Selective Stimulation of Cat Sciatic Nerve Using an Array of Varying-Length Microelectrodes

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Branner, Almut, Richard B. Stein, and Richard A. Normann. Selective stimulation of cat sciatic nerve using an array of varying-length microelectrodes. J Neurophysiol 85: 1585–1594, 2001. Restoration of motor function to individuals who have had spinal cord injuries or stroke has been hampered by the lack of an interface to the peripheral nervous system. A suitable interface should provide selective stimulation of a large number of individual muscle groups with graded recruitment of force. We have developed a new neural interface, the Utah Slanted Electrode Array (USEA), that was designed to be implanted into peripheral nerves. Its goal is to provide such an interface that could be useful in rehabilitation as well as neuroscience applications. In this study, the stimulation capabilities of the USEA were evaluated in acute experiments in cat sciatic nerve. The recruitment properties and the selectivity of stimulation were examined by determining the target muscles excited by stimulation via each of the 100 electrodes in the array and using force transducers to record the force produced in these muscles. It is shown in the results that groups of up to 15 electrodes were inserted into individual fascicles. Stimulation slightly above threshold was selective to one muscle group for most individual electrodes. At higher currents, co-activation of agonist but not antagonist muscles was observed in some instances. Recruitment curves for the electrode array were broader with twitch thresholds starting at much lower currents than for cuff electrodes. In these experiments, it is also shown that certain combinations of electrode pairs, inserted into an individual fascicle, excite fiber populations with substantial overlap, whereas other pairs appear to address independent populations. We conclude that the USEA permits more selective stimulation at much lower current intensities with more graded recruitment of individual muscles than is achieved by conventional cuff electrodes.

INTRODUCTION

Progress in the development of a widely accepted motor neuroprosthesis for paraplegics has been slow because of the difficulties in selectively generating and controlling muscle force by electrical stimulation and real-time monitoring of the kinematics of movement. Contemporary research in developing a motor neuroprosthesis has concentrated on addressing these two issues through the development of interfaces to the peripheral nervous system that do not damage the nerve, enhance access to kinematic information from sensory afferents, selectively stimulate multiple muscles, and provide graded control of muscle force.

Previous efforts directed at restoring motor function in paralyzed individuals used stimulation of the motor end plates or the nerve very close to the muscle with surface, percutaneous, or epimysial electrodes (Akers et al. 1999; Bhadra and Mortimer 1997; Bhadra and Peckham 1997; Cameron et al. 1998a,b; Ferguson et al. 1999; Haugland et al. 1999; Kobetic et al. 1999; Onishi et al. 2000). While these types of electrodes provide a straightforward means for selectively stimulating individual muscles, a number of issues have hindered successful implementation of motor prosthetic systems based on these electrodes. Principally, the currents required to produce graded contractions are high and can produce discomfort if used for periods of time at high current levels (Chae and Hart 1998). Surface electrodes can also cause pain, dermal irritations, and localized burns. Second, these electrodes cannot selectively stimulate different motor units in a muscle, so periods of use may be limited by fatigue. A final issue with percutaneous electrodes is that the muscle contractions often result in the eventual breakage, although with the improvement of percutaneous electrodes and their lead wires, these problems have become less frequent (Onishi et al. 2000).

Another approach is to stimulate the motor neurons in the peripheral nerve further away from the target muscle to generate contraction of the muscle (for a general overview, see Chapt. 5 in Agnew and McCreery 1990). This has a number of significant advantages. For example, the currents needed to generate action potentials in the motor neurons are orders of magnitude less than those needed for motor-unit stimulation. Also the density of motor fibers in a nerve is such that a single implant in the peripheral nerve can stimulate most if not all of the motor neurons going to a particular muscle, permitting complete and forceful contraction of the muscle.

Current research efforts have centered on two approaches: cuff electrodes that encircle the nerve and intrafascicular electrodes that are implanted inside individual fascicles.

