Recruitment Properties of Intramuscular and Nerve-Trunk Stimulating Electrodes

Kan Singh, Frances J. R. Richmond, and Gerald E. Loeb

Abstract—**Functionally useful reanimation of paralyzed limbs generally requires reliable, finely graded control of muscle recruitment and force with minimal fatigue. We used force and electromyographic (EMG) recordings in combination with myofibrillar adenosine triphosphatase activity and glycogen depletion analysis to investigate the recruitment properties of intramuscular (IM) and nerve cuff (NC) stimulating electrodes implanted acutely or chronically in cat hindlimbs. Overall, 32 muscles were submaximally stimulated with current intensities producing approximately 20% of maximal twitch force using 330 ms trains of pulses at 20 and 40 pps. Both the glycogen-depletion and fatigue-test results were found to be difficult to interpret because NC stimulation resulted in surprisingly unstable recruitment during such trains. Fluctuations of force and M-waves within trains of identical stimuli were significantly greater for NC than for IM stimulation. NC stimulation produced much steeper recruitment curves and a reduced tetatnus/twitch ratio compared to IM stimulation. IM stimulation produced more reliable and less fatigable recruitment of a mix of motor unit types that tended to be localized in neuromuscular compartments containing, or adjacent to, the IM electrode. We hypothesize that trains of submaximal stimulation applied through NC electrodes resulted in fluctuating recruitment because this electrode configuration magnifies the effects of refractoriness and small changes in axonal excitability during pulse trains.**

Index Terms—**Force, muscle, nerve cuff, recruitment, stimulation.**

I. INTRODUCTION

F UNCTIONAL electrical stimulation (FES) seeks to reanimate paralyzed limbs by replacing voluntary activation of muscles with electrical stimulation of their nerves. Muscle fibers themselves are difficult to excite electrically, but the large myelinated axons of the motoneurons that innervate them are readily stimulated with brief pulses. Thus, FES is most suitable for the treatment of paralysis due to lesions of upper motor pathways (e.g., stroke and spinal cord injury). In these conditions, the motoneurons, peripheral nerves and muscles remain relatively intact.

In order to perform many motor tasks successfully, it is important that the FES system achieve graded control of force with

resolution and reliability approaching that of natural neural control. Unfortunately, electrical stimulation of motor axons tends to result in a recruitment order opposite to that obtained by synaptic inputs [1], [2]. Normally, the smaller motoneurons innervating small numbers of slow, fatigue-resistant muscle fibers are recruited first [3]. They provide stable and finely controlled force for small, precise, and frequently needed movements. The larger motoneurons innervating larger numbers of fast, easily fatigued muscle fibers are held in reserve for occasional use in strong, brief efforts such as ballistic movements. Electrical stimulation, however, tends to depolarize and recruit axons more or less in proportion to their diameter [4]. The larger motoneurons have larger axons, so they tend to be recruited at the lowest stimulation intensities. Because the larger motoneurons innervate large numbers of fast-fatigable muscle fibers, their preferential recruitment results in relatively large increments of force with small changes in stimulus intensity. In addition, they are susceptible to fatigue, so that force tends to decline rapidly over successive contractions even when the number of recruited motor units remains constant. These problems are particularly severe when stimulation is applied to main nerve trunks and muscle nerves through nerve cuff (NC) electrodes because all of the motor axons are gathered together and subjected homogeneously to the electrical field created by the stimulating current.

When stimulation is applied via intramuscular (IM) electrodes, two factors influence recruitment in addition to the amplitude and duration of stimulus current.

1) *Distance from the Current Source:* When motor nerves enter a muscle, they distribute themselves into successively finer nerve branches that innervate individual compartments and fascicles of the muscle. An IM electrode is likely to lie close to some of these nerve branches but more distant from others that might contain the axons of different subsets of motoneurons. Because tissues act as volume conductors, the electrical field strength of an electrical current applied by such an electrode decreases rapidly as it spreads throughout the muscle. Due to this diminishing field strength, small axons that happen to run close to the IM electrode are more likely to be recruited than larger axons that course at a greater distance. These circumstances have been thought to underlie the shallow, often stepwise recruitment pattern of force typical of IM stimulation [5].

2) *Axon Branching Patterns:* Because the larger motoneurons innervate more muscle fibers distributed over larger territories [6] they must bifurcate more often to reach all of those fibers. As they divide, axons tend to become smaller in diameter. Thus, the diameters of the branches
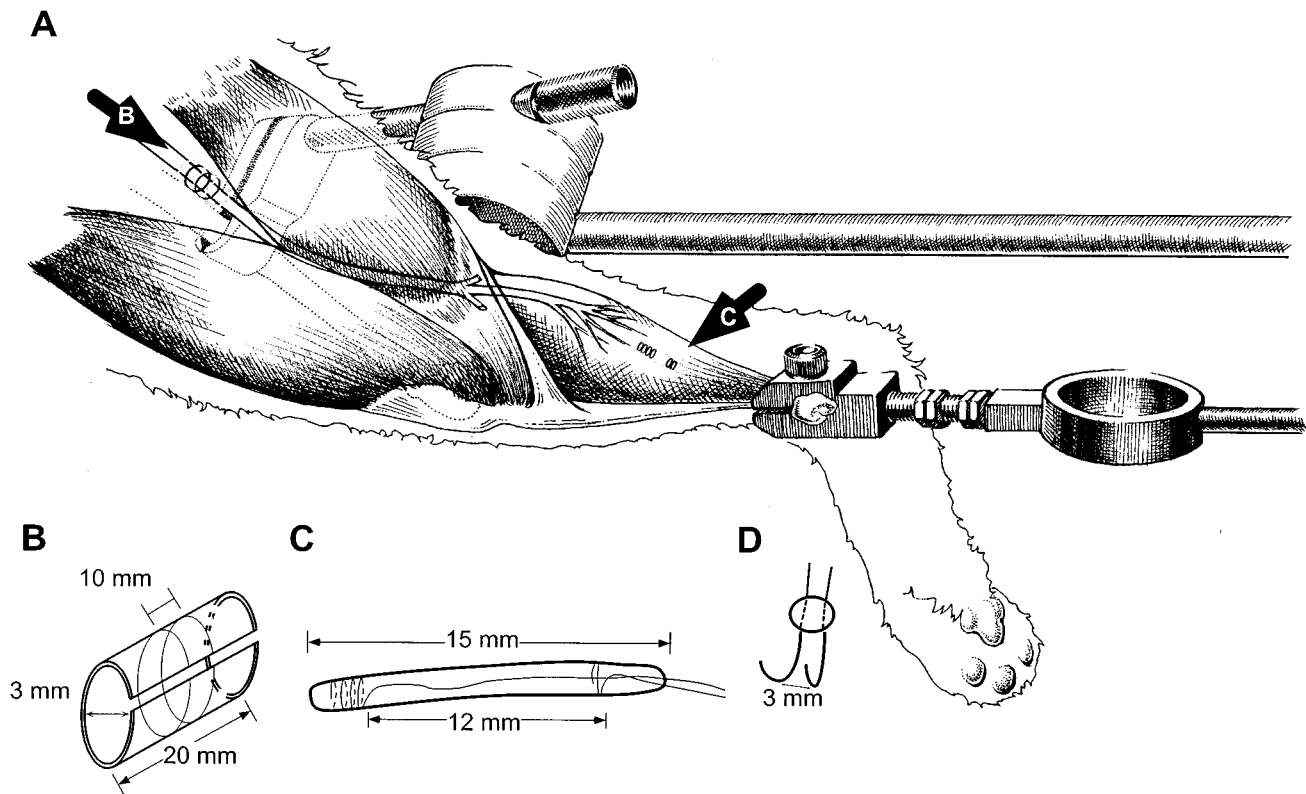
Fig. 1. (A) The experimental preparation used in the present series of experiments. (B) NC electrodes were implanted on the cut sciatic nerve and (C) IM electrodes were implanted in the MG muscle belly. (D) HK electrodes were implanted proximal to the NC electrodes. (B) NC electrodes consisted of bipolar contacts separated by 10 mm and molded into the inside wall of a 3 mm diameter silicone cuff. (C) IM electrodes consisted of bipolar contacts separated by 12 mm and wrapped around a silicone tube 2 mm in diameter and 15-mm long. (D) Hook electrodes consisted of bipolar, hemicircumferentially shaped, platinum contacts, each 0.2 mm in diameter and anchored 3 mm apart by a bead of epoxy.

