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Selective motor unit recruitment via intrafascicular multielectrode stimulation[1]

Daniel McDonnall, Gregory A. Clark, and Richard A. Normann

Abstract: Recruitment of force via independent asynchronous firing of large numbers of motor units produces the grace and endurance of physiological motion. We have investigated the possibility of reproducing this physiological recruitment strategy by determining the selectivity of access to large numbers of independent motor units through intrafascicular multielectrode stimulation (IFMS) of the peripheral nerve. A Utah Slanted Electrode Array containing 100, 0.5–1.5 mm-long penetrating electrodes was inserted into the sciatic nerve of a cat, and forces generated by the 3 heads of triceps surea in response to electrical stimulation of the nerve were monitored via force transducers attached to their tendons. We found a mean of 17.4 ± 4.9 (mean ± SEM) electrodes selectively excited maximal forces in medial gastrocnemius before exciting another muscle. Among electrodes demonstrating selectivity at threshold, a mean of 7.3 ± 2.7 electrodes were shown to recruit independent populations of motor units innervating medial gastrocnemius (overlap < 20%). Corresponding numbers of electrodes were reported for lateral gastrocnemius and soleus, as well. We used these stimulation data to emulate physiological recruitment strategies, and found that independent motor unit pool recruitment approximates physiological activation more closely than does intensity-based recruitment or frequency-based recruitment.

Key words: functional neuromuscular stimulation (FNS), microelectrode array, neuroprosthesis, intrafascicular multielectrode stimulation (IFMS).

Résumé : Le recrutement de la force par la décharge asynchrone indépendante d'un grand nombre d'unités motrices produit la grâce et l'endurance du mouvement physiologique. Nous avons examiné la possibilité de reproduire cette stratégie de recrutement physiologique en déterminant la sélectivité d'accès des nerfs périphériques à un grand nombres d'unités motrices indépendantes par une stimulation intrafasciculaire multiélectrodes (SIFM) des nerfs périphériques. Une matrice d'électrodes (*Utah Slanted Electrode Array*) contenant 100 électrodes de 0,5–1,5 mm de long ont été insérées dans le nerf sciatique de chats, et les forces produites par les trois chefs du triceps sural en réponse à une stimulation électrique du nerf ont été enregistrées à l'aide de capteurs de force fixés à leurs tendons. Une moyenne de 17,4 + 4,9 (moyenne + é.t.) électrodes ont excité de manière sélective les forces maximales dans le gastrocnémien médial avant d'exciter une autre muscle. Parmi les électrodes démontrant une sélectivité au seuil, une moyenne de 7,3 + 2,7 électrodes ont recruté des populations indépendantes d'unités motrices innervant le gastrocnémien médial (chevauchement < 20 %). Un nombre correspondant d'électrodes sont aussi signalées pour le gastrocnémien latéral et le soléaire. Nous avons utilisé ces de stimulation pour reproduire les stratégies de recrutement physiologique et nous avons constaté que le recrutement de populations d'unités motrices indépendantes s'approche plus étroitement de l'activation physiologique que le recrutement basé sur l'intensité ou la fréquence.

Mots clés : stimulation neuromusculaire fonctionnelle (SNF), matrice de microélectrodes, neuroprothèse, stimulation intrafasciculaire multiélectrodes (SIFM).

[Traduit par la Rédaction]

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[1]This paper is one of a selection of papers published in this Special Issue, entitled Nerve, muscle, and beyond: the R.B. Stein Symposium.
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Introduction

The state of the art of functional neuromuscular stimulation (FNS) has advanced beyond the demonstration of the feasibility of restoring motion to paralyzed muscles (Bhadra and Mortimer 1997; Hambrecht 1992). Specifically, the problems of fatigue, the apparent consequence of inverse recruitment stimulation order (Fang and Mortimer 1991; Mortimer 1981), and fine control of both low and high forces (Chizeck et al. 1988; Crago et al. 1980, 1981, 1996; Kofman 1984) remain neuroprosthetic challenges.

Physiological muscle activation generates graceful, yet powerful motions of the extremities. These motions are achieved by the asynchronous selective activation of populations of independent motor units that summate to produce a given force (Rack and Westbury 1969; Petrofsky 1979). The force generated by a muscle is a function of the size of this activated population, the type of the muscle fibers activated (fast-twitch or slow-twitch, oxidative (fatigue-resistant) or glycolytic (fatigable)), the size of individual excited motor units, and their firing frequencies (Rack and Westbury 1969; Burke et al. 1973; Monster and Chan 1977). Delicate motor movements are mediated by the activation of a small number of slow-twitch, relatively weak, oxidative muscle fibers at low frequencies. As more force is required, the frequency of stimulation of these fibers is increased, and more oxidative muscle fibers are recruited. For maximum forces, all muscle fibers (both oxidative and glycolytic) are recruited at high firing rates. Research directed at emulating this force recruitment strategy with FNS technologies has been directed at the confounding problem of inverse recruitment: these stimulation technologies activate fast, strong, rapidly fatiguing muscle fibers before activating low-force, fatigue-resistant oxidative muscle fibers, thus producing poor force gradation and rapid muscle fatigue.

The recent development of new electrode array architectures (Branner et al. 2001; Campbell et al. 1991; Hoogerwerf and Wise 1994; McCreery et al. 1997; Rutten et al. 1991; Tyler and Durand 2002; Yoshida and Horch 1993b) offers the potential to achieve highly selective muscle activation. This new generation of electrode arrays has been designed to provide enhanced access to the nerve fibers in the peripheral nervous system. Although these new designs cannot provide selective access to each of the motor units that innervate a muscle, they do allow greater access to subpopulations of motor units. Thus, these new electrode arrays are beginning to enable the control of muscle forces using asynchronous activation of independent subpopulations of motor units, resulting in the generation of fatigue-resistant, ripple-free motions (Yoshida and Horch 1993a; McDonnall et al. 2004). The effectiveness of this more physiologically based force recruitment strategy depends upon a number of factors, including between-muscle selectivity—that is, the number of electrodes that can independently activate a given muscle, without activating other muscles (i.e., without producing spillover)—and within-muscle independence—that is, the number of electrodes that can selectively activate different subpopulations of motor units to a given muscle, without activating the other motor units to the same muscle that are recruited by other electrodes.