Cuff electrodes

Cuff designs contain an electrode that is brought into contact with the peripheral nerve and held in place with a compliant sheath that is wrapped around the nerve. Over the past few decades, a large variety of nerve cuffs, some with shape-adapting features, have been designed and used acutely and chronically in animal experiments (Crampon et al. 1999; Grill...
and Mortimer 1998; Loeb and Peck 1996; Rodriguez et al. 2000; Slot et al. 1997; Strange and Hoffer 1999; Tyler and Durand 1997; Walter et al. 1997). To achieve some degree of stimulus selectivity, nerve cuffs have been designed with multiple electrodes distributed around the perimeter of the cuff. These “carousel” nerve cuffs permit “stimulus steering” (Deurloo et al. 1998; Koller et al. 1994; Sweeney et al. 1995; Veraart et al. 1993). Another cuff electrode has been designed to slowly reshape the nerve to get electrodes closer to individual fascicles and provide more selective stimulation (Tyler and Durand 1997). Problems with nerve cuffs can be compression injury and reduction in the blood supply to the nerve if the cuff fits too tightly. If the cuff is too loose, the electrode may not contact the nerve, and the recording properties of the cuff are compromised and stimulus selectivity is further degraded. However, peripheral nerve cuffs are being used clinically to provide some lower extremity restoration of function in paralyzed subjects (Hoffer et al. 1996; Rozman et al. 1994; Slot et al. 1997) in spite of eventual problems of poor muscle selectivity, steepness of the recruitment of force with stimulus intensity, and fatigue with prolonged muscle stimulation.

Intrafascicular electrodes

Many of the above-mentioned problems could be circumvented by the use of an electrode that directly stimulates a small number of motor neurons, but to do so, the electrode must penetrate the epineurium surrounding the entire nerve and the perineurium that surrounds each fascicle. While intrafascicular electrodes have been used extensively in acute research applications, their use in chronic experimentation has been limited due to their poor long-term recording and stimulating stability.

However, recently a new, highly flexible intrafascicular electrode wire design, the Longitudinal Intra Fascicular Electrode (or LIFE), has been used to stimulate and to record from peripheral nerves for weeks. While the selectivity of the LIFE is excellent, it is challenging to implant a few of these electrodes in different fascicles and, therefore, selectively stimulating fiber bundles to many different muscle groups (Malstrom et al. 1998; Yoshida et al. 2000). Other intrafascicular electrodes have been fabricated and tested (Bowman and Erickson 1985; Smit et al. 1999; Veltink et al. 1989), but their use in chronic applications has yet to be demonstrated.

Utah Electrode Array

Another intrafascicular electrode array has been developed that provides multichannel access to afferent and efferent fibers. The Utah Electrode Array, or UEA, has 100 1-mm-long needle electrodes that project out from a thin silicon substrate. This array and stimulation and recording results from its use in acute experiments have been described in more detail elsewhere (Branner and Normann 2000). In summary, it was shown that all 100 electrodes of the array can simultaneously be inserted into peripheral nerve using a pneumatic insertion device without significantly disturbing nerve function. Electrodes in the array are capable of selectively recording single-unit responses from mechanoreceptors, and digit switches can be evoked with current injections in the 1 to 20-μA range. Both the recording and stimulation were stable over the 36-h long periods of these experiments. However, the planar arrangement of the array (Fig. 1) is not optimal for recording from a large number of fibers in the nerve, as many electrodes along the axis of the nerve could contact a particular fiber.

To decrease the number of redundant electrodes, we designed an array with electrodes of varying length, which could provide access to most fascicles within the nerve. This new variant of the UEA architecture, the Utah Slanted Electrode Array, or USEA, could provide graded control of multiple individual muscles and acquisition of a large amount of sensory information from peripheral mechanoreceptors. In this paper, we have focused on a comparison of the stimulation selectivity of the new array geometry with cuff electrodes. We show herein that the USEA geometry provides access to large numbers of individual fibers in each fascicle and enhances graded recruitment of force in muscle groups in a highly selective fashion. We suggest that an array of this design might provide the foundation for not only a basic research tool, but also a peripheral nerve prosthesis.