of large motoneurons become more similar in size to those of smaller motoneurons as they approach their target muscle-fibers. Stimulation applied in the vicinity of these terminal axonal branches would then tend to be equally effective at recruiting small, fatigue-resistant motor units as large, fatiguable motor units.

The two factors described above led us to hypothesize that IM stimulation at submaximal force levels should result in contractions that have a lesser tendency to fatigue than similar contractions produced by NC stimulation. We tested this hypothesis by quantifying the rate of fatigue for such stimulation in cat medial gastrocnemius (MG), an ankle extensor with a simple unipennate architecture and a fairly typical mix of fiber types. We also attempted to confirm the underlying mechanism by examining the pattern of glycogen depletion following long periods of repetitive contractions. Unexpected difficulties with glycogen depletion following NC stimulation led us to study a surprising and perhaps more important difference between IM and NC electrodes related to the stability of recruitment during physiological trains of stimulation.

II. METHODS

A. Electrode Designs

The NC electrode was 3 mm in diameter and 20 mm long with a bipolar inter-electrode spacing of 10 mm [Fig. 1(b)]. The contacts were finely stranded stainless steel wire molded circumferentially into the inside walls of the silicone elastomer cuff, as described by Loeb and Peck [7]. Such electrodes have been used to produce precise activation of particular classes of peripheral nerve fibers in cutaneous, muscle and mixed nerves.

The IM electrode consisted of bipolar stainless steel wire contacts wound 12 mm apart onto the outside of a silicone tube 2 mm in diameter and 15-mm long [Fig. 1(c)]. This design provided a similar electrode geometry to an injectable, wireless microstimulator that is being developed for clinical FES applications [8], which was used previously to study muscle fiber recruitment by the method of glycogen depletion [5].

The hook (HK) bipolar electrodes consisted of malleable platinum wires 0.2 mm in diameter and anchored 3 mm apart in a small bead of epoxy [Fig. 1(d)]. These were formed into hemi-circumferential hooks onto which the sciatic nerve was set before covering the electrodes and adjacent nerve with low-melting point paraffin, the temperature of which was kept between 41–45°C prior to application.

B. Preparations

A total of 32 MG muscles from 16 cats of either sex ranging in weight from 2.7 to 6.9 kg were studied in acute terminal experiments under deep barbiturate anesthesia. Experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

In two chronic experiments, cats were anesthetized with sodium pentobarbital (to a level sufficient to abolish pedal reflexes) and aseptic surgical techniques were used to implant both NC and IM stimulating electrodes in each hindlimb three weeks before the acute experiments. The electrodes were implanted in the same manner as the acute experiments (see below) except that the leads were coiled and implanted in subcutaneous pockets so that they could be accessed without disturbing the electrodes during the acute experiment. Before and after the implantation procedure, the animals were given prophylactic doses of the antibiotic Tribrissen (Cooper's Agropharm, Ajax, ON) by subcutaneous injections.

In the 14 acute experiments, the stimulating electrodes were implanted at the beginning of the terminal experiment. In all terminal experiments, cats were anesthetized with sodium pentobarbital and cannulated for intravenous anesthetic and fluid delivery; a patent airway was ensured by tracheotomy. Core body temperature was maintained at 36–38 °C by a thermostatically controlled heating blanket.

In all animals, the NC electrodes were placed around the main sciatic nerve in the thigh (see Fig. 1). During the terminal experiment (three weeks after implantation in the case of the chronic experiments) the nerve was ligated and cut proximally at the level of the biceps femoris (at least 5 cm from the electrode) to prevent reflex responses. In eight preparations, HK electrodes were implanted proximal to the NC electrode and were stabilized by surrounding the nerve and hook contacts with low melting point (40 °C) paraffin. In all cases, care was taken to leave slack in the distal nerve to minimize the mechanical transmission of motion from the contracting MG muscle. The instrumented nerve was placed back in the cleft between the hamstring muscles to stabilize its temperature and prevent drying.

The MG muscle was dissected from the surrounding muscles and connective tissue, taking care to preserve its innervation and blood supply. Branches of the sciatic nerve supplying other muscles were cut. The IM electrode was implanted into the muscle by making a small incision in the distal part of the muscle belly near the aponeurosis on the dorsal surface. Blunt dissection was used to create a small pocket in the middle of the muscle belly into which the electrode was inserted parallel to the muscle fibers. A small suture was used to prevent the electrode from sliding back out of the muscle. The portion of the calcaneus onto which the MG tendon inserted was cut and clamped to a force transducer mounted on an adjustable positioner [9] [Fig. 1(a)]. The femur was clamped near the origin of the MG muscle and the positioner was used to hold the muscle at the length at which it produced about 4.9 N of passive tension initially (this baseline value tended to drift during the experiment). We determined in two experiments that this tension corresponds approximately to the mid-range of anatomical length for MG and to its optimal length for producing maximal isometric twitch force.