In the present studies, we address the number and independence of the motor unit pools accessed by one example of this new generation of electrode arrays, the Utah Slanted Electrode Array (USEA), as well as the functional consequences of this independence for force recruitment strategies. The USEA consists of 100 variable-length electrodes that can be implanted intrafascicularly into a peripheral nerve. Most of the implanted electrodes are expected to abut individual motor units, and thus should provide a vehicle for increased physiological force recruitment via intrafascicular multielectrode stimulation (IFMS). Experiments described herein were performed in the sciatic nerve of the anesthetized cat that had been implanted with a USEA. We first investigated the number of electrodes that could be used to stimulate a specific muscle without activating other muscles (between-muscle selectivity) as well as the ability to stimulate independent subpopulations of motor units within a given muscle (within-muscle independence), and explored how this between-muscle selectivity and within-muscle independence varied as a function of stimulus parameters. These stimulation data were then used to emulate physiological force recruitment strategies by (i) increasing the number of subpopulations of activated motor units via increases in pulse amplitude (intensity-based recruitment), (ii) increasing the frequency at which these motor unit pools are stimulated (frequency-based recruitment), and (iii) increasing the number of stimulation sites to increase the population of motor units active (independent motor unit pool recruitment).

Methods

Animal preparation and electrode implantation

IFMS arrays were implanted in the sciatic nerve of cats in accordance with procedures approved by the University of Utah Institutional Animal Care and Use Committee. Data from 7 cats are reported; however, not all experiments were performed in each cat. Specific number of cats and trials are listed for each experiment.

Anesthesia was induced with ketamine (10 mg/kg). The animals were intubated and general anesthesia was maintained by isoflurane (1.5%–2.5%). The electrocardiogram, heart rate, expired CO_2, blood pressure, and body temperature (rectal) were monitored to ensure anesthetic depth and stability of the anesthetic plane. Lactated Ringer's solution was administered intravenously at a rate of 8–12 mL/kg/h.

An incision was made on the left leg along the tendon of the gastrocnemius muscle. The tendons for medial and lateral gastrocnemius, soleus, and plantaris muscles were separated. The 2 heads of gastrocnemius, and in some cases, the head of soleus, were attached to Grass Force-Displacement transducers (FT03 and FT10, Grass Instrument, West Warwick, R.I.).

After dissection of the muscles, a skin incision was made along the left thigh from the vertebral column to the knee. The biceps femoris muscles were separated and retracted to expose the sciatic nerve. A machined small Lucite platform, layered with Sylgard® (Dow-Corning Corp., Midland, Mich.), was placed under the nerve to provide a stable support for implantation of the electrode array. All stimuli were delivered to the sciatic nerve via a Utah Slanted Electrode Array (USEA), which consists of a 10 × 10 square grid of variable length (0.5 to 1.5 mm) electrodes, spaced on 400

micron centers. The manufacturing processes for the USEA implanted in these experiments has been reported elsewhere (Branner et al. 2001). The USEA was positioned on the sciatic nerve from the lateral side of the animal, 1–3 cm proximal to the nerve's branching into tibial and fibular nerves. A high-velocity insertion technique, described elsewhere (Rousche and Normann 1992), was used to insert the array. After implantation, a Pt/Ir reference wire (20 IR2T, Medwire, Mt. Vernon, N.Y.) was placed in the fluids surrounding the nerve or in a neighboring muscle. The epineurium of the nerve was pinned to the Sylgard® on the supporting platform to isolate the nerve and array from movements resulting from stimulation. The nerve and muscles were kept moist with periodic applications of mineral oil throughout the duration of the experiment. The leg was restrained at the knee and ankle.

Stimulation and recording setup

Computer-controlled linear stimulus isolators (A395R-C, World Precision Instruments Inc., Sarasota, Fla.) were used to generate constant currents for electrical stimulation. The stimuli were biphasic pulses with a duration of 200 μs per phase and a 50-μs interphase interval. The muscle was preloaded to capture the initial phase of the twitch response. Responses were digitized with an A/D board (Win-30D, United Electronic Industries, Inc., Watertown, Mass.) installed in a 166 MHz Pentium PC. The computer triggered stimulation of the nerve and recorded the evoked forces. The recording was done in 1-s segments at a sampling rate of 500 samples/s. The recorded muscle forces were analyzed as the difference between a pre-stimulation baseline force and the average peak force evoked by each stimulus.

Between-muscle selectivity

After array implantation, we determined which electrodes stimulated which muscle. At the site of stimulation on the sciatic nerve, injected current could excite motor units innervating ankle flexors and extensors. Because we typically had strain gauges connected to both medial and lateral gastrocnemius muscles, we generally restricted our quantitative analyses to these 2 muscles. However, in a subset of 2 cats, we also monitored forces generated in soleus. The absence or presence of responses in other muscles was determined visually in conjunction with the collection of more quantitative data from the force transducers. The specific threshold current amplitude required to achieve a repeatable muscle twitch at a fixed amplitude was recorded for each electrode that stimulated triceps surae. A more stringent between-muscle selectivity test was performed via stimulation at 10% and 100% maximum force to explore the limits of muscle-specific stimulation.

Within-muscle independence

Although between-muscle selectivity was studied using the above mapping protocols, it cannot be assumed that each of the electrodes that evokes a particular muscle contraction is activating an independent subpopulation of motor units innervating that muscle. We determined the selectivity of stimulation for sites activating the muscle in each preparation with the largest number of stimulation sites. The interaction between electrodes was quantified (Rutten et al. 1991;

Yoshida and Horch 1993b; Branner and Normann 2000) by measuring the muscle forces evoked with stimuli delivered by 2 electrodes independently, then by comparing these with the force produced when current was passed via one of the 2 electrodes during the refractory period initiated by current injection via the other electrode (the 2nd electrode was stimulated 0.75 ms after the 1st). Stimulation overlap between a pair of electrodes was calculated as the difference between the sum of the peak forces evoked by each electrode independently and the peak force produced by stimulation of the 2 electrodes in rapid succession.

[1] $\%$ overlap $= 100 \times (F_1 + F_2 - F_{1\&2}) / F\text{mean}_{(1\&2)}$

F_1 = force evoked by electrode 1

F_2 = force evoked by electrode 2

$F_{1\&2}$ = force evoked by sequential stimulation via electrodes 1 and 2

$F\text{mean}_{(1\&2)}$ = mean force evoked by electrodes F_1 and F_2: $(F_1 + F_2)/2$

0% overlap would indicate activation of fully independent pools of motor units, and 100% overlap would indicate that the 2 electrodes activated the same subpopulation of motor units. The percentage of overlap for each electrode was evaluated as the average overlap between it and all other selected electrodes. The average electrode overlap for the muscle studied was then averaged across all other electrodes where overlap was studied in each animal.

