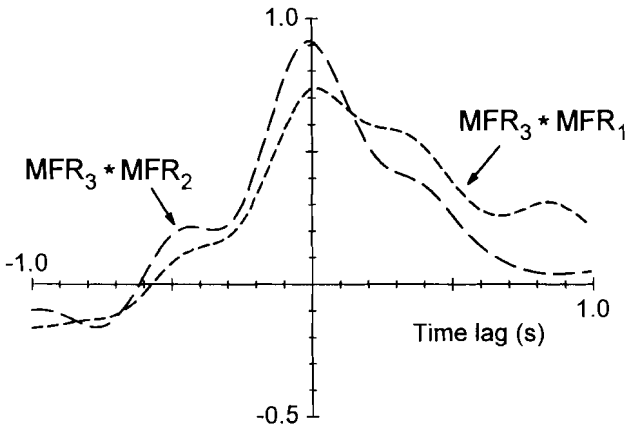

Fig. 5. Cross-correlation functions MU_3 * MU_2 and MU_3 * MU_1 for mean firing rate data of thyroarytenoid muscle in Figure 4 during the interval 1.6 to 6.4 seconds.

NeuroMuscular Research Center at Boston University. Segments were selected from the raw data and prepared into file formats required for the Precision Decomposition technique.[2,18] Precision Decomposition performs repetitive computer identification of motor unit signatures using firing rate probabilities, template matching, and dynamic template updating as MUAPs are identified. When two or more motor units fire simultaneously or when their MUAPs overlap in a manner that confound ready identification, as illustrated by the myoelectric event occurring at T_3 in Figure 2, successive attempts are made to identify the active motor units by the process of subtracting MUAP signatures of the statistically "most likely" motor units and resubmitting the MUAP residue to the identification procedure in iterative fashion. As a final step, all computer MUAP identifications are examined manually to resolve ambiguities.

RESULTS

The VEMG procedure described in previous paragraphs identified up to 10 motor units for each phonatory task. The results presented here represent consistent patterns that required minimal editing and that demonstrate dynamic motor control. The average length of recorded signals was approximately 8 seconds.

Firing Times

The interfiring interval (IFI) of a motor unit is the time between two incidences of motor unit firing. The bar plots in Figures 3A and 4A represent firing times for the high-pitch voiced /i/ task for subject no. 1 and subject no. 2, respectively. The identified motor units are labeled in the order of initial firing, MU_1, MU_2, etc. The bar plots effectively represent motoneuron activity (i.e., incidences of recruitment/decruitment and firing) for the identified motor units.

As an indication of gross muscle activity, a "pseudo force" signal, PF, was obtained through digital signal processing by rectifying and integrating the composite EMG signal using a 50-msec sliding von Hann window.[22] The signal thus obtained has been found to be representative of relative muscle force during moderate-level contractions for skeletal muscles of various size,[1] which is helpful to interpret results. The plots in Figures 3 and 4 also include the PF data derived from the composite EMG signal and the voice acoustic signal.

The firing time plot in Figure 3A demonstrates muscle tasking with minor but notable perturbation in PF and acoustic levels following initial vocal onset. Here, MU_1, MU_2, and MU_3 appear early in the task, approximately 0.3 seconds before onset of the acoustic signal. There were no additional motor units recruited after task onset, suggesting that fine regulation of force is accomplished by firing rate modulation. Further to note in this subject is that motor unit decruitment occurs in the reverse order of recruitment; that is, MU_3 was the last motor unit to become active and the first motor unit to become silent. This pattern is similar to that observed in limb muscles.

Precise data regarding the total number of motor units of laryngeal muscles are not available, but Faaborg-Andersen[3] estimated the number of motor units in both cricothyroid and thyroarytenoid to be approximately 100. Motor unit "size" (the average number of muscle fibers per motor unit, or *innervation ratio*) of laryngeal muscle is also not well understood, although investigators have presented estimates ranging from 3 to 60,[3,20,21] with "less than 10" being the modern estimate.[1] Muscles requiring fine adjustments in force, such as those controlling the ossicles in the ear and movement of the eye, are characterized by having small motor unit size, which provides for fine adjustments of force by the process of firing rate as well as motor unit recruitment. By contrast, skeletal muscles having large-size motor units (>500), such as deltoid, achieve fine force control principally through motor unit recruitment and have a more limited range of firing rate than muscles of small size.[1]

The bar plot in Figure 4A for subject no. 2 demonstrates laryngeal motor unit control during increasing force and acoustic pitch with varying acoustic intensity. While the target task was also high-pitch /i/, the subject was encouraged, for this repetition, to increase pitch throughout the course of task production in the fashion of glissando. In this case, motor units MU_1, MU_2, and MU_3 are recruited early in the task, whereas MU_4 and MU_5 begin to activate 3.4 and 4.2 seconds into the task, respectively. As seen in Figure 4, appearance of motor units MU_4 and MU_5 occur coincident with increased force demands of the thyroarytenoid muscle. This phenomenon illustrates the recruitment of individual motor units of the larynx during periods of increased force demands. Furthermore, while recruitment of motor units is observed during the period 1 to 4 seconds in Figure 4, the reverse phenomenon, motor unit decruitment, is seen to occur during the period 6 to 7 seconds, when force demands decrease.