In the last 6 preparations (including both chronically implanted animals), EMG recording electrodes were inserted across the MG belly to monitor M-waves produced by electrical stimulation. These electrodes consisted of two finely stranded stainless steel wires (Cooner Wire, #AS631) threaded through the muscle belly 10 mm apart and perpendicular to the muscle fibers. EMG signals were amplified differentially (Bak Electronics MDA-2, 50–5000 Hz bandwidth) and examined on a storage oscilloscope synchronized to the stimulator.

C. Physiological Tests

All electrical stimulation pulses consisted of electrically isolated, current-regulated symmetrical biphasic pulses (0.1 ms/phase; AM Systems Model 2100 stimulator; output error $<0.3\%$ of setting, $\pm0.5\%$ of range $\{0–100~\mu A, 0–1~mA,$ or $0–10~mA\}$). The NC electrode was used to determine the maximal isometric twitch force of the muscle by applying a supra-maximal stimulus and measuring the peak active twitch force on a digital storage oscilloscope. All subsequent stimulation through all available electrodes was set to the level that produced an active twitch force of 20% of this maximal twitch. The force was measured using a custom strain gauge and amplifier system with a bandwidth of 120 Hz.

To test the fatigability of the muscle at the 20% twitch recruitment level, we used the fatigue test developed by Burke et al. [10] in which trains of stimuli are applied at 40 pps for 330 ms every second. Fatigue tests using the IM electrode were carried out in one leg; either NC or HK electrodes were used to perform the fatigue test in the contralateral leg. Stimulation continued for at least one hour or until no contractions were visible. Force profiles were recorded by a Macintosh computer equipped with MacAdios real-time data acquisition hardware. Using custom software, force was digitized at 150 samples/s for 500 ms synchronized to the first stimulus of every fifth train.

The same stimulation equipment was used for other patterns described in the results. Force and M-waves were displayed on the storage oscilloscope and recorded by a Polaroid camera. In particular, we examined the detailed form of both the force profiles and M-waves produced by 330 ms trains of stimuli at 20 and 40 pps delivered by all electrode types. M-wave variability was assessed by measuring the amplitude of each M-wave in a given train and determining the ratio of the standard deviation to the mean amplitude for that contraction. Twitch-force recruitment curves for individual pulses delivered approximately 10 s apart were determined by gradually increasing stimulus strength from threshold to maximal for each electrode type available. Tetanus-to-twitch peak force ratios were also calculated in these preparations for the two stimulation rates.

D. Histological Analysis

The fatigued muscles were removed from the animals and cut into small blocks approximately 2-cm square. Non-fatigued MG muscles removed from three animals used for other purposes served as controls for the histochemical staining. Blocks were frozen and stored in liquid nitrogen. They were brought to -20 °C and 16 μm transverse sections were prepared on a cryostat. Mounted sections were stained routinely for haematoxylin and eosin, for mATPase activity after alkaline preincubation [11] and for glycogen content using the periodic acid-Schiff's (PAS) reaction [12]. Stained sections were examined under a light microscope and muscle fibers were considered to be depleted of glycogen if they were completely blanched. Fibers were further classified by fiber type according to the mATPase
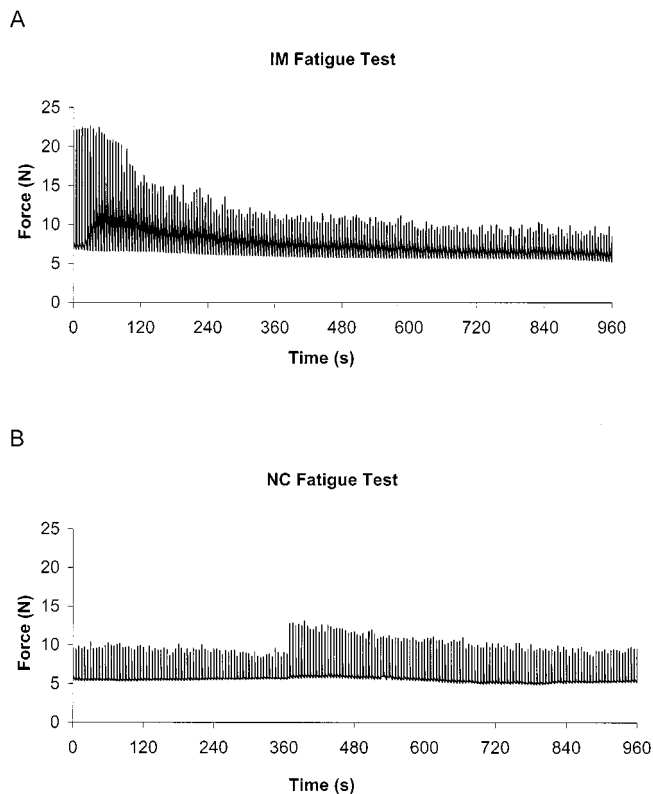
A

B

Fig. 2. (A) Changes in force during IM stimulation. Experiment ERC 07. During a typical fatigue test using an IM electrode (stimulation current = 1.84 mA), the force profile was observed to decline in a monotonic fashion to an eventual plateau level that was usually between 20–30% of the original force. (B) Changes in force during NC stimulation. Experiment ERC 07. This example of a force profile obtained during a fatigue test using a NC electrode (stimulation current = 160 μA) shows a large fluctuation in force that occurred with no visible movement of the preparation. Similar fluctuations (both increases and decreases) occurred on three occasions when NC electrodes were used.

activity of matching profiles on adjacent sections. Typically, histological analyses were made using two or three randomly selected populations of about 100 cells per section, and two to three sections were analyzed per muscle. For muscles stimulated with the IM electrode, the sections examined either contained the electrode or were adjacent to it.

III. RESULTS

A. Fatigability

In all eight muscles in which IM electrodes were used to test for fatigability, peak force declined fairly gradually during the first five minutes [e.g., Fig. 2(a)] and then remained stable at 20–30% of the initial peak force. The length of time to decline to 20% of the initial peak force ranged from 56 to 141 s (mean 101 s). Mean peak force after 15 min was 19.7% $\pm$ 5.5% of the initial peak force.