Results

Intrafascicular multielectrode stimulation (IFMS) technology has been reported to provide access to subpopulations of motor units via implantation of multiple electrodes within a fascicle (Rutten et al. 1991; Yoshida and Horch 1993b; Branner and Normann 2000). One goal of the present study was to determine the number of electrodes that can be used to stimulate a specific muscle without activating other muscles (between-muscle stimulation selectivity), and the number of electrodes that could stimulate independent subpopulations of motor units for a given muscle (within-muscle stimulation independence). These determinations were made using a particular intrafascicular multielectrode array, the USEA. Hence, our results are particularly germane to this and similar electrode array architectures. However, the conclusions drawn from the experiments are pertinent to all intrafascicular electrode designs.
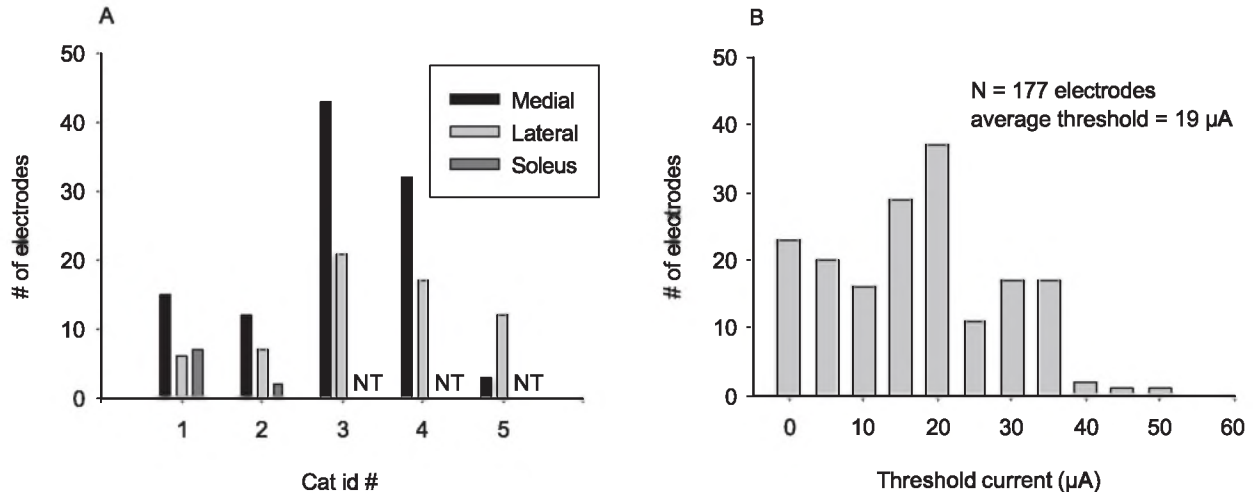
Between-muscle selectivity

Selectivity at threshold

After implantation of the 100-electrode USEA into the sciatic nerve, we mapped the muscles targeted by each of the implanted electrodes by injecting low currents into each electrode until a muscle twitch was evoked in the stimulated limb.

We found that we could selectively stimulate each head of gastrocnemius and soleus without activation of any other muscles. Figure 1a is a histogram showing the number of electrodes in each implanted cat ($n = 5$) that evoked thresh-

Fig. 1. Between-muscle selectivity at threshold. In 5 cats, electrodes in each implanted array were capable of selectively evoking perithreshold twitches in either head of gastrocnemius without activating other muscles. In the 2 animals in which force was recorded from soleus, electrodes were found to selectively activate this muscle as well. (A) The mean number of electrodes activating medial gastrocnemius was 21 ± 7 (mean ± SEM), the mean number of electrodes activating lateral gastrocnemius was 13 ± 2, and the mean number of electrodes activating soleus was 5 ± 1. NT indicates a muscle that was not tested. (B) Distribution of twitch thresholds for the 177 electrodes in (A).

old twitches initially in each of these 3 muscles, before activation of any other muscle determined either visually or via a force transducer (between-muscle selectivity). As indicated in the figure, soleus force was recorded with a force transducer in only 2 of the 5 cats. On average, 21.0 ± 6.5 (mean ± SEM) electrodes selectively evoked contractions in medial gastrocnemius; 12.6 ± 2.2 electrodes selectively activated lateral gastrocnemius, and 4.5 ± 1.2 electrodes activated soleus. In Fig. 1b, we provide a histogram of the threshold currents required to evoke these twitches in the same 5 cats (a total of 177 electrodes). The average current amplitude necessary to evoke repeatable threshold twitches in gastrocnemius or soleus was 19.3 µA ± 0.8 µA. The highest threshold current was 51 µA, but only 4 electrodes had thresholds greater than 40 µA. Currents passed through many other electrodes in the array could also evoke twitches in these 3 target muscles, but the currents evoked responses in other muscles at lower amplitudes, and so did not meet the criterion of between-muscle selectivity at threshold for the target muscles. Presumably, the other muscles that were activated at lower intensities had their own threshold independence, but this independence became compromised as stimulus intensities increased and our target muscles were recruited. We explicitly examine between-muscle selectivity using suprathreshold stimuli in subsequent experiments described below.

### Selectivity at higher forces

The penetrating nature of the USEA implies that some of its electrodes will be implanted intrafascicularly. Given the fascicular organization of fibers to a given muscle (Thomas et al. 1993), it might be expected that electrodes that excite a particular muscle would be grouped in the array, and that many of these electrodes would activate threshold twitches in one muscle before beginning to activate another muscle, as demonstrated above. We next investigated 2 more increasingly stringent indices of muscle selective stimulation: the

ability to recruit 10% of maximal force in one particular muscle without activating other muscles; and the ability to recruit maximal force (i.e., the full dynamic range of force generation) in one particular muscle without producing significant forces in neighboring muscles. In physiologically intact systems, cats do not require much force to stand, walk, or even run (Walmsley et al. 1978); thus, if contraction is summated in a muscle across the individual force contributions of multiple stimulation sites, then each stimulation site would rarely be required to evoke more than 10% of the muscle's maximum force.

We found that most of the electrodes tested could recruit a partial or even maximal force in one muscle without recruiting substantive force in other muscles. Between-muscle selectivity at maximum forces is illustrated in Fig. 2, where stimulation via 3 different electrodes in the same array are shown to selectively elicit maximum forces in medial gastrocnemius (Fig. 2a), lateral gastrocnemius (Fig. 2b), and soleus (Fig. 2c) without exciting the other muscles. The saturated plateau at the top of the force recruitment curve has been considered an indicator of complete muscle activation (Grill and Mortimer 1996). Independent activation of maximal force from lateral gastrocnemius and soleus muscles are of particular interest because fibers to these muscles typically lie within the same fascicle at this point in the sciatic nerve (Thomas et al. 1993). It might have been possible to achieve interfascicular stimulation selectivity without being able to achieve intrafascicular stimulation selectivity because the perineurium that surrounds the fascicle may provide a barrier to the passage of current between fascicles. In contrast, although the locations of our electrode tips have not been confirmed histologically, our data suggest it may indeed be possible to achieve nearly complete and selective activation of individual muscles even when the motoneurons that activate the muscle are in the same fascicle, in keeping with previous work (Yoshida and Horch 1993b).