Methods

Structure of the electrode array

The USEA used in this study is manufactured using similar methodology as used to fabricate the UEA; the manufacturing process used to build the UEA has been described elsewhere (Nordhausen et al. 1996). The needles are conductive due to the use of doped silicon and are electrically insulated with silicon nitride. Only the platinum-plated tips are exposed with an area of about 0.005 mm². The electrodes are regularly spaced on 400 μm centers and project out of the plane of the 0.2 mm thick silicon substrate to varying lengths (Fig. 2). The electrode lengths range from 0.5 to 1.5 mm with 0.1-mm difference in length between rows of neighboring electrodes. Each electrode is electrically isolated from its neighboring electrodes by a glass “moat” surrounding its base. Each electrode is approximately 80 μm wide at its base and tapers to a sharpened tip. Teflon-insulated Pt/Ir wires (10IR1T, Medwire, Mt. Vernon, NY) are soldered to bond pads deposited on the back of each electrode on the array. The lead wires are soldered to a connector that plugs into a data-acquisition system.
Animal preparation and electrode implantation

Experiments were done in seven cats. Anesthesia was induced with ketamine (10 mg/kg, Sanofi Winthrop Pharmaceuticals, Morrisville, PA) and maintained with halothane gas (0.9–1.5%, Halocarbon Laboratories, River Edge, NJ) during the experiment. Electroencephalographs (ECGs), expired CO₂, and rectal temperature were continuously monitored. Adequate depth of anesthesia was periodically assessed by absence of corneal and paw withdrawal reflex.

The right leg was shaved and a skin incision was made along the tendon of the gastrocnemius muscle. The tendons of the gastrocnemius, soleus, plantaris, flexor hallucis longus, and peroneus brevis muscles were removed from their attachments to the bone, and the muscles were separated and connected to Grass Force-Displacement Transducers (FT03 and FT10, Grass Instrument, W. Warwick, RI) using silk sutures. No attempt was made to separate the two heads of the gastrocnemius muscle. The skin was closed over the muscles, and an incision was made on the opposite side to expose tibialis anterior and extensor digitorum longus. Again both muscles were separated and connected to force transducers using silk sutures, and the incision was closed. Only six of the seven muscles were tied to force transducers at a time, and in one experiment, only four force transducers were used.

After dissection of the muscles, a skin incision was made along the thigh from the vertebral column to the knee. The biceps femoris muscles were separated and retracted to expose the sciatic nerve. A cuff electrode was placed around the sciatic nerve at the proximal end of the opening. The cuff electrode was made of silicone rubber tubing (Bio-Sil, 3.175 mm ID × 4.75 mm OD, Sil-Med, Taunton, MA). The cuff electrode construction followed the description by Davis et al. (1978). The cuff electrode had two Pt/Ir electrode wires (10IR3T, Medwire) sutured into the silicone rubber tubing with 1-cm spacing. The array was inserted into the nerve using a pneumatic impulse insertion technique that has been used successfully in cerebral cortex, and that has been described elsewhere (Rousche and Normann 1992). The impact inserter was positioned to apply a slight pressure on the array to facilitate insertion direction during the impact. After implantation, a Pt/Ir reference wire (20 IR2T, Medwire) was placed in the fluids surrounding the nerve or in a neighboring muscle. To protect the array from movement of the surrounding muscles, a 2 × 2 cm piece of polyethylene film was placed between the muscle and the nerve. The muscle was put back into place and the skin was closed over it. The leg was restrained at the knee.

All experiments were conducted according to National Institutes of Health guidelines for the use of animals.