By contrast, four out of six HK and all eight NC electrodes produced much less consistent results in which peak force often fluctuated abruptly and nonmonotonically in successive contractions. The problem was particularly severe for NC electrodes, as described below. For the four stable HK preparations that could be studied, peak force declined fairly rapidly to below 20% of initial values within the first three minutes and
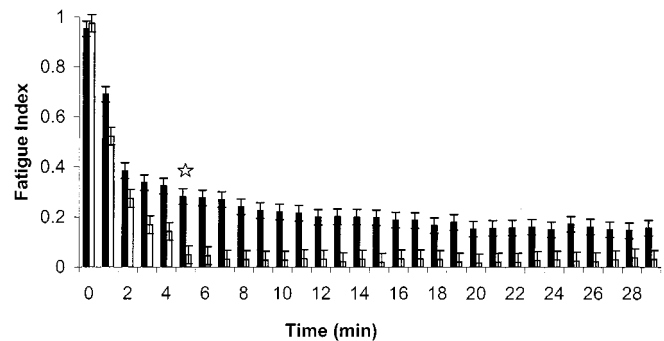

Fig. 3. Mean fatigue indices (ratio of force at a given time to maximal force achieved during the test) for IM (black bars, $n = 8$) and HK (white bars, $n = 4$) stimulated muscles (with standard error bars). The values for IM stimulated muscles are significantly greater than those for the HK stimulated muscles after 5 min (asterisk).

was generally below 5% within 6 minutes. Mean peak force after 15 minutes was $4.0 \pm 2.1\%$ (significantly different from IM values, $p < 0.001$). Averaged fatigue patterns for the 8 IM preparations and 4 HK preparations are shown in Fig. 3. These fatigue index values were calculated by normalizing the peak force of the first contraction of each minute to the maximum peak force achieved during a given fatigue test.

Fatigue tests with 4 other HK and all NC electrodes were highly inconsistent and essentially uninterpretable. In three cases, the records were also marred by abrupt changes in force production. Instead of declining gradually over time, force fluctuated continuously during the fatigue test, sometimes increasing and decreasing gradually and/or abruptly several times as shown in Fig. 2(b). Such fluctuations were observed even though no mechanical motion of the nerve with respect to the NC electrode was apparent. Similar problems were encountered with the chronically implanted NC electrodes, which appeared to be well stabilized on the nerve by the usual foreign body response of connective tissue enveloping the NC electrode. When force profiles elicited during individual trains were examined closely, their individual waveforms fluctuated randomly within and between contractions as described below.

B. Stability

The difficulty in obtaining stable, monotonically declining force values over time when using NC electrodes led us to suspect that we were not recruiting the same population of motor axons consistently during each submaximal stimulation pulse. We examined recruitment stability during individual stimulation trains by recording M-waves from the stimulated muscle and relating them to the characteristics of the resulting force profiles. The results for 40 pps stimulation of acutely and chronically implanted NC and IM electrodes are shown in Fig. 4. For the same preparations, we examined similarly the recruitment stability at 20 pps, which is closer to the sorts of repetition rates likely to be used for FES; these results are shown in Fig. 5.

Stimulation with all NC electrodes was associated with M-waves whose amplitudes fluctuated markedly (Table I). The variability (ratio of standard deviation to mean amplitude) was relatively high for the NC experiments (0.32 ± 0.11 and 0.31 ± 0.09 for 20 and 40 pps, respectively). The first few M-waves in
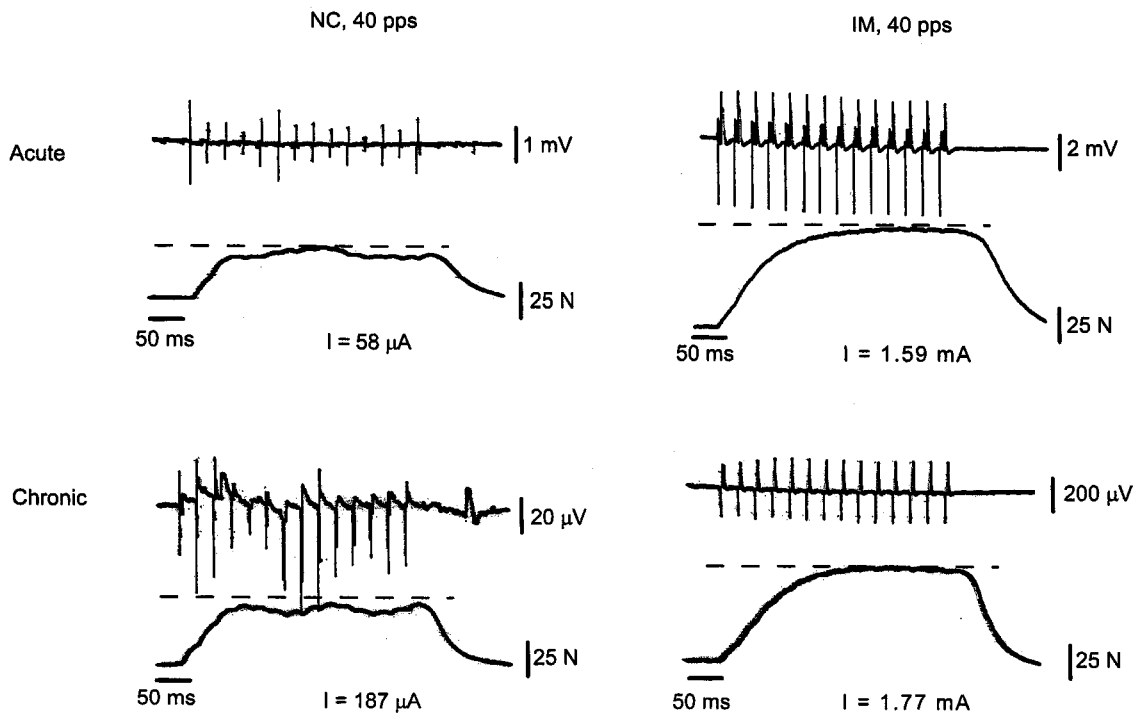
Fig. 4. Acutely and chronically implanted NC and IM electrodes were used to stimulate muscles at a rate of 40 pps while force and M-wave profiles were recorded. During NC stimulation, M-wave amplitude varied widely from pulse to pulse and the corresponding force profiles also fluctuated considerably. The results from IM stimulation demonstrated consistent M-wave amplitudes and stable force profiles. (Traces digitally enhanced from original oscilloscope photos.)
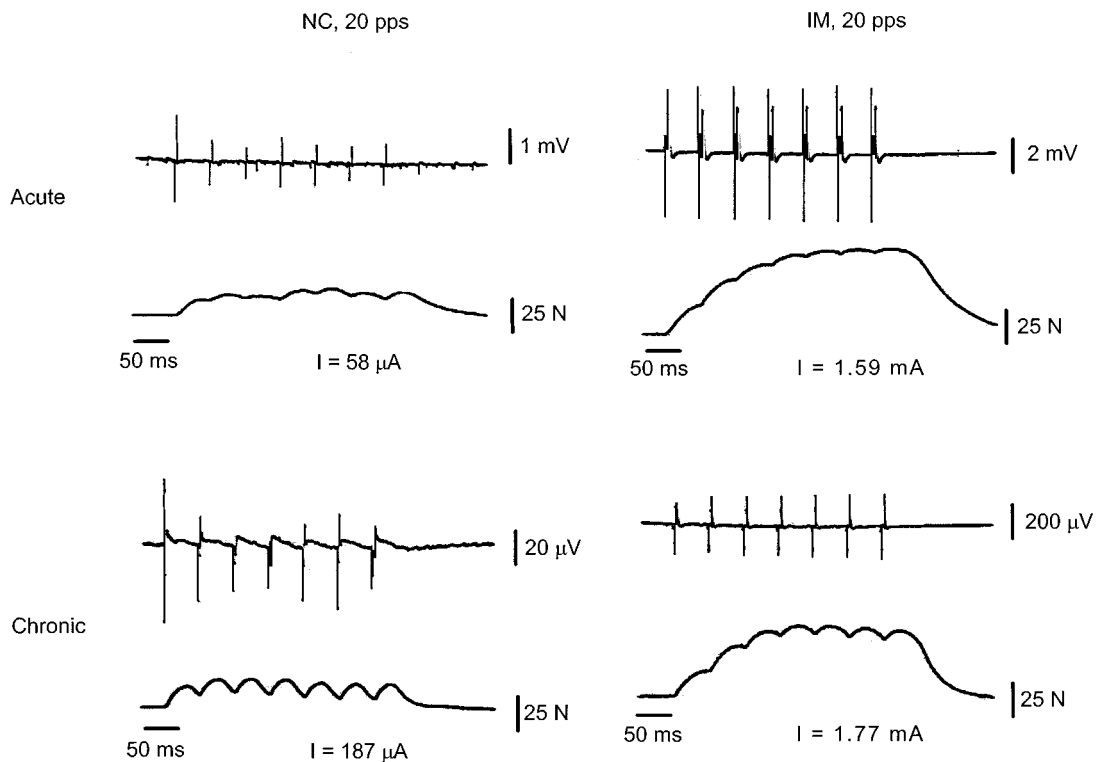

