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Nonlinear muscle recruitment during intramuscular and nerve stimulation

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Abstract—Future progress in neuromuscular prostheses will depend on developing techniques for stimulating paralyzed muscle, especially utilizing neuromuscular stimulation. We have found nonlinear force versus stimulus amplitude characteristic (recruitment) curves in the gastrocnemius-soleus-plantaris muscle group of the cat in response to stimulation of the tibial nerve near the muscle entry point. Such response characteristics are undesirable in neuromuscular control systems. Nonlinear recruitment curves usually consisted of two regions in which force increased linearly with stimulus amplitude, separated by a "plateau" region in which force was relatively constant. The two linear regions were associated with activation of separate neuromuscular compartments (lateral or medial gastrocnemius, plantaris, soleus, or subdivisions of those muscles). When the stimulated myoelectric responses from these compartments were plotted versus stimulus amplitude, the region of recruitment between threshold and saturation often did not appreciably overlap for different compartments, suggesting that the axons innervating those compartments were physically segregated within the nerve from axons innervating other compartments. Correlation coefficients between force and stimulated myoelectric response were very high (up to $R^2 = 0.99$) when using a composite curve produced by averaging myoelectric response curves recorded from each of the active compartments. By dividing the tibial nerve into its component bundles or fascicles and stimulating each in turn, it was possible to show that individual bundles innervate non-overlapping groups of muscle compartments, and that recruitment of the nerve bundles over different threshold ranges could account for the nonlinear force/stimulus response curves initially observed. The presence of separate innervation of muscles or compartments by fascicles should be an important factor in designing functional neuromuscular stimulation (FNS) systems.

Key words: *EMG, fascicle, force, functional neuromuscular stimulation, FNS, muscle, nerve, recruitment.*

INTRODUCTION

In studies involving peripheral nerve stimulation, it is usually assumed that the axons in the nerve are more or less randomly distributed with respect to size. When the distance between electrodes and axons are similar, the largest axons, which have the lowest input impedance, are recruited at the lowest stimulus strengths (22,32). These larger axons tend to innervate more and/or larger muscle fibers. Since the fast-fatigable fibers tend to be larger, they are recruited at the lowest stimulus thresholds (3). As stimulus amplitude is increased, smaller axons and hence smaller muscle fibers, which generally have greater oxidative capacity and are therefore more fatigue-resistant, are recruited. This orderly recruitment of large to small axons/muscle fibers is the reverse of that arising from natural activation of the motoneurons (14,21).

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This reverse recruitment phenomenon has important implications for functional neuromuscular stimulation (FNS) studies investigating the control of paralyzed muscle by electrical stimulation (31,38).

In FNS studies, muscular responses to electrical stimulation are often characterized using a recruitment curve of muscle force and/or myoelectric activity in response to graded amplitude stimuli. In such cases, stimulation may be applied via surface (37) or intramuscular electrodes (25,26), or directly to the nerve via nerve cuff (2,16,21) or hook electrodes. Most reports in the literature of muscle electrical and contractile responses to stimulation show simple sigmoidal recruitment curves which usually have a region which can be approximated by a linear (first order) equation (5,28,29).

Some studies have also reported higher order or piece-wise linear relationships containing "plateaus" (1,5,13,20). In such cases, with increasing stimulus amplitude there is an initial linear increase in muscle force, followed by an intermediate plateau or leveling off of force, and then a second increase in force, followed by a final plateau or saturation. Such nonlinear recruitment curves pose a problem for FNS control systems in that they may represent non-unique solutions for obtaining a given output, and therefore require more complicated equations for transforming the command input into desired force output. One solution that has been used is to break the force curve into piece-wise linear segments, each represented by a first-order equation. Such discontinuities increase the difficulty of implementing FNS control systems and may reduce their reliability (5,33).

In our own FNS experiments, we became concerned about variabilities in the correlation between myoelectric and contractile responses to stimulation of the gastrocnemius muscle, and differences in recruitment curve shapes in different preparations in general. On the basis of intramuscular recordings in response to whole nerve or intramuscular stimulation (13,20), we theorized that the nonlinearities between force, myoelectric response, and stimulus amplitude might be caused by independent activation of the individual ankle extensors innervated by the tibial nerve. It has been shown, for example, that the lateral gastrocnemius (LG) can be decomposed into four subdivisions, or compartments, innervated by separate primary nerve branches (8,9,10,17). These nerve branches or fascicles can continue for several cm proximal to the gastrocnemius, allowing the tibial nerve to be surgically subdivided into discrete bundles innervating various regions of the lateral and medial gastrocnemius (MG) and other ankle extensors.

To test whether the organization of the axons into discrete fascicles is responsible for the intermediate plateaus seen in force and myoelectric activity, we made intramuscular recordings from the LG, MG, plantaris, and soleus muscles of the cat hindlimb while stimulating isolated bundles of the tibial nerve near the entry point of the gastrocnemius muscle. The following findings are reported here: 1) a high degree of correspondence between stimulation of individual bundles and stimulated myoelectric responses (SMR) in one or more of the ankle extensors or their compartments; 2) that nonlinear force curves resulting from whole nerve stimulation could be decomposed into segments correlated with activation of different extensors as identified by myoelectric activity; 3) compound action potentials (CAP) recorded from a single bundle during whole nerve stimulation were found to be highly correlated to SMR of a single extensor muscle; and, 4) when individual SMR from each of the LG, MG, and soleus are averaged together, the correlation between ankle force and average SMR is significantly higher than between force and the SMR from any single muscle.

Since there may be some ambiguity in the use of the term "fascicle," we will define our usage of this and related terms. Peripheral nerves are surrounded by a connective tissue sheath, the epineurium. They are usually further subdivided into fascicles (or "bundles" of axons) each surrounded by another distinctively staining connective tissue sheath, the perineurium (34). Within each fascicle, the axons are embedded in a connective tissue matrix, the endoneurium. Finally, perineurial septi course through the fascicles, further segregating the axons. In this paper, the term fascicle will be reserved for *the smallest group of axons surrounded by perineurium at a given point on the nerve,* while a bundle may represent one or more fascicles. Fascicles can be readily identified histologically, but not always surgically. It is important to note that the definition of "fascicle" is a dynamic one, varying along the length of the nerve. Axons become more clearly segregated into fascicles towards the periphery. As Truex and Carpenter (34, p. 169) note:

"These fascicles do not run like isolated cables, but may split at acute angles and connect with adjacent fascicles for an interchange of fibers." The fascicles which ultimately innervate neuromuscular compartments are termed "primary nerve branches" (8,9).

METHODS

These experiments involved 13 cats. The stimulating and recording protocols varied slightly for different experiments due to our desire to focus on different aspects of the nonlinear recruitment phenomenon and our ability to define different muscular compartments. The illustrations shown below are derived from experiments on 5 representative cats. The details of the stimulation and recording protocols are provided along with the results.

For the purposes of this paper, we will consider the LG to consist of lateral, intermediate, and medial subdivisions (LGl, LGi, and LGm respectively). These subdivisions can usually be delineated by visual inspection (8,9). It should be noted that four neuroanatomical compartments of the LG (LG1, LG2, LG3, and LGm) can be defined on the basis of innervation by primary nerve branches (8,9). Since this distinction is not crucial to our purpose, and we could not specifically distinguish LG1, LG2, and LG3, we will use the anatomical nomenclature.