Experimental setup for stimulation

The cuff electrode and the electrode array were both used for stimulation of the nerve. The cuff electrode was placed proximally to the implantation site of the electrode array. A computer-controlled WPI Linear Stimulus Isolator was used to provide a constant current for electrical stimulation. The stimuli were single biphasic pulses with a width of 200 μs per phase and a 100-μs interphase interval. Muscle forces were recorded using Grass Force-Displacement Transducers, which were connected to strain gauge amplifiers (CP122, Grass Instrument). Responses were digitized with an A/D board (Win-3D, United Electronics Industries, Watertown, MA) installed in a Pentium PC. Stimulation of the nerve and recording of force were triggered simultaneously by the computer and recording was done in 800-ms segments at a sampling rate of 500 Hz.

The recorded muscle recruitment was analyzed in two ways; calculating the maximum force amplitude (N) and the integrated force (Ns). During each measurement, the stimulation threshold current needed to produce a muscle twitch was determined for each electrode. Stimulation was started at 10 μA and increased in steps of 5 μA if no muscle twitch could be detected. As soon as a muscle twitch was detected using visual and tactile cues, the current was decreased in 1-μA steps until threshold was reached. For the stimulation with cuff electrodes, we started with a current of 50 μA, increased it in steps of 20 μA and decreased it in steps of 10 μA. This method was used to determine a map of stimulation thresholds and stimulated muscles for the electrode array. Of the 100 electrodes, up to 18 were picked for further analysis based on their stimulation thresholds and positions.
Recruitment curves for the USEA compared with cuff electrodes

Using recruitment curves it can be shown how the forces increase in a muscle with increases in stimulation current. Recruitment curves were recorded by starting stimulation at a current below threshold and increasing it in steps of 1–30 μA depending on the steepness of the curve. We determined recruitment curves for both the selected electrodes of the USEA and cuff electrodes and compared the two. Supramaximal muscle recruitment with the cuff electrode was used to calibrate maximal twitch force. The recruitment curves were fit to a sigmoid function using Eq. 1:

\[
y = \frac{a}{1 + \exp\left(-\frac{x-x_0}{b}\right)}
\]

Three parameters characterize this function: The peak of the recruitment curve in N is given by the parameter \(a\). Its steepness is determined by the current parameter \(b\), and the current required to reach 50% of the maximum is given by parameter \(x_0\). To make the shape of the curve independent of the absolute value of the threshold, the width of the recruitment curve \(W\) is calculated as the difference in log currents between 10 and 90% of maximum recruitment force. The mean values for the USEA and the cuff were compared for each muscle group using one-tailed independent samples t-tests without assuming equal population variances.

Selective muscle activation and stimulation of two electrodes

The cat sciatic nerve activates several different muscle groups in the lower leg. The nerve fibers for a particular muscle are located in the same fascicle. However, motor neurons of several muscles are bundled in one fascicle. Because we implanted 100 electrodes at different locations in the nerve, we should be able to selectively stimulate nerve fibers in several fascicles and, therefore selectively activate different muscles. During each experiment, we monitored the forces in up to six different muscles produced by stimulation of motor neurons through various electrodes.

We first determined which muscle groups were activated at the lowest current threshold for each electrode in the array. Then for selected electrodes in the array, the stimulation current was slowly increased to generate a recruitment curve for that muscle and to investigate the spread of current within the nerve. For the latter, we monitored what other muscle groups were also activated by motor fibers stimulated at higher currents and the similarities of the stimulation properties of two electrodes. To study the interactions between the two electrodes (\(e_1\) and \(e_2\)), the second stimulus is given 0–5 ms after the first. The stimulation currents were chosen to produce about 25% of maximal force for both electrodes so that the total force produced was smaller than the maximal force in that muscle. The total force \((s_1)\) produced in the muscle as a result of that stimulation was compared with the forces produced by stimulation of each electrode alone \((f_1\) and \(f_2)\). It is assumed that the force produced by stimulating \(e_1\) \(f_1\) is greater than \(e_2\) \(f_2\). The overlap was calculated using an interstimulus interval of 0.5 or 1 ms (Eq. 2), when fibers stimulated by the first stimulus will be refractory.

\[
\% \text{ overlap} = 100 \frac{f_1 + f_2 - s_1}{f_2}
\]