Fig. 5. Stimulation at 20 pps in the same preparations as Fig. 4, showing similar variability of M-wave amplitude and force production when muscles were stimulated with NC electrodes in both acutely and chronically implanted animals. Stable, predictable force profiles and M-waves were produced in response to IM stimulation.

the train were usually different from each other in amplitude but in a consistent manner from train to train. The first M-wave was often but not always the largest. The later M-waves in the train tended to fluctuate less predictably. The changing EMG records were accompanied by fluctuating force levels during the trains. IM electrodes produced more consistent results using both 20

TABLE I

TOP: VARIABILITY (STANDARD DEVIATION, sd, DIVIDED BY MEAN, x) OF M-WAVES PRODUCED DURING TRAINS AT 20 AND 40 pps FOR VARIOUS ELECTRODE CONFIGURATIONS (NC, IM AND HK) IN FIVE PREPARATIONS. BOTTOM: RATIOS OF PEAK TETANIC TO TWITCH FORCE (tet/tw) FOR STIMULUS TRAINS OF 20 AND 40 pps FOR THE SAME ELECTRODES AND PREPARATIONS. BOLD TYPE INDICATES SIGNIFICANTLY DIFFERENT VALUES ($p < 0.05$) FOR IM STIMULATION VERSUS THE OTHER TWO CONFIGURATIONS

cat #	side	NC - M-wave		IM - M-wave		HK - M-wave	
		sd/x, 20	sd/x, 40	sd/x, 20	sd/x, 40	sd/x, 20	sd/x, 40
11 Acute	R	N/A	N/A	N/A	N/A	N/A	N/A
	L	N/A	0.26	N/A	0.04	N/A	0.28
12 Acute	R	0.30	0.35	0.04	0.02	0.47	0.90
	L	0.48	0.40	0.13	0.04	0.23	0.31
13 Acute	R	0.20	0.22	0.01	0.11	N/A	N/A
	L	N/A	N/A	N/A	N/A	N/A	N/A
15 Chron	R	0.16	0.17	0.08	0.05	N/A	N/A
	L	0.39	0.29	0.11	0.11	N/A	N/A
16 Chron	R	0.38	0.37	0.11	0.10	N/A	N/A
	L	0.30	0.43	0.04	0.08	N/A	N/A
mean+/-std		0.32+/-0.11	0.31+/-0.09	**0.08+/-0.05**	**0.07+/-0.04**	0.35+/-0.17	0.50+/-0.35

cat #	side	NC - force		IM - force		HK - force	
		tet/tw 20	tet/tw 40	tet/tw 20	tet/tw 40	tet/tw 20	tet/tw 40
11 Acute	R	N/A	N/A	N/A	N/A	N/A	N/A
	L	N/A	14.29	N/A	15.00	N/A	12.14
12 Acute	R	3.99	17.28	11.90	13.23	N/A	N/A
	L	4.52	9.04	9.95	12.66	6.35	10.88
13 Acute	R	4.76	7.94	7.41	8.47	N/A	N/A
	L	N/A	N/A	N/A	N/A	N/A	N/A
15 Chron	R	6.59	10.26	11.76	15.97	N/A	N/A
	L	7.79	12.99	11.69	16.88	N/A	N/A
16 Chron	R	4.02	8.71	13.78	16.84	N/A	N/A
	L	5.10	8.67	N/A	N/A	N/A	N/A
mean+/-std		5.25+/-1.42	11.15+/-3.35	**11.08+/-2.17**	**14.15+/-3.00**	6.35	11.51+/-0.89

and 40 pps pulse rates (Table I). Relatively little fluctuation in M-wave amplitude was observed during the trains, as evidenced by the significantly lower variability measure (0.08 ± 0.05 and 0.07 ± 0.04 for 20 and 40 pps, respectively; $p < 0.05$). The partially fused force traces produced by IM stimulation had profiles that were consistent with regular recruitment of a stable subpopulation of motor units at the given stimulus frequency. The largest fluctuations seen for any IM electrode occurred in the chronically implanted preparation illustrated in Figs. 4 and 5. There was a gradual shift in the shape of the M-wave during the first five stimuli at 40 pps, but this change was not apparent in the force record and there were no M-wave oscillations later in the train. Such a shift would be consistent with the inevitable change in shape of the muscle during the rising phase of the contraction, which could affect the recruitment properties of the IM electrode and/or the recording properties of the EMG electrodes.