Mean firing rate. The mean firing rate (MFR) of each motor unit is determined by applying a sliding von Hann window of 400 msec to the IFI data and obtaining the inverse of the mean value. Figures 3B and 4B are the MFR versus time records for the firing time data appearing in Figures 3A and 4A, respectively.

For subject no. 1 in Figure 3B, the maximum firing rate is 42 pulses per second (pps) and the average of motor units MU_1 and MU_2 is 30 pps (during the period 1–9 s). For subject no. 2 in Figure 4B, the maximum firing rate is

26 pps and the average for MU_1, MU_2, and MU_3 is 20 pps (range, 2–5.5 s).

It is evident from the MFR plot in Figure 3B that the firing activity of MU_2 more closely mirrors the firing activity of MU_1 than that of MU_3. MU_3 appears early, but the MFR decreases to 7–13 pps after initial activation onset. The activity of MU_3 rises again at 4.0 seconds, a moment of increased force, only to subside again soon afterward, around 5.3 seconds. The relatively steady firing rate of MU_1 and MU_2 and the fluctuating activity of MU_3 exemplify two different types of motor unit behavior during this phonatory activity.

A second observation concerns the "onion skin" phenomenon: the ordered correspondence between recruitment hierarchy and firing rate. Successively recruited motor units typically have lower firing rates than those previously recruited according to the order in which they appear, resulting in a layering of motor unit firing rates as illustrated in Figure 3B. This phenomenon has been demonstrated in other musculature to a significant degree.[1,18]

The motor unit firing patterns of subject no. 1 may be contrasted to that of subject no. 2 appearing in Figure 4B. During the period of 1.5 to 5.5 seconds, the firing rates of MU_1, MU_2, and MU_3 are changing in unison; that is, the peaks and valleys are respectively aligned across these motor units. This phenomenon would seem to imply a high degree of coupling, at some level of the central nervous system, in the manner that these motor units are being fired during this testing paradigm. A related but different phenomenon observed during this period is that the MFR of these motor units are each steadily increasing along with apparent increases in force demands and acoustic intensity. These motor units appear to be driven in a common fashion by controlling centers at hierarchical levels above the motoneuron. In other words, there is a high degree of *common drive* for these active motor units under the current task paradigm for this subject.[23]

Cross-correlation* between MFRs of MU_3 and MU_2 and between MU_3 and MU_1 during the period of their mutual activations was performed. The results are shown graphically in Figure 5. As suspected, the cross-correlation function reveals a high degree of common drive among the three motor units, indicated by the high (>0.5) correlation peaks near time lag equal to 0 seconds.

A third observation that can be made concerns the fluctuation of motor unit firings. In addition to the examples provided here (which admittedly do not likely exhibit sustained periods of isometric muscle contraction), motor unit firing rate is never steady, although it appears ordered. The modulation of firing rate is in the fashion of a feedback-controller mechanism, which may relate to coupling characteristics of the efferent–afferent structure of the (extralaryngeal) vocal motor and auditory sensory systems.

As a final observation of Figure 4B, increasing task demands result in the recruitment of motor units MU_4 and MU_5 in successive order. At approximately 5.7 seconds, this subject's phonation transferred into falsetto

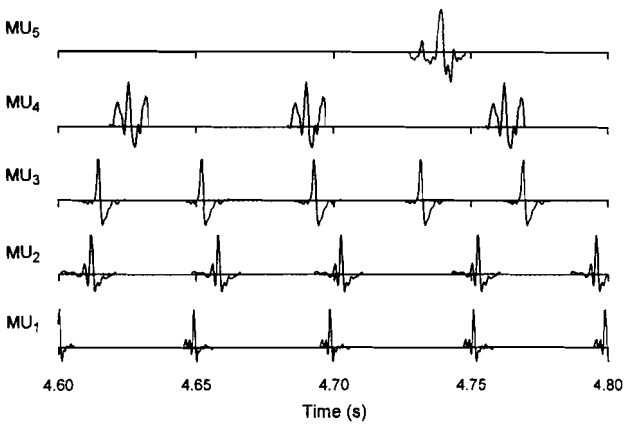

Fig. 6. Motor unit action potentials for five motor units (MU) of thyroarytenoid muscle in Figure 4 during a selected segment.

register for the duration of phonation (as verified by frequency analysis of the acoustic signal). During this period, all motor units reach their maximum firing rate, with MU_5 seeming to dominate the increase in force by rapidly increasing from 3 to 26 pps. Motor unit firing rate and recruitment for subject no. 2 was more dynamic in achieving thyroarytenoid muscle control than subject no. 1.