These more stringent assessments of between-muscle

Fig. 2. Between-muscle selectivity demonstrated at maximum forces. Between-muscle selectivity is illustrated by 3 different electrodes in one implanted array. These electrodes can elicit maximum forces in (A) medial gastrocnemius, (B) lateral gastrocnemius, and (C) soleus without eliciting noticible excitation to other muscles. (D) Group data. Most of the electrodes capable of selectively evoking perithreshold twitches were also capable of evoking maximum twitch forces in each of the muscles without activating other muscles. The mean number of electrodes activating medial gastrocnemius was 17.4 ± 4.9 (mean $\pm$ SEM), the mean number of electrodes activating lateral gastrocnemius was 10.9 ± 1.6, and the mean number of electrodes activating soleus was 1.5 ± 1.1. NT indicates a muscle that was not tested.
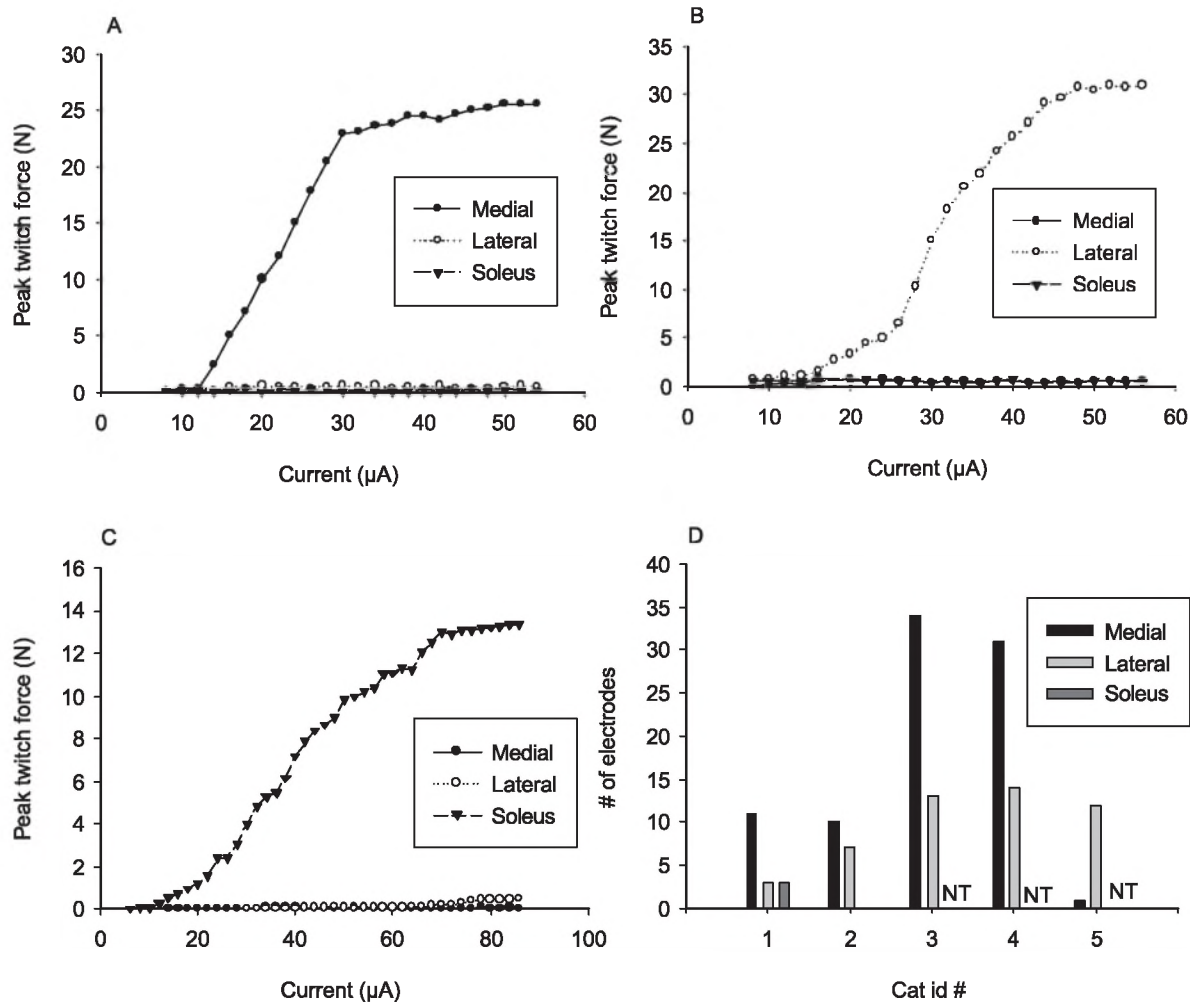

stimulation selectivity were performed on all electrodes in cats with multiple stimulation sites in multiple muscles. The group data for the electrodes that demonstrated selective maximum activation are shown in Fig. 2*d*. Of the 177 total electrodes that demonstrated between-muscle selectivity at threshold, 162 electrodes passed the stricter assessment of between-muscle selectivity at 10% maximal twitch force, and 139 of 177 passed the assessment of between-muscle selectivity at maximum forces. Specifically, at 10% maximal force levels (a force level employed in other experiments reported later herein), we found that an average of 19.2 ± 4.9 electrodes per cat activated medial gastrocnemius selectively (i.e., without activating any other muscles); 13.1 ± 1.7 electrodes per cat activated lateral gastrocnemius selectively; and 3.0 ± 1.4 electrodes per cat activated soleus selectively. At maximal force levels, only slightly fewer electrodes showed between-muscle selectivity (Fig. 2*d*). A mean of 17.4 ± 4.9 electrodes per cat fully activated medial gastrocnemius without producing observable responses in

other muscles. Similarly, an average of 10.9 ± 1.6 electrodes per cat were capable of evoking maximal lateral gastrocnemius forces selectively, and 1.5 ± 1.1 electrodes per cat were capable of evoking maximal soleus forces selectively. Injecting still higher currents (above those needed to achieve maximal contraction for a given muscle) began to activate additional muscles, thus providing a positive control for muscle viability.

Spatial distribution of fibers that activate a given muscle

The electrodes that activated either lateral or medial gastrocnemius were generally localized to a specific, grouped region of the array. Further, there was a sharp border between the activation areas for the two muscles, where threshold activation values rose dramatically. This pattern can be seen in Fig. 3, which is a greyscale plot of the currents required to evoke a twitch in medial gastrocnemius (Fig. 3*a*) or lateral gastrocnemius (Fig. 3*b*) as a function of the posi-