C. Histological Analyzes

The glycogen depletion results were consistent for IM stimulation and in agreement with the hypothesis that a mix of fiber types might be activated by IM stimulation. Fig. 6(a) shows the typical pattern of glycogen depletion following prolonged IM stimulation. Many, but not all, fibers in a sharply delimited territory were depleted; the depleted fibers had histochemical profiles characteristic of all three fiber types. The sharply delimited territories depleted by IM stimulation provided a clear contrast to other parts of the same section that contained strongly stained fibers and thus served as controls for staining. In the region con-

taining the highest density of PAS-negative fibers in one muscle, for example, 50% of type I, 45% of type IIa and 27% of type IIb fibers were depleted. In another muscle, the percentages were 30% of type I, 74% of type IIa and 67% of type IIb fibers. By contrast, depletion patterns following NC and HK stimulation were inconsistent and difficult to interpret. In one case, type IIb PAS-negative fibers could be found scattered throughout the section [Fig. 6(b)]. More commonly, when force profiles were unstable, all fibers throughout the section had variable but positive PAS reactivity. In these cases no analysis of the histological results was carried out. It should also be noted that a wide range of glycogen content in control, nonstimulated type I fibers made it difficult to ascertain whether or not the lightly stained profiles scattered throughout some sections of stimulated muscle reflected partial glycogen depletion.

D. Recruitment

There was a consistent difference between the recruitment slope of IM electrodes versus nerve stimulation elicited by either HK or NC electrodes, as illustrated in Fig. 7. Only very small increases in current (less than 5% of the available range of current intensities) were required to progress from twitch threshold to maximal twitch force when the nerve trunk was stimulated. Such a pattern was observed regardless of whether the threshold was very low (typical of most NC electrodes) or somewhat higher (typical of HK electrodes). The difference between NC and HK electrodes was probably due to the inter-contact spacing at 10 mm for NC versus 3 mm for HK electrodes. By contrast, IM stimulation never reached maximal recruitment
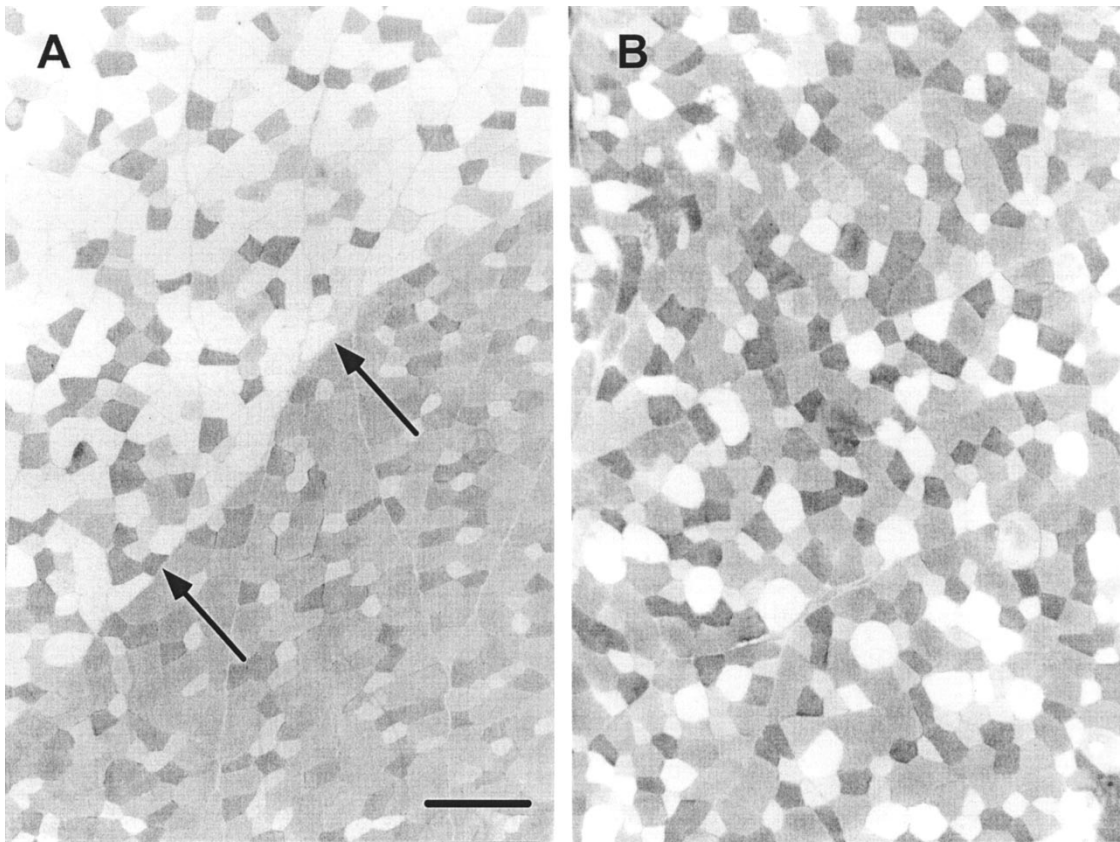
Fig. 6.   Histological sections, reacted for glycogen content (PAS reaction), were obtained from muscles stimulated with IM (A) and HK (B) electrodes. A clearly delimited region of depleted fibers (marked by arrows) was observed in sections obtained from muscles stimulated with IM electrodes. The depleted fibers corresponded to a mix of fiber types. Sections obtained from muscles stimulated with HK and NC electrodes demonstrated less consistent results. Occasionally, depleted fibers were scattered throughout the section and were identified as type IIb. More frequently, no fibers were clearly depleted in response to prolonged NC or HK stimulation. Scale bar $= 200 \, \mu$m.
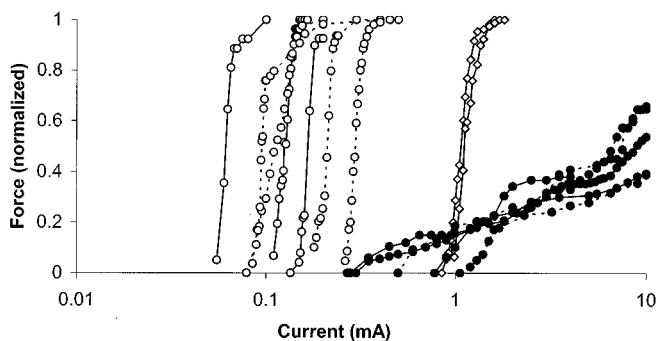

Fig. 7.   Normalized muscle twitch force was plotted as a function of current to demonstrate the recruitment characteristics of the different electrode types. Acutely (broken lines) and chronically (solid lines) implanted NC (open circles), HK (open diamonds), and IM (filled in circles) electrodes were studied. HK and NC electrodes demonstrated high rates of recruitment; maximal twitch force was achieved with small increases in stimulus amplitude above threshold. IM electrodes could only achieve a fraction of maximal twitch force at the maximal stimulus current available.

even when current amplitude was increased by a factor of 10–20 times threshold.