If the two electrodes are stimulated synchronously, a more than linear summation can occur as a result of a subthreshold interaction zone (Fig. 3). In other words, there is an area containing neurons that are depolarized by each electrode to a subthreshold level. When the stimulus to both electrodes is applied, the depolarization of these fibers is now sufficient to produce an action potential and hence additional force. The size of the subthreshold interaction zone in percent can be represented by Eq. 3. The sum due to a synchronous stimulation is \(s_0\).

\[
\% \text{ interaction} = 100 \frac{s_0 - s_1}{s_1}
\]

When two stimuli are separated by a longer interval e.g., 2 ms, many of the fibers will have recovered from refractoriness and can be stimulated by the second stimulus to the same electrode (doublet) (Burke et al. 1976; Stein and Permigliani 1979). Then the forces generated by the sum \(s_2\) is given by

\[
s_2 = s_1 + \frac{\% \text{ overlap}}{100} (d_2 - f_2)
\]

The response can be increased by the amount of extra force generated by a doublet stimulation of electrode \(e_2\). This extra force arises because some of the nerve fibers have recovered from the refractory period, and the resulting force \(d_2\) is then larger than \(f_2\).

The two electrodes can also excite independent populations of motor neurons (no overlap) or the collection of fibers stimulated by \(e_2\) is completely subsumed in the collection of fibers stimulated by \(e_1\) (100% overlap).

**RESULTS**

We conducted seven acute experiments in cat sciatic nerve. The muscle recruitment using the electrode array was compared with recruitment obtained with cuff electrodes. We also compared these electrodes in terms of selectivity of muscle activation and studied their interaction by stimulating more than one electrode simultaneously.

Recruitment curves for the USEA compared with cuff electrodes

The goal of this study was to determine the differences in muscle recruitment when using the USEA and cuff electrodes. Recruitment curves for different muscles were recorded for both electrode configurations. In Fig. 4 examples of typical recruitment curves for tibialis anterior (A) and soleus (B) are shown. The curves were plotted logarithmically to eliminate their dependency on the threshold current. We looked at two different parameters; the width \(W\) of the recruitment curve and the current that evoked a 50% maximal force \((x_0)\). In this particular experiment, for tibialis anterior the USEA required 10.6% of the current needed to produce a half-maximum force using the cuff electrode (4.4% for soleus). On the logarithmic scale, the USEA had a 4.3 times broader recruitment curve for tibialis anterior than the cuff electrode (2.8 times for soleus).

These results were consistent between experiments and muscle groups. We summarized the data for seven different mus-
cles in six different experiments in Fig. 5. Both the mean values in recruitment width \( (P < 0.01) \) and current at 50% force \( (P < 0.005) \) differ significantly between the USEA and the cuff (1-tailed independent samples t-test). On average the USEA recruitment curves are 4.1 times broader \( (W) \) and require one-tenth the current to generate them \( (x_0 \text{ in Eq. 1}) \). The ratio of the width of the recruitment curve is smaller (2 times) for the slow-twitch soleus muscle.

**Selectivity between different muscles**

To make a functional stimulation map of the muscles accessed by the electrodes of the USEA, we passed currents through each electrode in the array and examined target muscles for twitches. In Fig. 6 an example of a typical stimulation map is shown. Both plots represent locations of the stimulation electrodes in a 10 by 10 electrode grid. In Fig. 6A the muscles activated by the motor neurons that were stimulated by each electrode at threshold can be seen, whereas the corresponding threshold stimulation currents are shown in Fig. 6B. One can see a fascicular, somatotopic organization in these measurements, with efferent fibers that target single muscles all localized in one region of the nerve. The presumed fascicles were coded in shades of gray and their presumed boundaries were indicated by black lines. In this particular example, 94 of the 100 electrodes in the array were able to evoke motor responses in at least one of the muscles that were examined [biceps femoris, tenuissimus, soleus, flexor hallucis longus (FHL), tibialis anterior, lateral and medial gastrocnemius, plantaris, extensor digitorum longus]. The data suggest that the electrode tips of the array were located in different fascicles throughout the nerve. The minimum single-pulse current amplitude at which a muscle twitch could be detected (threshold) was determined for each electrode. The lowest threshold was 4 \( \mu \text{A} \). Stimulation thresholds were found to be particularly high along the borders of fascicles, which indicates that some electrodes were probably implanted between fascicles. The black regions represent electrodes where no muscle twitch could be evoked even at maximum current. The maximum stimulation current used here was 200 \( \mu \text{A} \) but most electrodes had a threshold below 30 \( \mu \text{A} \). As a histological analysis of this particular sciatic nerve was not performed, the anatomical basis for this functional organization could not be validated.