Fig. 3. Spatial distribution of threshold responses for gastrocnemius. Electrodes with low threshold currents for excitation of (A) medial gastrocnemius and (B) lateral gastrocnemius were localized within specific regions of sciatic nerve. Both grids show expected geometric locations (not to scale) of electrode tips from the same array within a cross-section of sciatic nerve in one animal. The intensity bar on the left shows current thresholds necessary to evoke the muscle twitches. Low stimulation currents are represented by darker squares and high stimulation currents are represented by lighter squares. Markers "*" indicate electrodes capable of evoking threshold twitches without exciting another muscle.
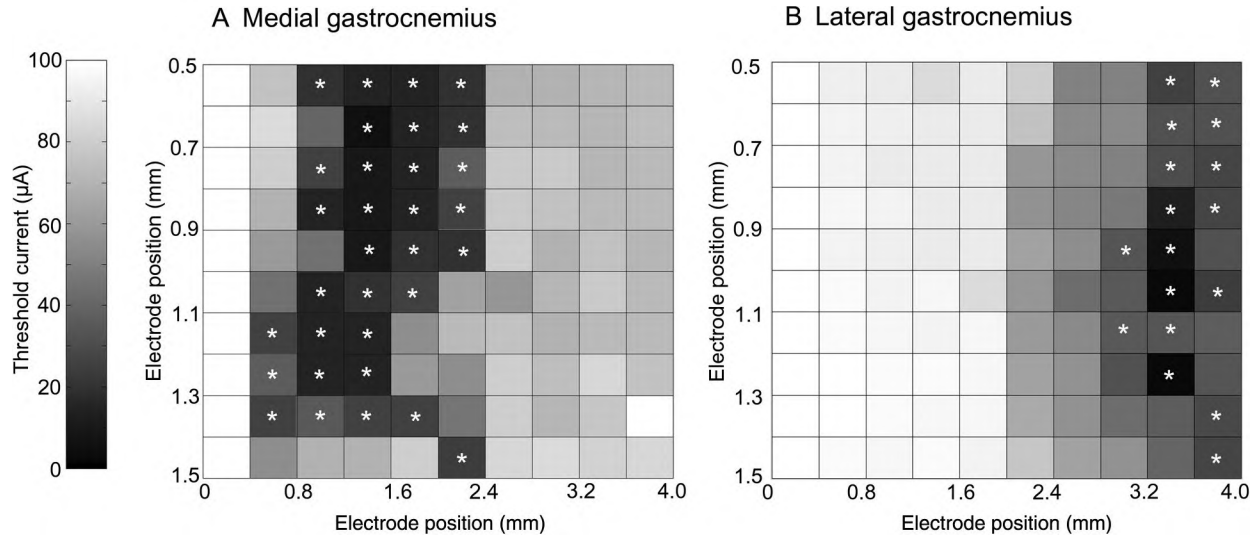
tion of the electrode in the array. The greyscale intensity bar on the left shows current thresholds necessary to evoke the muscle twitches. Low stimulation currents are represented by darker squares and high stimulation currents are represented by lighter squares. Medial gastrocnemius had electrodes with low thresholds grouped on one side of the nerve. Thirty-two electrodes were capable of evoking twitches in medial gastrocnemius before exciting another muscle (marked with "*"). In contrast, lateral gastrocnemius was innervated by motor units excited by 17 low-threshold electrodes demonstrating between-muscle selectivity at threshold. These were all grouped on the opposite side of this nerve (Fig. 3b) where threshold independence is indicated by "*".

Within-muscle independence of stimulation

Electrodes chosen for overlap testing must first have passed the between-muscle selectivity at threshold test. From this pool of electrodes, we tested a subset of electrodes for stimulation overlap. On average, we tested overlap among 9 electrodes per preparation. Although more electrodes were available for testing, the increase in number of combinatorial possibilities made the testing of all possible electrode pairs not feasible in practice. Each of the 9 selected electrodes was parametrically assessed to determine the overlap between it and each of the other 8 electrodes, thus producing an average of 9 average overlap values for each cat, 1 for each electrode. These average overlap values were averaged across the other electrodes to determine the within-muscle independence of electrodes within each cat. Figure 4a provides a histogram of these average overlap values. The average electrode overlap was found to be 16%. The negative average overlap value for Cat #3 likely reflects the presence of subthreshold summation (Branner et al. 2000). This is because of a subthreshold depolarization pro-

duced in fibers surrounding the excited fibers that are brought fully to threshold only when the second test electrode injects current at nearly the same time. This cat was classified as an outlier (defined as those values that fall more than 3 interquartile ranges above the 3rd quartile or below the 1st quartile), and as such was not included in the reported 16% average overlap ($n = 4$).

What is the number of independent stimulation sites obtained within a muscle? Because this number will vary with stimulus intensity and overlap criteria, we first computed the percentage of electrodes tested that exhibited relatively low (<20%) overlap with other electrodes at 10% of maximal twitch force. This 20% overlap value was chosen as an intermediate value between the 10% and 40% overlap values that were used to generate fatigue curves in other experiments reported later herein. We found that half of electrodes tested at 10% maximum twitch force exhibited low overlap. We applied this percentage of low-overlap electrodes to the total (typically somewhat larger) number of electrodes that exhibited complete between-muscle selectivity at 10% maximal twitch force for each cat. A histogram of this number of electrodes per cat is provided in Fig. 4b. This approach yielded an estimate of 7.3 ± 2.7 relatively independent sites of stimulation for medial gastrocnemius ($n = 4$ cats), 5.1 ± 1.0 sites for lateral gastrocnemius ($n = 4$ cats), and 1.5 ± 1.0 sites for soleus ($n = 2$ cats), respectively.

Stimulation protocols taking advantage of IFMS technology

Fine force gradation is achieved physiologically by a combined strategy of modulating both the firing rate of motor units and the numbers of motor units activated, with smaller, weaker motor units activated first (the size principle, Denny-Brown, D., and Pennybacker, J.B. 1938, or "Henneman" principle, Vilensky, J.A., and Gilman, S. 1998).