Surgical procedures

At the beginning of each experiment, the animal was anesthetized with pentobarbital i.v. and maintained on saline drip with supplemental pentobarbital administered as necessary to suppress pinna and toe pinch reflexes. The animal was placed on a table heated by circulating water. The water temperature was adjusted to maintain body (rectal) temperature at 37 degrees Celsius. To expose the triceps surae, the biceps femoris was reflected back after cutting its insertion along the tibia. The sciatic nerve was dissected free of connective tissue for 5 cm proximal to its entry into the gastrocnemius and bathed in saline. The leg was immobilized by first inserting bone taps at each end of the shaft of the femur and clamping the taps to a metal frame mounted on the table. The distal end of the tibia was affixed to the same frame using a three-pronged clamp. The foot pad (metatarsals) was taped securely to a force transducer (Revere, Wallingford, CT, Model FT-70, 10 kg max). Tendon forces were calculated by taking the ratio of the lever arms from the calcaneal insertion of the gastrocnemius to the center of the ankle joint and from the joint center to the force transducer.

The sciatic nerve was divided into peroneal and tibial branches approximately 4 cm proximal to the gastrocnemius. The peroneal nerve was cut along with the branch of the tibial nerve to the biceps femoris. In six experiments, a bipolar helical nerve cuff electrode was wrapped around the intact tibial nerve at the muscle entry point. This electrode was made of a 1 cm piece of silastic tubing (Dow Corning #601-325, 2.64 mm ID, 4.87 mm OD) cut into a helix with turns 3 mm apart. A pair of multi-stranded SS wires (0.25 mm OD, teflon coated; Med Wire Inc., Mount Vernon, NY) with the insulation stripped 1 cm were inserted through adjacent turns at the center of the helically-cut tubing and then wound in opposite directions one complete turn along the inside wall. The ends of the wires were then led out through the silastic wall and the entry and exit points coated with silastic rubber (Dow Corning #382 medical grade elastomer).

In 7 cats, the tibial nerve was subdivided into smaller bundles of axons between 1 and 3 cm proximal to the gastrocnemius. Fine dissecting scissors were used to cut the epineurium and the bundles were teased apart with fine forceps and glass probes. This sometimes involved rupturing the blood vessels running alongside and between the bundles, although the blood supply was maintained as much as possible. Ordinarily, the tibial nerve immediately separated into 1 large and 2 smaller bundles. Depending on the preparation, these were then further subdivided into a total of about 7–8 bundles. After separation, the bundles were isolated for purposes of electrical stimulation and ease of handling by placing plastic polyethylene strips between them. Nerve bundles were stimulated by gently lifting and placing them on a pair of tungsten or platinum hook electrodes, 3 mm apart, and raising them out of the saline for the period of stimulation (1–2 minutes). This procedure did not appreciably damage the axons, since bundles could be re-stimulated at later times during the experiment with little change in muscle response, although stimulus threshold usually changed due to differences in electrode positioning. Bundles could also be

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stimulated while immersed in saline, albeit with higher threshold. To insure that current spread was not activating adjacent nerve fascicles, the proximal ends of the bundles were cut in two animals and then re-stimulated. Since a differential isolated stimulator was used, cutting one end of the nerve proximally limited the stimulus current to that end, greatly reducing the possibility of stimulating any other bundles. In such cases, no change in the pattern of muscle activation was seen.

Muscle and nerve recording

Intramuscular recording electrodes were made from the same multistranded wire as the nerve cuff electrodes. The electrode wire was bared of insulation at the tip for 1 mm (yielding approximately 0.9 mm^2 surface area) and inserted into the muscle using a 23-gauge hypodermic needle. The end of the electrode was first inserted into the beveled end of the needle so that approximately 1 mm of insulation was inside the barrel. The rest of the wire was then bent back alongside the shaft and the needle inserted approximately 3 mm into the muscle and withdrawn while holding the wire, leaving the electrode "hooked" inside the muscle. In order to determine the extent of crosstalk in the intramuscular recordings, a bipolar pair of electrodes similar to those used for recording, was connected to the stimulator outputs and inserted 3 mm deep into the LG approximately 3 mm lateral to a pair of differential recording electrodes (also in the LG). Both differential pairs of electrodes were spaced 2 mm apart along the main axis of the muscle. A 200 mV (60μA pk-pk), 500 Hz sine wave was then applied to the stimulating electrodes which did not result in any visible contraction. The signal seen at the recording electrodes was less than 2 mV peak-to-peak. Thus, little crosstalk should be expected at the recording electrodes due to localized current sources more than a few mm distant.

Recordings were made from the LG, MG, plantaris, flexor hallucis longus (FHL), and soleus muscles. As shown on pages 6–9 in **Figures 2, 3, and 4**, recordings were made at three equally spaced sites along the LGl, LGi, LGm, MG, and soleus muscles. For monopolar recordings, the reference electrode was a 23-gauge needle inserted subcutaneously at the base of the tail. The animal was grounded to the table via a separate needle electrode and via the bone-taps connected to the table frame. Myoelectric signals were amplified using a X100 instrumentation amplifier with a bandpass of 10 Hz to 20 kHz and an input impedance reduced to 1 MΩ. Twelve myoelectric signals were simultaneously digitized by a Data Translation #DT 2821-F-16SE A/D converter at 8250 samples per sec for 11 msec. The force transducer signal was digitized at 267 samples per sec for 350 msec. Data was acquired, stored, and displayed on an IBM-AT computer. Stimulation and recording was automatically controlled by the computer. A series of clock-timers were programmed to initiate acquisition and at a latency of 1 msec, deliver a biphasic pair of pulses 200 μsec positive followed by 200 μsec negative. These pulses were multiplexed with an analog voltage provided by the DT2821 D/A converter and fed to a WPI Linear-Iso III voltage-to-current stimulus isolation unit which provided the stimulus pulses. To generate a recruitment curve, the threshold and saturation points of the muscle or nerve were determined, and then approximately 20 pulses with an interpulse interval of 5 sec were delivered within this amplitude range. The series was sometimes repeated using reversed polarity or decreasing amplitude pulses. There was no difference in the curves using increasing versus decreasing stimulus pulses.

Data analysis

Data analysis consisted of calculating the peak-to-peak amplitude of the SMR and nerve bundle compound action potential during the 10 msec interval following the end of the stimulus artifact. Average SMR curves were generated by computing the mean of the peak-to-peak SMR values for the muscles concerned at each stimulus amplitude. The peak force, which typically occurred at about 30 msec, was also computed. Data were transferred to a Macintosh computer for plotting and analysis. Correlations between force, SMR, and nerve CAP amplitudes were performed using commercial statistics programs.

Histological analysis

At the end of the experiment, the animals were sacrificed by nembutal overdose. The ankle extensors and attached tibial nerve were removed and placed in 10 percent neutral formalin. The muscles were later dissected and electrode positions checked by visual inspection. In some animals, the tibial nerve was embedded in paraffin, sectioned at 12μ, and stained with Luxol Fast Blue.

RESULTS

Presence of intermediate plateaus in force and SMR recruitment curves

Stimulation of the tibial nerve with hook or cuff electrodes often produced sigmoidal force and SMR curves similar to those shown in **Figure 1A**. In this experiment, SMR were recorded differentially using two monopolar electrodes in different parts of the gastrocnemius. These curves contain regions which can be approximated by linear equations. In **Figure 1A**, for example, between 530 and 630 μamps, the slopes of the force and SMR curves are 29 g/μA and 0.25 mV/μA respectively.