**Selectivity between different electrodes**

The goal of this experiment was to investigate whether individual electrodes of the USEA stimulate motor fibers that innervate more than one muscle. Selectivity of the electrodes at threshold can be seen in the stimulation map (Fig. 6). For neuroprosthetic applications, it is important to stimulate selectively, even for larger forces. To investigate selectivity for suprathreshold stimulation, we simultaneously monitored forces in up to six different muscles and recorded force recruitment for stimulation with a single electrode.

In Fig. 7 an example of six different electrodes with each row representing data from the same experiment is shown. The simultaneous recording of the generated muscle forces evoked by stimulation of each of the electrodes in Fig. 7 can be seen in Fig. 8. The used current is indicated by the dashed vertical line. The first electrode \( (A) \) strongly activates both the motor fibers of the gastrocnemius and soleus muscles, as can be seen in both Figs. 7 and 8. Although the response in gastrocnemius did reach a maximum, it only reached about 65% of the force produced by stimulation with the cuff electrode. The nerve...
fibers that innervate the lateral gastrocnemius and soleus muscles form a common nerve that branches from the sciatic and presumably formed a discrete fascicle at the level that the sciatic nerve was studied. The second electrode (B) mainly activated gastrocnemius and produced about 80% of maximal force. While the integrated force in soleus is negligible, about 15% of the maximal force was produced. The third (C) and fourth (D) electrodes both stimulated the motor neurons of flexor hallucis longus and plantaris. The third electrode (C) could produce almost maximal force in FHL before plantaris was activated. In contrast, the fourth electrode (D) could produce almost half-maximal force in plantaris before FHL was activated. The last two electrodes (E and F) activated the motor neurons to peroneus brevis, tibialis anterior, and extensor digitorum longus. For the fifth electrode (E) there was almost no selectivity between the three muscles, whereas the sixth electrode (F) could produce maximal force in peroneus brevis with only about 50% force in extensor digitorum longus and about 25% force in tibialis anterior. Within the recruitment range of

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**FIG. 6.** Functional stimulation maps of sciatic nerve using the USEA. The presumed boundaries between fascicles are indicated by black lines. A: map of muscles activated at threshold by each electrodes of the USEA. B: single biphasic pulse, twitch current thresholds for each electrode.

**FIG. 7.** Recruitment curves for 6 different electrodes. For each plot, the area (Ns) was plotted against the stimulation current (µA). The tips of some pairs of electrodes (C and D, E and F) were presumably located in the same fascicle. The intersections of the curves with the dashed lines represent discrete data points that have been used in Fig. 8.

**FIG. 8.** Recorded muscle forces over time for 6 different electrodes at the currents indicated by the dashed lines in Fig. 7. The data were normalized to the maximum muscle force produced by stimulation with the cuff electrode.
these muscles, no other muscles were activated by the two individual electrodes as can be seen in Fig. 8.

In some cases, other muscles seemed to relax when others are stimulated. This could either be due to reflex responses or mechanical interactions between muscles. Assuming that the typical current to produce maximal force was 50 μA, this translates to a maximum of 10 nC/phase or 200 μC/cm². Generally, agonist muscles were co-activated in a pattern that is consistent with the distribution of motor neurons in a given fascicle. There was no evidence of spread to neighboring fascicles in the current ranges studied.