Fig. 4. Within-muscle selectivity at 10% maximum twitch force. (A) The mean overlap between electrodes in each cat stimulating a specific muscle at 10% maximum twitch force ($n = 5$ cats). Cat #3 has a negative overlap value, which means that when the average 2 electrodes were stimulated, one immediately after the other, the resulting force was greater than double the force produced by the stimulation of one electrode alone. However this cat was found to be an outlier from the distribution of overlap as seen in the other animals. The mean overlap percentage in the remaining 4 animals was 16%. (B) Number of independent stimulation sites. The ratio of electrode pairs with low overlap was multiplied by the number of electrodes in each cat that were capable of selectively eliciting a 10% maximum twitch to calculate the number of low overlap electrodes per muscle per cat as shown in this histogram. The mean number of independent electrodes stimulating medial gastrocnemius, lateral gastrocnemius, and soleus were 7.3 ± 2.7 (mean ± SEM), 5.1 ± 1.0, and 1.5 ± 1.0, respectively. NT indicates a muscle that was not tested. *, Cat #3 was excluded from this analysis.
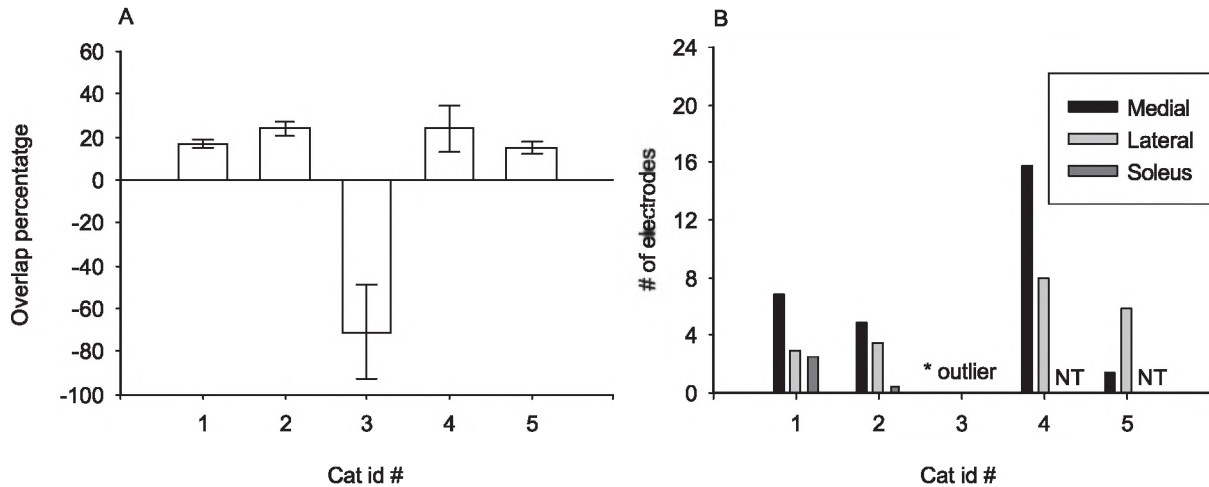
The USEA allows for access to 2 of these 3 mechanisms: population recruitment (via either intensity-based recruitment or independent motor unit pool recruitment) and frequency-based recruitment. The inverse recruitment order inherent to FNS limits access to the fatigue resistant control of force via stimulation of small diameter motoneurons over large diameter motoneurons. Changes in the number of motor units recruited with external stimulation could, in principle, be achieved either by increasing the intensity of stimulation at a given site (e.g., intensity-based recruitment), or by stimulating increasing numbers of independent stimulation sites. In the present experiments, we examine the use of the USEA to implement the recruitment strategies of: (*i*) intensity-based recruitment, (*ii*) frequency-based recruitment, or (*iii*) independent motor unit pool recruitment.

Intensity-based recruitment

We investigated the effectiveness and potential limitations of increasing stimulation pulse amplitude across IFMS electrodes as one method of population recruitment. A problem with this approach may arise when current injected via multiple electrodes within a fascicle is increased at each stimulation site to levels at which a significant population of motor units are activated by overlapping stimulation from multiple electrodes. The magnitude of this potential loss of independence was investigated by stimulating 3 separate pairs of electrodes from each cat ($n = 5$) at currents sufficient to achieve 10%, 20%, 40%, 60%, and 80% maximum twitch forces with each electrode. Overlap data were collected at each force level to assess any changes in the independence of stimulation sites. As each electrode injected higher currents, it stimulated a larger percentage of motor units within the fascicle, and the number of motor units activated by overlapping stimulation from both electrodes in-

creased (Fig. 5*a*). An ANOVA trend analysis on this data shows a significant ($p < 0.001$) linear trend, with no significant nonlinear trends. Thus, although it is possible to achieve maximum twitch forces with 2 electrodes by increasing pulse amplitudes, it comes at the cost of a loss of the independence between stimulation sites.

As described above, this loss of independence may have serious consequences on the ability of IFMS to achieve fatigue resistance via interleaved activation. We have previously documented that producing higher force levels via intensity-based recruitment yields a trend toward higher fatigue (McDonnall et al. 2004), but it was unknown whether differences in fatigue were due to differences in stimulation overlap, or other factors arising from higher pulse amplitudes. To investigate explicitly the consequences of overlap on fatigue, we used interleaved stimulation with different electrode pairs selected to have 10%, 40%, and 80% overlap at similar force levels when stimulated at a frequency of 10 Hz (thus removing the potential confounds of other consequences associated with increased stimulus levels). These fatigue trials were compared with a 100% overlap control tested by stimulating each electrode at 20 Hz (Fig. 5*b*). It is clear that one consequence of stimulation overlap during IFMS is increased fatigue. The low overlap (10%) pairs of electrodes demonstrated significantly more fatigue-resistance than pairs with 40% and 80% overlap or the 100% overlap control. An ANOVA with repeated measures with a Tukey post hoc test showed a significant ($p < 0.001$) difference in fatigue between 10% overlap pairs and higher overlap pairs. No significant difference ($p > 0.4$) was found among 40%, 80%, and 100% pairs.