Reversing the stimulus polarity in this experiment resulted in substantially different characteristic curves as shown in **Figure 1B**. Here, there are two linearly increasing regions in the SMR and force curves separated by an intermediate plateau between 530 and 610 μamps.

This plateau phenomenon is not unique to nerve stimulation as seen in **Figure 1C**. Here, in a different experiment, force and SMR were recorded during intramuscular stimulation of the gastrocnemius. Differential stimulating electrodes were located in the LGl at the level of the motor nerve entry zone, while a pair of differential recording electrodes were placed in the LGl approximately 1 cm distally, near the middle of the muscle. An intermediate plateau can clearly be seen in the force, but not the SMR, curves in this case. (The SMR curve has been scaled to show that it follows the force curve during the first rise.) SMR and force curves are shown for both increasing and decreasing amplitude stimulus series to demonstrate that hysteresis is not causing the intermediate plateau in the force curve. The force plateau phenomenon appeared to be due to successive recruitment of different portions of the muscle at different stimulus thresholds. In order to explore this possibility, multiple simultaneous recordings were made of the cat ankle extensors during nerve and intramuscular stimulation.

Segregation of recruitment thresholds during nerve stimulation

In the experiment shown in **Figure 2**, bipolar intramuscular recordings were made from the LGl, LGi, LGm, MG, soleus, plantaris, and FHL. The tibial nerve was stimulated using a helical cuff

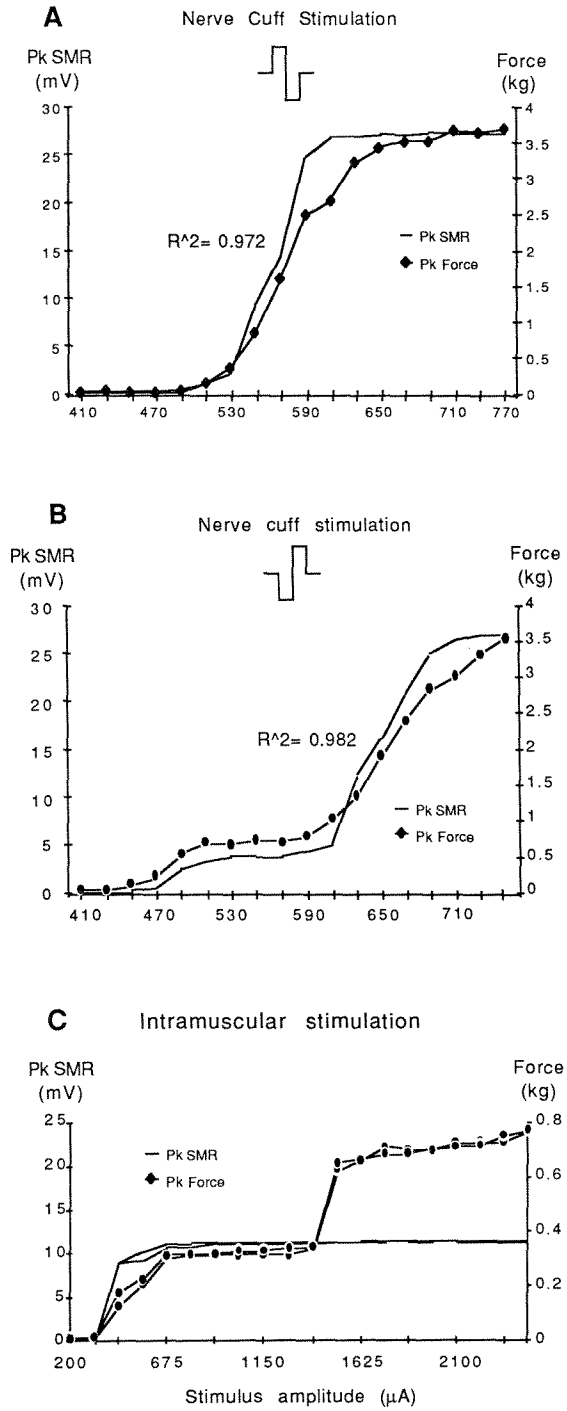

Figure 1.
Examples of force and myoelectric waveforms recorded in response to nerve cuff and intramuscular stimulation. A) Typically, sigmoidal curves are seen in response to stimulation, with a rather steep slope in relation to stimulus current. B,C) In both nerve cuff and intramuscular stimulation, nonlinear responses can also occur in which there are two or more "linear" regions separated by plateaus. If the recording involves only one neuroanatomical compartment, the SMR is normally sigmoidal.

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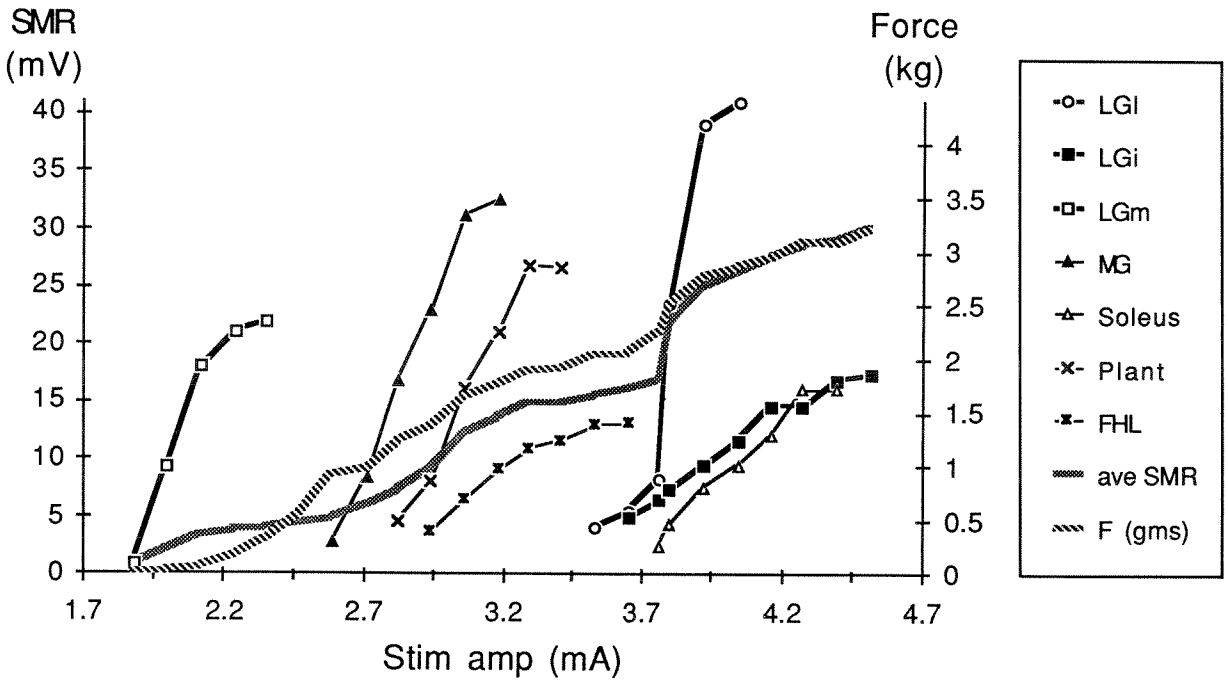

Figure 2.
Stimulation of the tibial nerve near the muscle entry point, while recording from several neuroanatomical muscle compartments, reveals considerable variation in recruitment threshold and slope. The curves appear to be divided into three groups, between which there is minimal overlap of their effective stimulus ranges.

placed adjacent to the belly of the gastrocnemius. In order to assess the effects of electrode geometry and stimulus waveform, both monophasic and biphasic stimuli of both polarities and monopolar and bipolar configurations were used. **Figure 2** shows the simplest case with monopolar (cathodic) monophasic pulses applied. For clarity, only the portions of the SMR recruitment curves between approximately 10 percent and 90 percent of saturation are shown. It can readily be seen that there are at least three clear threshold groups. Moreover, there is little overlap of recruitment range between the three groups. Similar results were obtained with monopolar biphasic, bipolar monophasic, and bipolar biphasic stimulation, although thresholds were lowest with the last [see also (8)]. There is little evidence of a plateau effect in the force curve due to the relatively continuous recruitment of compartments as a whole. Recruitment of LGl produced a marked increase in both average SMR and force curves, suggesting it was a relatively strong part of the muscle.