Table I shows the tetanus-to-twitch force ratios for the three different electrode types at the two stimulation rates examined. The mean ratio for NC stimulation was 11.15 ± 3.35 and 5.25 ± 1.42 at 40 and 20 pps, respectively. For IM stimulation, the ratios were 14.14 ± 3.00 and 11.08 ± 2.17. HK stimulation pro-

duced ratios of 11.51 ± 0.89 and 6.34, but the HK sample size was small ($n = 2$ and 1 for 40 and 20 pps, respectively). The NC and IM ratios were significantly different ($p < 0.05$). It should be noted that the peak tetanic tension was used to calculate the tetanus-to-twitch force ratios. The tension was rarely stable during NC stimulation, usually exhibiting several local maxima during a single train of stimuli (i.e., Figs. 4 and 5). Conversely, the peak tetanic tension measured during IM stimulation was achieved by about the third or fourth stimulus and was maintained throughout the rest of the train.

IV. DISCUSSION

A. Fatigue Resistance

In order to restore functional use of a paralyzed limb, it is necessary to obtain precise control over the force generated in response to electrical stimulation of many muscles. One obstacle to this control is fatigue of the activated muscle fibers. The problem is more problematic for FES than for natural use of an intact limb for several reasons:

1) electrical stimulation tends to recruit the largest diameter and most fatigable motor neurons first [13];
2) chronically paralyzed muscles tend to become more fatigable than normal as a result of disuse;
3) prosthetic control systems lack much of the robust sensing and reflex control apparatus that permits

biological systems to adapt gracefully to fatigue and other perturbations.

We hypothesized that FES applied in the muscle (IM) would produce less fatigue at a given force level because IM stimulation would produce less selective recruitment of fatigable units than stimulation applied to peripheral nerves (NC and HK). This would be predicted if intramuscular motor axons are more homogeneous in diameter and more widely dispersed as they branch within the muscle than they are in the nerves. We observed that force output from muscles stimulated IM decayed more slowly toward a higher plateau than when stimulated by HK electrodes (Fig. 3). These fatigue test profiles for IM stimulation are consistent with recruitment of a mix of motor unit types, including fatigue-resistant, fast and slow units presumably responsible for the persistent force plateau. This conclusion must be tempered by the larger problem of recruitment instability (see below), which caused us to reject all of the NC fatigue-test results.

The hypothetical mechanism for fatigue resistance was supported by glycogen depletion and fiber-typing in IM-stimulated muscles (Fig. 6). These showed the predicted glycogen depletion of a mix of different fiber types in a clearly demarcated compartment that we presume corresponded to the innervation zone of an intramuscular bundle of motor axons. The obverse finding that we expected in NC-stimulated muscles was selective glycogen depletion of fast, fatigable muscle fibers. Such results were found in a few muscles. However, we were hesitant to accept these data uncritically because inconsistent recruitment might exercise the more fatigue-resistant fibers insufficiently to deplete them.

B. Stability of Recruitment

In situations where recruitment was fluctuating, we saw inconsistent fatigue [e.g., Fig. 2(b)], incomplete glycogen depletion [Fig. 6(b)] and reduced tetanus/twitch ratios (Table I). If fluctuations in axonal excitability caused stimuli subsequent to the first pulse to recruit a reduced and shifting population of units (as suggested by Figs. 4 and 5), these units would then actually be firing at various subharmonics of the intended stimulus frequency. This fluctuating and asynchronous recruitment would actually be somewhat more physiological and perhaps advantageous in terms of producing less occlusion of the blood supply and less fatigue. However, the unpredictability of the force fluctuations would pose problems for fine control. Interleaved stimulation at several intramuscular sites could achieve the same goal but with better control.

We were surprised that instability of NC recruitment had not been noted before in the well-documented development of this technology. Several previous studies have examined the recruitment properties of different types of electrodes, but these have focused either on responses evoked by single stimulation pulses [7], [14], [15] or on peak forces during submaximal trains without examining M-wave or force fluctuations during those trains [13]. NC electrodes are commonly used in neurophysiological experiments to assure homogeneous supra-maximal stimulation [16], in which case a steep recruitment curve can guarantee the stable, complete recruitment of an entire muscle.

The present series of experiments suggests that our particular NC electrode design would be less effective than IM electrodes to produce consistent activation of a muscle at steady submaximal force levels. If the mechanism that we propose below is correct, then this problem is likely to be shared by most nerve cuff electrodes in which the stimulation contacts surround the nerve and produce homogeneous current densities within the nerve. Designs with small contacts distributed around the circumference of the nerve have been developed to permit the selective stimulation of nerve fascicles supplying individual muscles [17]. These more sophisticated electrodes might be expected to have a larger spatial gradient of current density and, hence, shallower and more stable recruitment curves. These designs are more like the asymmetrical interface produced by HK electrodes, which had higher thresholds but still produced steep and unstable recruitment patterns like those produced by NC electrodes in our experiments. Even designs that use intraneural wire electrodes produce steep recruitment curves in the middle (5–30%) of the force range [18], suggesting that they may experience similar instability problems when used for typical FES applications.

C. Fluctuations in Excitability

The M-wave and force fluctuations that we observed from pulse-to-pulse within brief trains of NC stimulation (Figs. 4 and 5) suggest a different mechanism for recruitment instability from those that have been considered previously in FES applications. The fluctuating nature of the recruitment is similar to, although somewhat less regular than that described recently for high frequency intracochlear stimulation of the auditory nerve [20]. In both systems, the first evoked potential of the train was usually the largest, followed by alternating or otherwise cyclically modulated amplitudes in successive pulses of the train. In the cochlea, this nonuniformity was attributed to the relative refractory period of myelinated neurons when stimulated with interpulse intervals of 0.5–2.0 ms.