Frequency-based recruitment

Modulation of stimulation frequency was also investigated

Fig. 5. Within-muscle independence decreases at higher stimulation currents. (A) Force recruitment via increases in pulse amplitude produce maximum twitch forces at the expense of within-muscle selectivity. Overlap data obtained from pairs of electrodes in each cat stimulated at currents sufficient to achieve 10%, 20%, 40%, 60%, and 80% maximum twitch forces. Each electrode pair demonstrated a linear increase in overlap at high twitch forces. (B) Consequences of overlap at 10% maximum force. Responses to 10 Hz, interleaved stimulation of pairs of electrodes that exhibited 10%, 40% and 80% overlap. The rate of fatigue was compared between pairs of electrodes with varying percentages of overlap and a control of single electrode 20 Hz stimulation. Pairs of electrodes with high overlap fatigued more than did pairs of electrodes with low overlap.
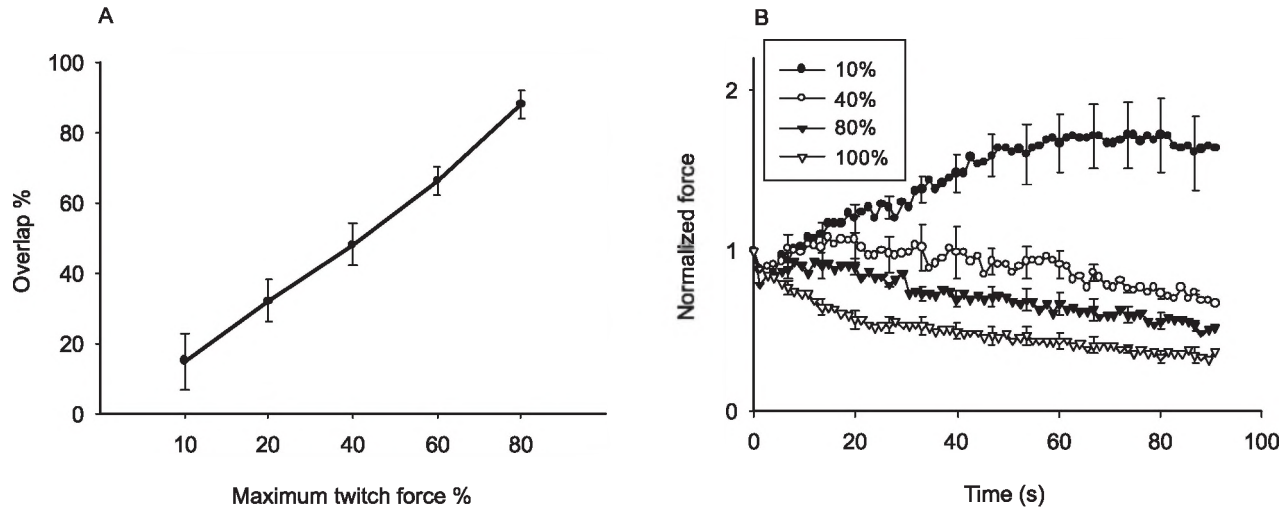
Fig. 6. Frequency-based recruitment. Force recruitment via increasing stimulation frequency with a single electrode was assessed at 10, 20, 30, and 40 Hz, relative to a single electrode stimulus pulse (0 Hz). Recruitment saturates after a two-fold increase in force, only offering a limited range of force recruitment. The saturation in force with increasing frequency seen in Fig. 6 has also been seen in additional experiments and averaged data from 5 cats are shown in Fig. 7b.
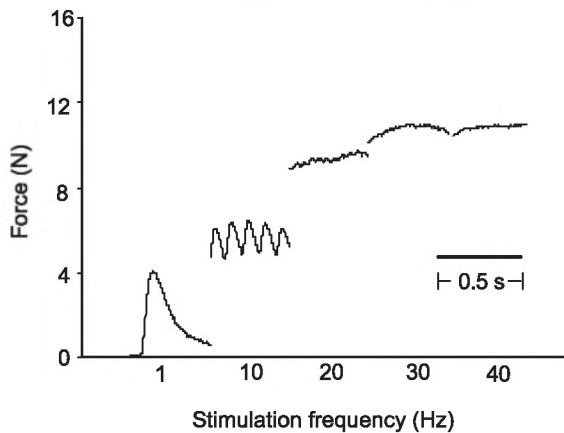
as an alternative activation strategy to recruit additional muscle force while avoiding a loss of stimulation site independence arising from increases in the area of excitation from each electrode. We stimulated individual electrodes with enough current to produce 10% maximum twitch force from each electrode, and examined the consequences on force production of stimulating an electrode at 10, 20, 30, and 40 Hz (Fig. 6) for 0.5 s. This experiment was performed with 3 electrodes in each of 5 cats. As the stimulation frequency was increased, ripple in the unfused tetanus decreased and there was about a 2-fold increase in force before

recruitment saturated. If the additional constraint of having ripple-free responses is imposed (<10% ripple), occurring between 10 and 20 Hz, then the range of forces available via frequency modulation would be even less. These data indicate that frequency-based recruitment by itself does not provide an efficient alternative IFMS force recruitment strategy, because force does not increase very much when motor units are stimulated at higher frequencies.

Independent motor unit pool recruitment

The finding that the USEA offers several independent sites of stimulation within a given muscle allows for a third possible strategy for force recruitment: activation of increasing numbers of stimulation sites. For this strategy to be maximally effective and produce linear summation of forces across all electrodes, each and every stimulation site must demonstrate within-muscle independence with all other stimulation sites, a criterion far more demanding than simple pair-wise independence.

To investigate the independence of stimulation that IFMS can achieve across multiple electrodes, we examined the muscle forces evoked by stimulation via 1, 2, 3, and 4 USEA electrodes (Fig. 7a) that activated a given muscle. Each electrode was stimulated at 10 Hz, and the current level in each electrode was adjusted to produce a 10% maximal twitch force when delivered in isolation. As additional electrodes were included in the stimulation, the stimulus phases were adjusted to interleave the stimuli of each additional electrode. Stimulation with a single electrode at 10 Hz produced significant ripple in the force. Adding a second, interleaved electrode produced approximately twice the force, along with a major reduction in ripple. Adding the third and fourth interleaved electrodes tripled and quadrupled the net force and further reduced the ripple. The independence of stimulation of motor units is evident in the linear superposi-

Fig. 7. Independent motor unit pool recruitment. (A) Force recruitment via interleaving increasing numbers of independent subpopulations of motor units innervating a muscle. The abscissa is labeled as composite stimulation frequency to denote the stimulation frequency experienced across the fused contraction, compared with a stimulation pulse (0 Hz). Each electrode was stimulated at an interleaved 10 Hz. The summation of multiple asynchronous unfused tetanic contractions produces ripple-free muscle forces. The linear nature of this summation provides further evidence that each stimulation site has a high degree of independence. (B) The averaged data from frequency-based recruitment and independent motor pool recruitment experiments. Both strategies produce similar increases in force going from 1 electrode stimulated at 10 Hz to 20 Hz composite stimulation. However, the independent motor pool recruitment strategy allows for greater increases in force production as composite frequency is increased to 30 Hz and 40 Hz.
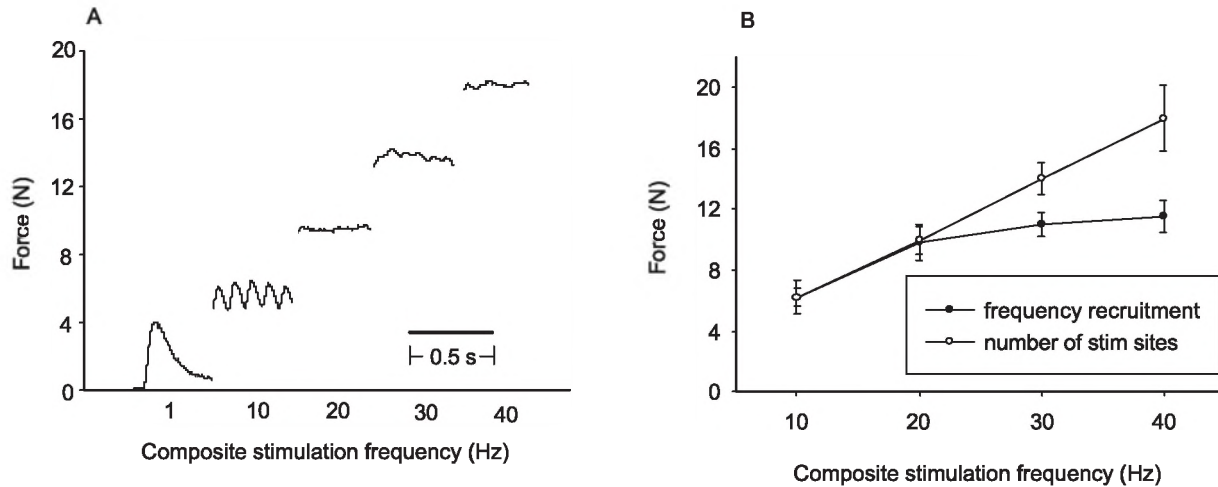
tion of the forces evoked by the addition of each electrode. A curve fit analysis on the group data (Fig. 7b) indicated that force increased as a linear function of the number of stimulation sites ($F_{1,4} = 37.0$, $n = 5$, $p < 0.01$), with no significant nonlinear components (p's > 0.79), providing additional support for linear summation of forces and the independent stimulation capabilities of USEA-based IFMS. In contrast, frequency-based recruitment resulted in a nonlinear increase in force, with a significant quadratic component ($F_{1,4} = 122.1$, $n = 5$, $p < 0.01$), and the slopes of the curves for the 2 recruitment strategies were reliably different (recruitment strategy by composite frequency interaction, $F_{3,12} = 23.3$, $n = 5$, $p < 0.01$). The forces evoked in frequency-based recruitment were comparable at 10 Hz and 20 Hz composite stimulation (t tests, p's > 0.2). However, the forces were significantly greater for increasing stimulation sites at 30 Hz ($p < 0.05$) and 40 Hz ($p < 0.05$).