Correspondence between nerve fascicles and muscular compartments

Figure 3A shows the results of an experiment in which the tibial nerve was stimulated using tungsten hook electrodes and biphasic stimulus pulses. The recording conditions were as described for **Figure 2**. The recruitment curve for each extensor muscle is slightly different, and the MG shows a significantly lower threshold. The force curve does not resemble any of the individual recruitment curves. When the average of the four individual SMR recruitment curves was calculated, it was found to be highly correlated to the force curve. The linear correlation coefficient between force and average SMR recruitment curves was $R^2 = 0.99$ for a first order curve fit.

To determine whether the individual extensor muscles were indeed being recruited by separate fascicles, the tibial nerve was then divided into three major bundles: B-I, B-II, and B-III, which were stimulated independently. These results are shown in **Figure 3B**. Here raw SMR and force curves are shown during stimulation of each bundle. Bundle

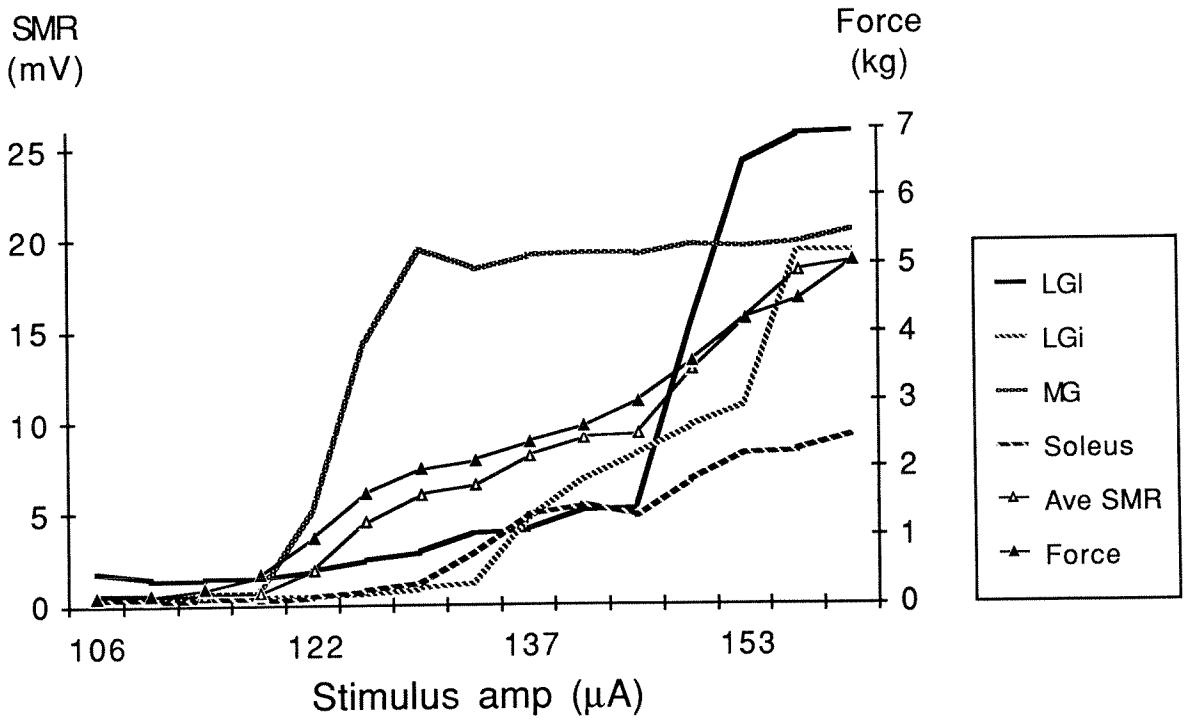

Figure 3A.
In this experiment, whole nerve stimulation revealed a much lower threshold for activation of the MG than for LGl, LGm, or soleus in which the MG was completely recruited before threshold for the other compartments was reached.

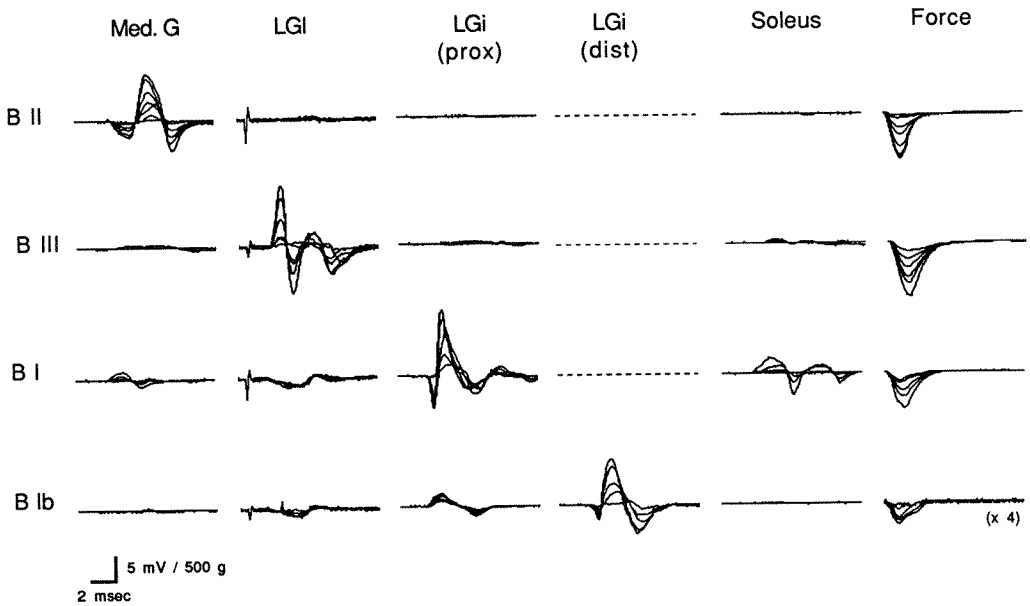

Figure 3B.
SMR from the individual compartments in response to stimulation of individual nerve bundles. Note that each bundle selectively activates one, or in some cases, two compartments. Bundle B-Ib is a fascicle within bundle B-I. The force curves in response to stimulation of bundle B-Ib are magnified 4×.