Motor unit action potential trains. The exact firing times of motor units obtained from the process of Precision Decomposition provide a base of motor unit data that may then be used to obtain time-series plots of the motor unit action potential trains. The "mean" MUAP shapes of each motor unit are readily obtained by the process of "spike train averaging" the composite EMG signal with firing time data using digital signal processing.[18] The results of this procedure are shown in Figure 6 and correspond to data in Figure 4B for subject no. 2 during the interval from 4.6 to 4.8 seconds. Figure 6 is a constructed time-series, assembled by placing the average MUAP shapes at the firing times that have been precisely determined from the previous procedure. The resulting data provide for various analyses to be performed that are based on morphologic features of multiple MUAPs.

As a final observation, the longer-duration and more complex shapes of MU_4 and MU_5 in Figure 6 likely indicate motor units of successively larger size (i.e., larger number of muscle fibers) compared with the previously recruited units. In other words, the spatial–temporal summation of additional MUAPs of individual muscle fibers result in broader composite MUAP shapes with greater absolute area. This phenomenon is in accord with the *Henneman size principal* observed for spinal nerves,[24] which states that smaller-sized motor units are recruited before larger-sized units in the hierarchy of motoneuron activation. It must be noted, however, that the shapes of MU_4 and MU_5 in Figure 6 are approximated to an unknown degree because few firings are available to the spike train averaging procedure compared with the other units. A more accurate approximation would be obtained by performing muscle contractions for a longer period of time or by averaging across tasks. Nonetheless, the results are included here to illustrate the method.

*Although most authors refer to this procedure as *cross-correlation*, it is more accurately the measure of *cross-covariance* because MFR data are de-trended by high-pass filtering (~1 Hz) prior to correlation.

Vector laryngeal EMG has thus demonstrated a technique whereby all useful information contained in laryngeal myoelectric signals can be obtained.

CONCLUSION

This article describes a method of obtaining firing rate and recruitment patterns of multiple, simultaneously active motor units in intralaryngeal muscles. Results that are representative of the technique were provided for thyroarytenoid muscle of two normal subjects. While activities of up to 10 motor units were identified by the procedure, a representative subset was reported here to facilitate introduction of the technique and discussion of results. Although findings are preliminary and few, some comments can nonetheless be made regarding our observations to date. These include the phenomena of common drive, firing rate regulation, and ordered recruitment of laryngeal motor units.

Like with conventional electromyography, the motor unit activity identified by the VEMG technique represents only those units within the detection proximity of the electrode. However, given the relatively small size of intralaryngeal muscles, and therefore the relatively small total number of motor units, it can be conjectured that findings are representative of the collective behavior of motor units in the muscle under test. Use of the quadrifilar needle electrode and the Precision Decomposition technique has been demonstrated in other muscle to be highly reliable (>95% accurate).[25]

In 1992, Chanaud and Ludlow[26] reported on a crude and unreliable method of examining the firing rates of three normal motor units of thyroarytenoid and cricothyroid muscles during respiration, derived by successive filtering of the composite EMG signal recorded with a bipolar electrode. In a manuscript summarizing laryngeal EMG state-of-the-art in 1994, authors reported on using the same technique in a cricothyroid muscle of a normal subject during a speech task.[27] In both cases, the investigators suggested that laryngeal motor unit firing rates were heterogeneous and unpredictable, concluding that an "inherent complexity in muscle activation" resulted in "motor units within a muscle firing independently." The remarkable conclusion of these two laryngeal studies, limited by the available technology, is contradicted by extensive findings of a great many investigations of neurology, kinesiology, and myography during the last 30 years for numerous muscles and by the preliminary findings reported here.

The technique of vector laryngeal EMG provides, for the first time, the ability to "look upstream" to the level of the laryngeal motoneuron. It is anticipated that experiments can be crafted that provide further insight into how the nervous system, including both efferent and afferent components, is providing the signal array that controls laryngeal muscles of the normal population, and to investigate how disease affects the neural control of laryngeal muscles in certain pathologic populations. These investigations should include the identification of activation patterns that facilitate prognosis of laryngeal paresis and patterns that facilitate early diagnosis of neurogenic diseases such as Parkinson's disease.

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