Discussion

The purpose of this study was to investigate the capability of IFMS technology to selectively activate muscle via stimulation of a large number of independent subpopulations of motor units. We have applied increasingly stringent criteria of between-muscle independence and within-muscle independence to determine the number of selective stimulation sites for the muscles of triceps surae.

We found a mean 17.4 ± 4.9 (mean ± SEM) electrodes selectively exciting maximal forces in medial gastrocnemius before exciting another muscle (between-muscle selectivity). Among the electrodes that demonstrated selectivity at threshold, a subset was shown to be highly independent. As a result, we report 7.3 ± 2.7 electrodes that demonstrate within-muscle independence for medial gastrocnemius. Corresponding numbers of electrodes are reported for lateral

gastrocnemius and soleus as well. We used these stimulation data to emulate physiological recruitment strategies and found that independent motor unit pool recruitment approximates physiological activation more closely than intensity-based recruitment or frequency-based recruitment.

Selectively activating motor units in peripheral nerve is a continuing challenge for FNS approaches. Selective interfascicular stimulation has been demonstrated with multiple contract cuff electrodes with steering currents (Grill and Mortimer 1996), multigroove electrodes (Koole et al. 1997), slowly penetrating interfascicular cuff electrodes (Tyler and Durand 1997) and flat interface electrodes (Tyler and Durand 2002; Hoffer et al. 2000). These electrode architectures have proven the feasibility of selectively generating dorsiflexion and plantarflexion via access to tibial and peroneal branches of sciatic nerve in cats. Intrafascicular stimulation has been shown to allow even further selectivity via access to pools of motor units within a fascicle. Yoshida and Horch (1993b) achieved preferential activation of one muscle before another by placing a pair of wire electrodes in the fascicle that innervated both muscles. Branner et al. (2001) demonstrated between-muscle selectivity at thresholds for lateral gastrocnemius and soleus with an array of electrodes implanted intrafascicularly. In this study we have extended the investigation of selective stimulation and determined the number of independent stimulation sites that are accessible with the given architecture of the USEA. By applying increasingly more stringent tests of selectivity on our pool of available electrodes, we report a procedure for determining the independence of multiple stimulation sites that is applicable to any IFMS approach.

The goal of FNS systems is to restore as much as possible the grace and endurance of physiological motion to paralyzed limbs. We have proposed that implementing asynchronous, low-frequency, ripple-free IFMS protocols will provide

an important step toward realizing this goal because this strategy closely approximates the mechanisms of physiological force recruitment. Specifically, having a large number of electrodes implanted within a fascicle that accesses different populations of motor units innervating a given muscle allows different motor units to be stimulated asynchronously at low, fatigue-resistant frequencies. The more independent electrodes available, the lower the stimulation frequency possible on each electrode, which will slow the rate of fatigue.

However, the most effective method for controlling the distribution of charge across these electrodes to produce a fatigue-resistant target force level is not clear. The comparison of recruitment strategies of intensity-based recruitment, frequency-based recruitment, and independent motor pool recruitment outlined in this study demonstrates that increasing the number of stimulation sites most closely approximates the advantages of physiological recruitment. To implement this strategy at low forces, only a small number of electrodes would be used to produce the targeted force. The actual frequency used to stimulate each electrode would be determined by dividing the tetantic fusion frequency (the stimulation frequency that allows no more than 10% ripple) by the number of independent electrodes selectively activating the muscle. Additional force that may be required because of increasing force demands from a change in task (i.e., transition between standing and walking or from walking to running) or from fatigue resulting from inherent FNS inverse motor unit recruitment would be produced by interleaving increasing numbers of stimulation sites, thereby stimulating a larger population of motor units innervating the muscle. The problem with using this approach alone is that the resulting force production from each additional site will produce low-resolution, quantized recruitment. If, for example, the targeted force is balanced across 4 electrodes, then by definition each electrode would be responsible for 1/4 the tetantic force. A fifth electrode would have to inject the same amount of current as each of the other electrodes to avoid ripple in the fused contraction. Adding this fifth electrode would increase force production by an ungainly 25%. To elicit a more finely graded increase in force would require additional strategies. A combinatorial approach incorporating intensity-based recruitment or frequency-based recruitment may be used to provide a smoother transition from 4 electrode stimulation to 5 electrode stimulation. This additional recruitment strategy would be more crucial for transitions between small numbers of electrodes used at small forces because the addition of each stimulation site represents a large percentage of total tetantic force.

To further improve the IFMS approach to stimulation, more electrodes could be implanted within a fascicle using higher density electrode arrays. The electrodes used in this study on the USEA range in length down a column from 0.5 to 1.5 mm with 0.1 mm difference in length between rows of electrodes. Across each row, the electrodes are spaced at 0.4 mm intervals. A higher density array with electrodes spaced at 0.2 mm intervals across the rows of electrodes in an array could be used to double the number of electrodes placed within a fascicle. This array would permit even lower stimulation frequencies and presumably even finer control of muscle activation allowing the restoration of more graceful motion and even greater endurance. It is likely that the placement of a larger density of electrodes would improve force control because the independence of each stimulation site would be expected to be maintained. Rutten et al. (1991) investigated optimal IFMS spacing by implanting electrodes with 10 exposed stimulation surfaces each spaced 50 μm apart along a silicon shaft into the peroneal nerve in rats. They report that an electrode spacing of 200 μm to 250 μm provides maximal selectivity at minimum stimulation levels. This population of electrodes could be used to generate delicate, fatigue-resistant forces, and as greater forces are required, additional electrodes could be recruited.

The development of new generations of electrode array architectures will bring us even closer to achieving truly physiological recruitment of muscle force. These new architectures, coupled with the stimulation strategies described herein, are expected to offer great improvements in the control of the musculature driving paralyzed limbs.

Acknowledgements

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