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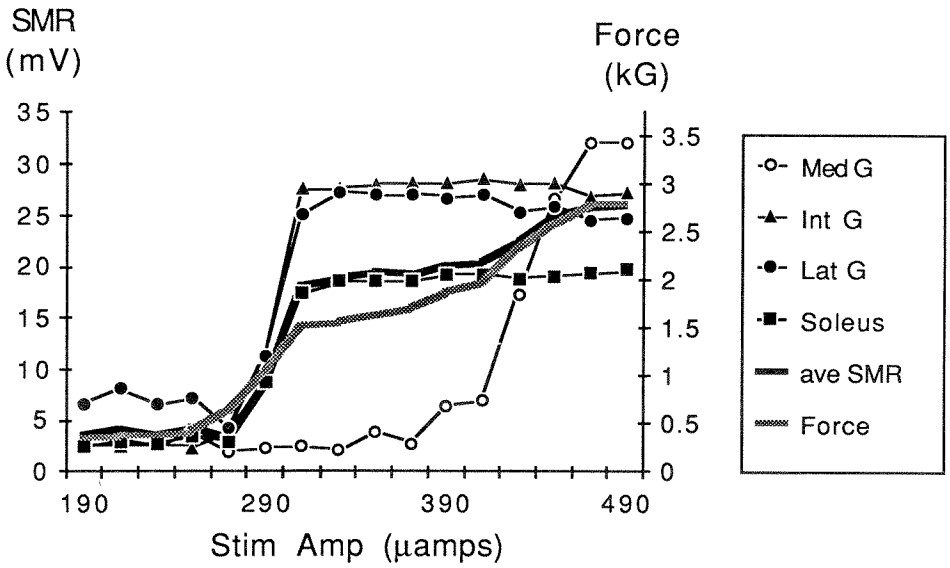

Figure 4A.
Another experiment in which whole tibial nerve stimulation resulted in sequential activation of compartments. Here, MG threshold is much higher than LGm, LGl, or soleus. Division of this nerve into bundles allowed isolation of one bundle.

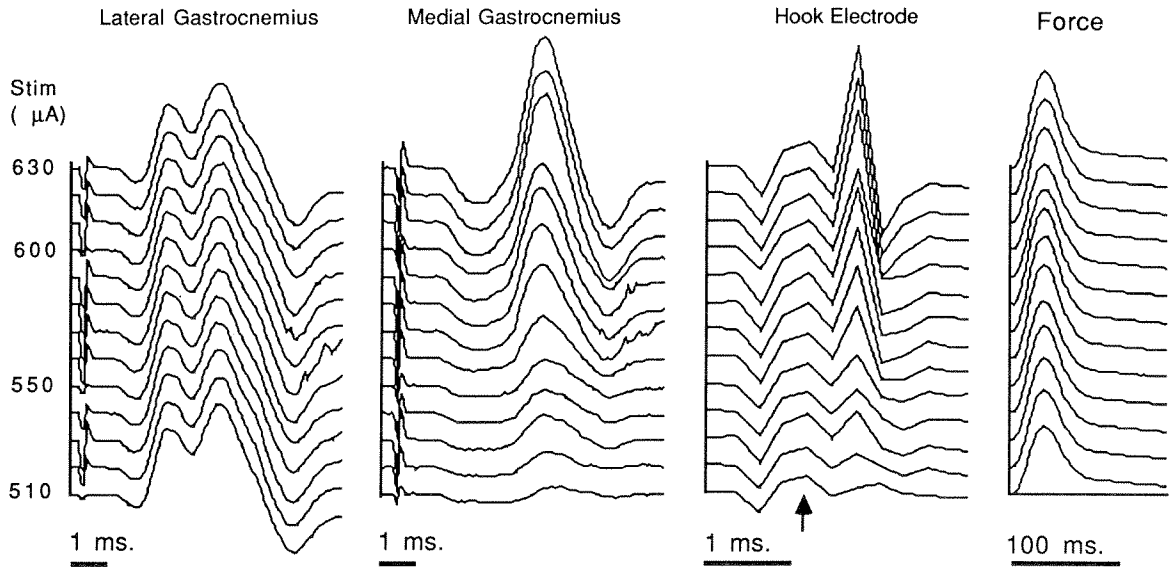

Figure 4B.
One bundle of the nerve which selectively activated MG was isolated and placed on hook electrodes. The whole nerve was then stimulated and the nerve CAP from the isolated "MG" bundle compared with activation of MG and LG. Note different time scales for CAP and force; arrow under CAP indicates end of stimulus artifact.

B-III selectively innervates the LGl, B-II the MG, and B-I the LGi and soleus. During stimulation of B-I, there appears to be either some myoelectric crosstalk or else incomplete segregation of axons resulting in a small degree of activation of LGl and MG. Bundle BI, which was the largest, was further subdivided into three smaller bundles. Stimulation of one of these sub-bundles produced a response similar to that of the whole bundle (not shown), while stimulation of another sub-bundle (BIb) produced a noticeable twitch with little activity in any of the recording electrodes. By probing the muscle with needle electrodes during stimulation, a localized area within the distal half of the LGi was found which showed a sizeable myoelectric response **(Figure 3B)**. The activation of proximal and distal

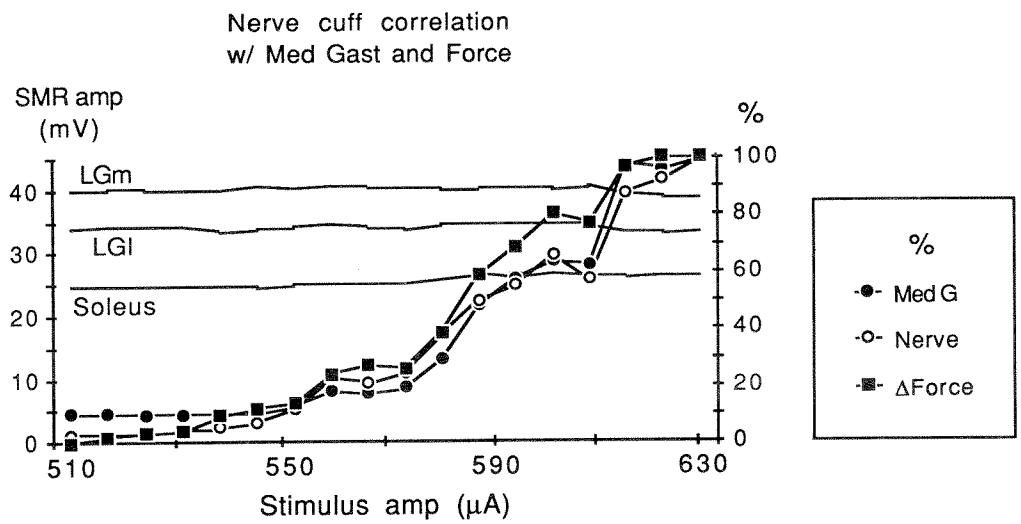

Figure 4C.
Recruitment of LG and soleus (solid lines, scale left) versus MG SMR, MG bundle CAP ("nerve"), and muscle force on expanded stimulus scale to show the high degree of correlation between bundle activation, MG activity, and muscle tension. Muscle tension (△ Force) shown as increase in force from onset of MG activity (1.72 kg). MG SMR and nerve activity shown as percent max.