Changes in the excitability of myelinated peripheral nerve axons have been reported to last as long and even longer than the inter-stimulus intervals used in the present study (25 and 50 ms). A subtle but prolonged refractoriness was noted to affect repetitive electrical stimulation near threshold as early as 1933 [1], but the mechanism remains unclear. Likely candidates include local accumulations of extracellular potassium [21], [22] and perhaps intracellular fluctuations of other ions such as sodium and calcium. The mechanisms of ion diffusion and clearance by Schwann cells and capillary perfusion could have the long time constants implicit in the slow oscillations of the M-waves that we saw at 40 pps. Fluctuations in oxygenation and glucose supply may also be important.

Kiernan *et al.*[23] have examined the recovery cycle of motor and sensory axons in the human median nerve. In this cycle, the usual axonal refractory period is followed by periods of supernormal excitability (occurring between 5–20 ms after the initial impulse) and subnormal excitability (from 20 to 100 ms after the initial impulse). The shortest inter-pulse interval that we used was 25 ms, which is within the range of time during which previously stimulated axons may be somewhat less excitable.

Bostock *et al.* [24] have reviewed experiments in which axonal excitability thresholds were found to vary following single impulses. They attributed the changes in excitability to changes in membrane potential that arise as a result of ion channel kinetics. In addition, Stys and Waxman [25] suggested that increases in K^+ conductance and activation of the Na^+–K^+–ATPase could contribute to increases in the threshold for excitation.

The long lasting changes in excitability described above might be expected to occur during intramuscular stimulation of axons, but their effects are likely to be greatly attenuated over those in densely packed peripheral nerves. The axon branches that are stimulated by IM electrodes are dispersed within a well-perfused muscle, which would tend to reduce ion and metabolite accumulations that might affect their excitability. Even if the fluctuations in excitability were the same in nerve and in muscle, they would still cause much larger effects on recruitment in the nerve. Nerve cuff electrodes with circumferential contacts are designed to produce homogeneous current densities throughout the nerve, resulting in the steep recruitment curves that we and others have reported. With most of the axons operating near threshold, small changes in excitability will cause large changes in the total number of recruited axons. Intramuscular stimulation results in a shallow recruitment curve because the motor axons to be recruited sit at varying distances from the stimulating electrodes. At a given level of submaximal recruitment (e.g., the 20% used in our experiments), axons very close to the electrode will be well above threshold and those far from it will be well below threshold. Small changes in excitability should therefore affect recruitment of only those few axons that happen to be located at the edge of the recruitment zone and are operating near threshold.

D. Steepness of Recruitment

NC stimulation is known to be associated with steep recruitment curves [19]. This has lead designers of FES stimulators to specify fine control of stimulus current and pulse duration in order to achieve fine control of recruitment [15]. Fluctuations in stimulus intensity were not a problem in the precisely current-regulated laboratory stimulator that we used in these experiments, but this might be a factor in portable FES equipment for use with NC electrodes. Steep recruitment curves are also known to be associated with vulnerability of recruitment levels to small movements of the electrode with respect to the nerve [5], suggesting the importance of careful placement, surgical anchoring and connective tissue fixation of chronically implanted NC electrodes. In our acute experiments, the relatively large distance between the nerve stimulation site and the contracting muscle plus the measures taken to stabilize the nerve should have minimized this problem, although inadvertent motion may have contributed to abrupt changes in force level in the middle of some of our fatigue tests [e.g., Fig. 2(b)]. Contractile motion does not seem to be a plausible explanation for the continuously fluctuating force and M-wave amplitudes during trains of steady stimulation in all of our HK and NC preparations, both acute and chronic (Figs. 4 and 5). The present study suggests that steepness of NC recruitment (Fig. 7) is associated with instability of recruitment that is independent of the precision of the stimulator or the mechanical stability of the electrode on the nerve. It is not clear if or how this problem might be overcome.

Steep recruitment does not appear to be a problem when stimulating with IM electrodes constructed and implanted as described here. If such electrodes were to be implanted closer to the entry point of the muscle nerve, however, recruitment would become steeper and, we would predict, less stable. Grandjean and Mortimer obtained shallow recruitment when they positioned monopolar epimysial electrodes opposite the nerve entrance in the cat soleus but they obtained steep recruitment curves similar to NC stimulation when the same electrodes were placed over the nerve entry zone [14].

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Frances J. R. Richmond received the Ph.D. degree in neurophysiology from Queen"s University, Kingston, ON, Canada, in 1976.

She completed her Postdoctoral studies at the National Institutes of Health and the Université de Montreal. She was on faculty at Queen"s University for 25 years, where she most recently held a position as Professor and Director of the Medical Research Council Group in Sensory-Motor Neuroscience. In 1999, she moved to the University of Southern California, Los Angeles, where she presently holds the position of Director of Clinical and Regulatory Science in the Alfred E Mann Institute for Biomedical Engineering and Adjunct Professor in the School of Pharmacy. Her research interests include both basic and applied studies of movement systems. She has experience in animal and clinical trials of implantable devices and consults on regulatory aspects of medical product development. She is currently establishing a Master's level program in Regulatory Science at the University of Southern California.

Kan Singh was born in Brampton, Canada, in 1974. He received the B.Sc.H.degree in life science in 1997 and the M.Sc. degree in physiology in 1999, both from Queen's University, Kingston, ON, Canada. He is currently working towards the Ph.D. degree in the Department of Anatomy and Cell Biology at Queen's University, where he is studying human reaching movements and adaptation to novel dynamic environments.

His undergraduate research project involved examining the effects of chronic electrical stimulation of muscle. His graduate work examined the recruitment properties of nerve cuff and intramuscular stimulating electrodes. His other research interests examine morphometric and histochemical properties in neck and arm musculature.

Gerald E. Loeb received the B.A. and M.D. degrees from the Johns Hopkins University, Baltimore, MD, and surgical training at the University of Arizona, Tucson.

From 1973 to 1988, he was an Intramural Research Scientist and Chief of the Section on Neurokinesiology in the Laboratory of Neural Control, NINDS, National Institutes of Health. He was Professor of Physiology and Director of the Biomedical Engineering Unit at Queen"s University, Kingston, ON, Canada, until 1999, when he moved to his present position as Professor of Biomedical Engineering at the University of Southern California, Los Angeles, and Director of the Medical Device Development Facility in the affiliated A.E. Mann Institute for Biomedical Engineering. He has consulted widely in the medical device and instrumentation industry and served as Chief Scientist to Advanced Bionics Corporation, Sylmar, CA. His research activities are in sensorimotor neurophysiology and neural prosthetic medical devices.