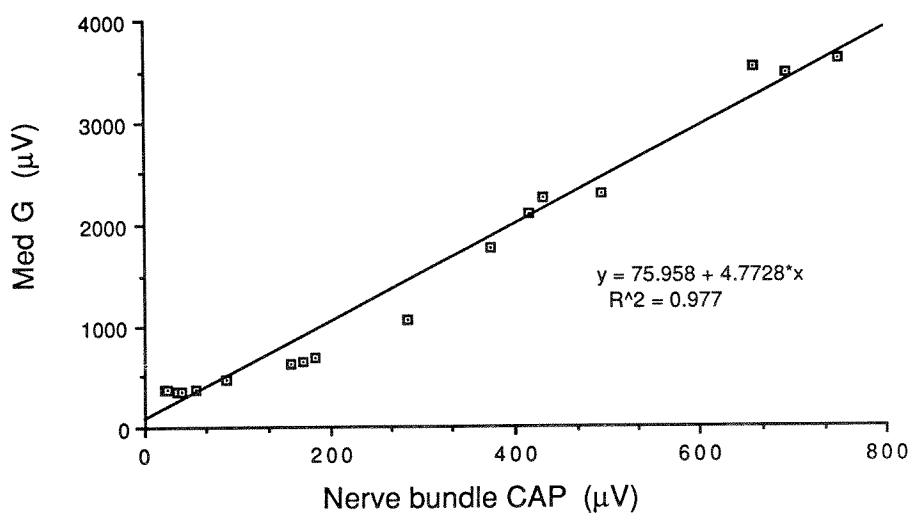

Figure 4D.
Linear correlation between nerve bundle CAP and MG SMR activity.

portions of LGi by separate bundles is in agreement with the findings of English and Weeks (10), who divided LGi into corresponding LG2 and LG3 neuroanatomical compartments, each with its own primary nerve branch.

The foregoing results suggested that intermediate plateaus in the force curves resulted from the recruitment of fascicles at different thresholds during nerve stimulation, with each fascicle activating a different compartment. In order to confirm that axons in an individual fascicle could be selectively recruited apart from axons in other fascicles during whole nerve stimulation, experiments were performed in two cats in which the whole tibial nerve was stimulated with a nerve cuff electrode while recording from isolated bundles proximal to the cuff. A helical nerve cuff electrode, in which two wires each completely encircle the nerve, was used in order to produce a more uniform current flow through the epineurium. The results from one of these experiments are shown in **Figures 4A-D**. During whole nerve stimulation (**Figure 4A**), the

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MG was found to be recruited at a significantly higher threshold than the other ankle extensor muscles. The correlations between force and SMR for LGl, LGm, MG, and soleus over the full stimulus range were 0.83, 0.88, 0.53, and 0.91 respectively. By stimulating each tibial nerve bundle in turn, using small hook electrodes, a single bundle was found which activated the MG. The antidromic nerve CAP in that bundle was then recorded using the same hook electrodes while the whole nerve was stimulated. The relationship between the MG bundle CAP and SMR was then examined over a narrow stimulus range (**Figures 4B,C**). The stimulus range was slightly different from that shown in **Figure 4A** due to rinsing of the nerve with saline. The raw records shown in **Figures 4B,C** demonstrate that while the LGl, LGm, and soleus were fully recruited at 510 μA, the MG and the nerve CAP showed only a very small response at that amplitude. Increase in the force curve and recruitment of MG bundle CAP and SMR then occurred in parallel up to 625 μA where saturation occurred. Although the digitizing rate was lower than desirable for the nerve CAP, the signal was adequate for determining the peak-to-peak amplitude. The recruitment curves produced from this data are shown in **Figure 4C**. The MG and nerve CAP responses are shown as percent of maximal response (100 percent = 36.2 mV for MG and 7.5 mV for nerve CAP). Force is plotted as a percentage of the maximal response over the stimulus range 510-630 μA ($\triangle$ Force; 0 percent = 1.72 kg, 100 percent = 3.17 kg). The MG SMR, MG bundle CAP, and $\triangle$ Force curves all appear to be highly correlated. In particular, the dip at 610 μA (the cause of which is unknown) did not occur in the LGl, LGm, or soleus. A linear regression was performed between MG SMR and MG bundle CAP (**Figure 4D**), and a highly significant correlation was found ($R^2 = 0.977$; $p < 0.001$, t-test).

The nerve from this experiment was sectioned just distal to the point where it was surgically divided into bundles and stained to determine the relative positions of the fascicles. A representative section is shown in **Figure 5** in which the compartments activated by each of the fascicles observed in the photomicrograph are noted. When surgically dividing this nerve, it first separated into three bundles (A, B, and C), one of which (A) then further subdivided into three sub-bundles (A1, A2, and A3). The connective tissue surrounding and separating bundles A, B, and C was not specifically detected histologically at the level of this section. Bundles A1 and A3 each appear to consist of two fascicles. At the time of the experiment, it was not apparent that either bundle A1 or A3 was surgically divisible, possibly because they had not completely separated into fascicles at that point. The prominent spaces in fascicles A1b and A2 are artifact, apparently where axons have separated along the perineurial septi due to distortion of the nerve prior to embedding. Bundle C appeared to innervate LGl, LGm, and soleus. It is noteworthy that the MG fascicle is somewhat removed from that carrying axons to the other compartments. A magnified (8.6×) image of the edges of fascicles A2 and B2 and the intervening connective tissue is shown to the right. The dark-staining perineurium ("p") around each bundle is easily identifiable, while the connective tissue in between the fascicles appears more loosely organized. Axons in the fascicles can be identified as donut-like structures in which the outer ring is the myelin sheath.

DISCUSSION

While investigating muscle recruitment by nerve and muscle stimulation, we quickly became aware that the ankle extensor muscles were not being uniformly activated in many cases, resulting in plateaus in force and SMR recruitment curves. The force plateau in **Figure 1C**, produced by intramuscular stimulation, can be explained as follows. First, the LGl is fully recruited between 0.2–0.7 ma. Next, another head of the gastrocnemius is recruited beginning at about 1.3 ma. Activity produced by the second head presumably lies outside the recording range of the LGl electrodes, so that the second rise in force is unaccompanied by an increase in myoelectric activity.

The similarities between the intermediate plateaus in both nerve cuff and intramuscular stimulation suggested that they shared a common mechanism, probably involving the discreet innervation of different ankle extensor muscles or muscle subdivisions by separate primary nerve branches (7,8,9,10,17). These primary branches form separate bundles or fascicles prior to entering the muscle. Therefore, given the proper stimulus conditions, it seemed likely that individual muscle compartments could be independently activated.

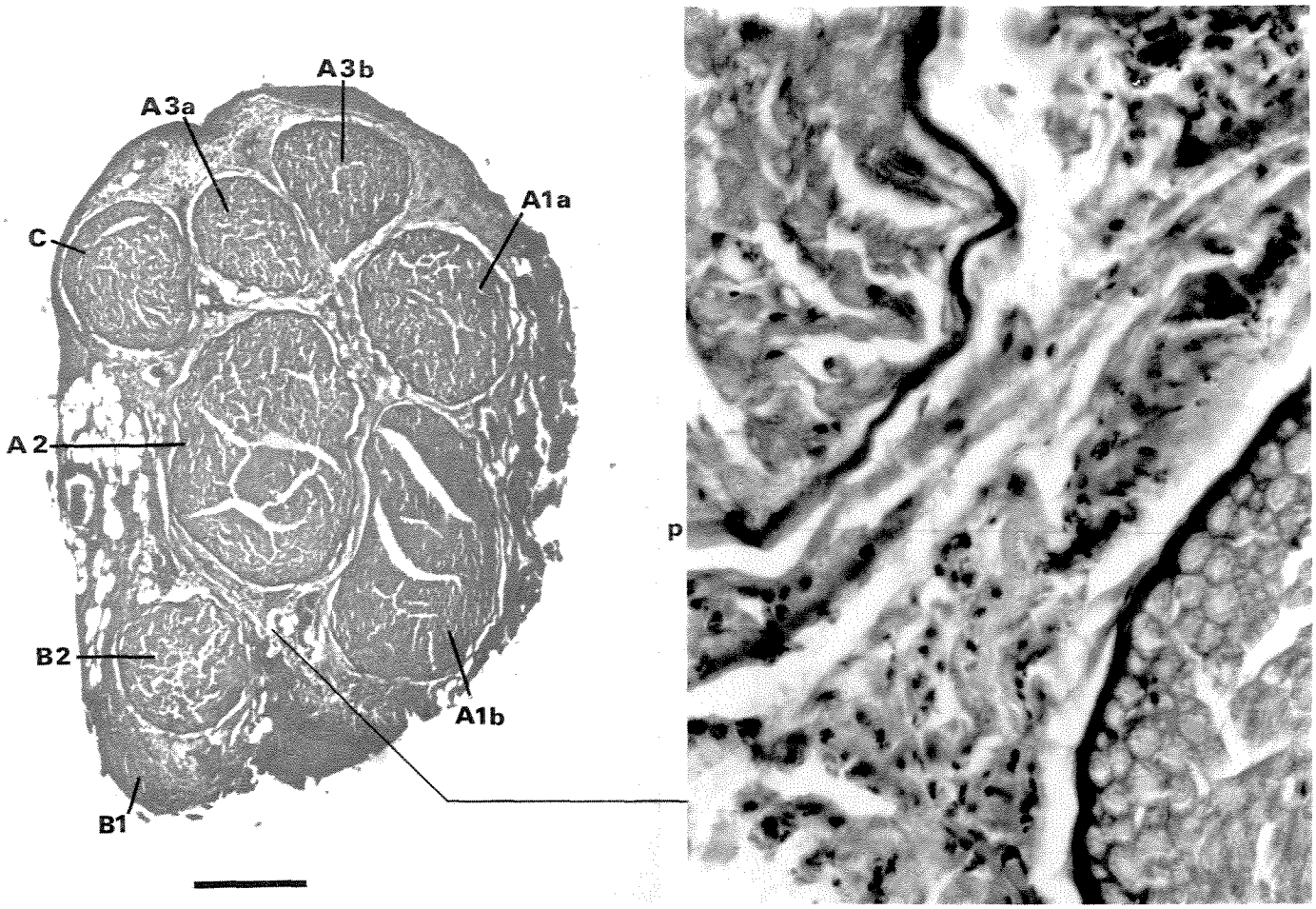

Figure 5.
Section of the nerve from the experiment in **Figure 4** showing the location of the bundle innervating the MG (B2). Section was cut just distal to the level at which bundles were isolated for stimulation. In this animal, LGl, LGm, and soleus were all activated by bundle C. Note that bundles A3a, A3b, and A1a, A1b, appeared to be single bundles at the point of nerve dissection, while in the histological section, they are each clearly subdivided into two fascicles. Scale at bottom = 0.5 mm. A magnified view (8.6×) of the space between fascicles A2 and B2 (rotated 90 degrees clockwise) is shown at right. "P" = perineurium of fascicle B2. The donut-shaped structures in the fascicles are axons.

McNeal and Bowman (23) had previously shown that the organization of axons into fascicles does affect their threshold to stimulation. In that study, cuff electrodes were placed around the sciatic nerve before it separates into the peroneal and tibial branches. The ankle flexor and extensor muscle groups, which those branches respectively innervate, could be individually activated by appropriately placed electrodes within the cuff, confirming that the position of the electrodes with respect to the nerve bundles was an important factor in axonal recruitment. This factor could explain our observations of selective activation of ankle extensor muscles during tibial nerve stimulation. However, the anatomical organization of the tibial nerve is more complex than the case of the peroneal versus tibial branches, involving up to 9 fascicles in at least 3 "levels"—the tibial nerve, bundles (of fascicles), and fascicles (**Figure 5**). It was not evident that electrode/fascicle geometry alone could completely account for such selectivity.

To examine the selective activation of muscles and compartments during nerve stimulation, we separately stimulated the fascicles of the tibial nerve several cm prior to entering the gastrocnemius. Single fascicle stimulation (as in **Figure 4**, bundles

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B2 and C) did not produce intermediate plateaus in the force and SMR recruitment curves. Furthermore, intermediate plateaus were not observed in SMR curves when recordings were localized within a single neuromuscular compartment. The small amount of myoelectric "crosstalk" seen in some cases could be anatomical or electrical in origin, but it is unlikely to have resulted from stimulus current spread to other bundles (see METHODS). Similar levels of crosstalk were reported by English and Weeks (10). These results suggested that plateaus are not a phenomenon associated with stimulation of individual fascicles. Rather, they support the supposition that virtually all the axons within a single fascicle (i.e., the smallest anatomically definable group of axons) innervate a separate portion of the muscle which is not innervated by axons outside that fascicle (8,9,10).

During whole nerve stimulation, on the other hand, we often observed distinct, non-overlapping regions of recruitment (from threshold to saturation) for different muscles and/or compartments, which resulted in intermediate plateaus in the force curves (**Figure 4A**). We will use the term "fascicular recruitment" (FR) below to refer to this phenomenon. SMR recruitment curves will show intermediate plateaus when electrodes "see" more than one muscle or compartment, as when bipolar recording electrodes span different compartments, or a monopolar electrode is located between two compartments. The high correlation between bundle CAP and SMR in **Figure 4D** indicates that nearly all the axons in that bundle were recruited within the stimulus range of 550-630 μA, while all axons innervating the LGm, LGl, and soleus were already recruited (**Figure 4C**). This demonstration, in view of the known innervation of different muscles by individual fascicles, strongly suggests that intermediate plateaus in force curves result from recruitment of fascicles at different thresholds during nerve stimulation, and therefore the fascicular organization of axons has a significant effect on their electrical recruitment threshold.

Although many previous studies have involved nerve and intramuscular stimulation, plateau-type recruitment curves are rarely reported (see, however [1,5,13]). This is apparently because most such experiments either record from a surgically-isolated muscle or compartment innervated by a single fascicle, such as MG or TA (11,28,29,38), stimulate a single fascicle, or the nerve is stimulated centrally before fascicles to the individual compartments have formed (28,29). One must be careful, therefore, in using such results to predict the effects of whole nerve stimulation in an intact limb in which multiple heads or compartments are innervated by that nerve. In particular, the use of reverse-recruitment techniques to obtain normal recruitment order (28,38) may be compromised.

Mechanism of fascicular recruitment

The foregoing results imply that axonal recruitment is not uniform over the entire nerve, but is dependent upon both the organization of the axons into separate bundles, and upon electrode geometry, which together determine the pattern of current flowing through the nerve (22,23,25,32). Even though two bundles may have the same axonal number and size distribution, they may be recruited over substantially different stimulus ranges.

FR is almost certainly caused to some degree by the geometrical arrangement of electrodes and fascicles. Specific electrode geometries and/or stimulus waveforms can induce variations in current density in different portions of the nerve, which might selectively activate axons in one fascicle before those in a neighboring fascicle (22,23). For example, in **Figure 1A/B**, reversing stimulus polarity (effectively switching electrodes) significantly altered the recruitment pattern. Also, in **Figure 5**, fascicle B2, which activated MG, was relatively far from fascicle C, which activated LGl, LGm, and soleus. Generally, we did not find FR to be associated with any particular stimulus electrode configuration—hook or cuff, monopolar or bipolar. While the characteristics (threshold, range) of FR could be altered by changing the stimulus waveform (i.e., monophasic versus biphasic), FR could be elicited using all combinations of monopolar/bipolar and monophasic/biphasic stimulation (cf. **Figure 2**). In fact, our results suggest that intermediate plateaus do not result *solely* from the geometrical arrangement of fascicles and electrodes. If such were the case, especially with a circumferential electrode (as used in these experiments), one would expect a randomly scattered distribution of recruitment curves, such as shown in the simulated recruitment curves of Veltink *et al.* (35,36). (In those simulations, fascicular organization was not taken into account, although a variable for connective tissue impedance

was included.) Rather, we nearly always found a dichotomous activation of muscles/compartments in which the range of stimulation from threshold to saturation for each "group" was generally much less (30–50 percent) than the separation in thresholds between groups (**Figures 1, 2, 3A, and 4A**). This occurred even though helical coil electrodes were used to minimize variations in current flow around the circumference of the nerve in later experiments.

If fascicle/electrode geometry is insufficient to completely account for the observed patterns of FR, the next most likely candidate is tissue inhomogeneities which produce variations in intraneural current density—i.e., perineurium, epineurium, and associated connective tissue which separates and groups axons into bundles and fascicles. If this connective tissue represents a high resistance to current flow relative to the intrafascicular tissue, one would expect exaggerated differences between thresholds of axons in different fascicles. A fascicle could, for example, be modeled using passive cable equations (22,35). Variations in the relative values of perineurial and intrafascicular resistance due to differences in fascicular diameter or tissue sheath thickness could affect voltages and/or currents inside the fascicles and thereby significantly affect recruitment. As **Figure 5** shows, fascicles did differ by up to 100 percent in circumference. However, we do not yet have sufficient correlative data between fascicle size, location, and recruitment characteristics, or the specific resistances of epineurial, perineurial, and intrafascicular tissues, to test this hypothesis.

Other factors which can influence the expression of FR include the stimulus location. As noted above, fascicular organization varies along the nerve. Segregation of efferent axons into fascicles proceeds from proximal to distal, so that the closer a fascicle is to an individual muscle, the easier it is to isolate (10). FR also varies between animals (8). For example, MG had a relatively low threshold in **Figure 3**, a high threshold in **Figure 4**, and an intermediate one in **Figure 2**. Also, LGm was recruited independently in **Figure 2**, but with LGl and soleus in **Figure 4**. These variations probably depend on the locations at which axons to the different muscle compartments segregate to form their own fascicles or primary nerve branches.

The afferent/efferent composition of the fascicles in a nerve might also affect FR. Approximately half of the bundles in the tibial nerve had no effect on muscle contraction when stimulated, indicating that they primarily contained cutaneous afferents. These afferent fascicles may be interposed between other fascicles containing motor axons, affecting their relative thresholds. Fascicular recruitment of afferent fascicles has not been investigated. However, nerve electrode positioning has been used to selectively stimulate motor axons while minimizing the activation of sensory (e.g., pain) fibers in human studies (32).

Intramuscular stimulation and fascicular recruitment

The intermediate plateaus seen during intramuscular stimulation may be related to FR in the following manner. Consider a stimulating electrode in the vicinity of the point at which the tibial nerve enters the triceps surae, where the nerve fascicles divide into "primary branches," innervating the LG, MG, and soleus and their compartments. It has been established that intramuscular stimulation first recruits muscle fibers by activating the fine nerve branches or terminals in the muscle, rather than the muscle fibers themselves, as the latter have a higher threshold (25). In the case of the triceps surae, this implies that the physical separation of the primary branches might cause them to be successively recruited by the applied stimulus current according to their distance from the electrode. This would result in activation of different muscles or compartments over different stimulus ranges, and thus nonlinear force recruitment.

Practical implications

FR has practical relevance to many applications involving neuromuscular stimulation and/or recording, particularly in functional electrical stimulation (electrically-stimulated exercise, electrical stimulation feedback, neuromuscular assist, and pain control by electrical stimulation), and FNS (6,24,37). FR also has general relevance to physiological studies of force and myoelectric activity during voluntary contraction, including studies of task group organization of motor function (8,19). FR does not appear to be an isolated phenomenon, and should be found in nerves to other compartmentalized muscles, such as the sartorius (19), semitendinosus (15) and biceps femoris (4). The tibialis anterior does not seem to be compartmentalized.

When paralyzed muscle is activated by whole nerve stimulation, FR may cause earlier recruitment of some compartments or heads of a muscle, producing irregular stimulus/tension relationships. Such variation in recruitment of synergists may also produce undesirable patterns of joint tension. Nonlinear force curves may also require complex stimulus control equations or algorithms to compensate for them (5,33). Our results suggest that the degree to which nonlinear muscle recruitment can be expected in a given muscle should be a function of its neuromuscular innervation pattern: specifically, the degree of innervation of separate muscles and/or compartments by individual nerve fascicles at the point of stimulation. The benefits which might be achieved by stimulation of individual fascicles are presently not worth the risk of irreversible nerve damage introduced by attempting to surgically separate and place electrodes around individual fascicles. Specially-designed nerve cuff electrodes capable of applying current at specific points around the circumference of the tibial nerve might achieve selective stimulation of fascicles with minimal risk of nerve injury (23,29).

High degrees of correlation (up to $R^2 = 0.99$) were often found between SMR and force in the triceps surae when SMR from several major components of the triceps were averaged together. The fact that high SMR/force correlations were obtained, even without introducing different weighting factors for each muscle in these experiments (cf., **Figures 3A, 4A**), probably results from the fact that each electrode (LGl, LGm, MG, and soleus) effectively represented muscle partitions of approximately equal maximal force capability. In some experiments (cf., **Figure 2**), weighting factors would have improved the correlation between average SMR and force. Many studies of electrically- and voluntarily-activated muscle contractions have found complex and even nonlinear relationships between EMG measures and force (cf., [27]). If synergistic muscles or muscle compartments are recruited at different levels of effort during voluntary contractions or during normal behaviors such as walking (3,15,18,30), then one should not expect any single partition to accurately represent activity of the whole muscle. Since surface (and especially intramuscular) electrodes can be quite selective in their recording field, non-uniform muscle activation, by natural or electrical means, would tend to produce weaker correlations than when all relevant muscles and/or compartments are sampled and appropriately weighted. The worst-case situation for SMR is when differential recording electrodes are implanted such that they sample different muscles or compartments which are activated over non-overlapping stimulus ranges. In such a case, diminution and even reversal of the SMR waveform is likely (20). In view of these findings, low correlations between EMG measures and force may indicate fractionated activation of a muscle rather than a complex relationship between myoelectric activity and force generation.

Due to inherent limitations of surface or intramuscular stimulation, it seems likely that direct nerve stimulation will ultimately be the technique of choice for neuromuscular prostheses (12). As McNeal and Reswick (24) noted, "One can foresee a peripheral nerve implanted with a number of electrodes, combinations of which will control individual movements. . . ." While the finding of separate innervation of muscles or compartments by fascicles was not unexpected, the significance of the dependence of axonal threshold and motor recruitment on fascicular organization has received little attention. A more detailed understanding of the organization of motor innervation may lead to significant improvements in the external control of paralyzed muscles.

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