Stimulation of two electrodes

One useful feature of the USEA results from having multiple electrodes in fascicles that innervate individual muscle groups. These multiple electrodes could enable the generation of a broader and highly selective recruitment of muscle force in the innervated muscle to control muscle force more precisely, and it could reduce muscle fatigue. This would be possible if two electrodes could independently excite subsets of fibers that innervated the same muscle group. To investigate this capability, we monitored the forces generated in a given muscle when excitation is evoked by currents passed through two individual electrodes. We have also modeled the degree of independence of the fiber excitation in terms of the muscle force generated by paired electrode excitation (see METHODS). Applying the model to these data allows us to determine the degree of independent excitation that is possible with the electrodes of the USEA.

One way to enhance selectivity further would be to stimulate two or more electrodes that innervate the fibers to the same muscle at currents that produce less than maximal force. The effect of stimulating a single muscle via two electrodes under several temporal conditions is shown in Fig. 9A. Stimulation was performed either simultaneously or with an interval between the two stimuli. Stimulation amplitudes were adjusted such that peak twitch forces generated with electrodes 1 (○) or 2 (●) alone were about 25% of maximal peak force in that muscle (in this case 1 N). If the forces evoked by the two stimuli summed linearly, — in Fig. 9A would result: it would reach a force peak of about 2 N. Instead, simultaneous stimulation via the two electrodes (▼) produced nearly 6 N of peak force, indicating that many fibers were located in a subthreshold interaction zone that surrounded the fibers excited by each electrode; i.e., stimulation via either electrode alone depolarized nerve fibers in this zone but not to threshold. With simultaneous stimulation, many of these nerve fibers were depolarized to threshold and fired action potentials. Also superimposed are the results of stimulating with the same two electrodes, but with a small interval (0.5 ms, ▲) or a longer interval (5 ms, ▲) between stimuli. The data are closer to the linear prediction but still deviate below or above the predicted line.

The interactions between two stimulating electrodes seen in the peak force data were also observed in the integrated force data. The total integrated force for the interstimulus intervals described in the preceding text (and a few others) are plotted as a function of the interstimulus interval in Fig. 9B. As was shown for simultaneous stimulation, there is a large increase in the integrated force above the value predicted for linear summation (−) of the individual twitches. The extent of the subthreshold interaction zone can be calculated using Eq. 3 in METHODS. For a zero interstimulus interval, the integrated force was 152% greater than that produced by using short, nonzero stimulus intervals. Note also that at interstimulus intervals less than 2 ms, the observed integrated forces are less than the linear prediction. Presumably, this reflects a subset of fibers that are stimulated by either electrode when the stimuli were given separately. When the stimuli are given with a small interstimulus interval, this overlapping set of stimulated fibers will be refractory and unable to produce a second twitch in response to the second stimulus. The extent of the overlap can also be computed using Eq. 2 in METHODS and was 49% for the pair of electrodes illustrated in this figure. To verify that the neurons were refractory, we applied two stimuli (doublets) to each electrode separately at various interstimulus intervals and then summed the results (●). With interstimulus intervals of 1 ms or less, the integrated force was the same for two stimuli as for one, confirming that the nerve fibers were refractory. A final point is that at longer interstimulus intervals (3 ms or more) the doublets give more than twice the expected response. This potentiation in the force output with doublets is well known (Burke et al. 1976; Stein and Parmiggiani 1979). To the extent that some fibers overlap the excitation fields of the two electrodes and are stimulated twice, this potentiation can account for the greater than linear summation shown in Fig. 9A and B, at longer intervals.

In Fig. 10A, calculated values of overlap for 39 pairs of electrodes that excited various muscle groups are shown. All values are normalized in terms of the maximum peak twitch force produced by the stimulated muscle. Note that with small twitches (less than 20% of maximum), the amount of overlap could be small or large, presumably dependent on the relative positions of the electrodes within a fascicle. However, when the stimulus was strong enough to generate a large percentage
The electrodes were on average 0.88 mm (±0.53 mm) apart. A, (array, the USEA, that provides substantial access to sensory as little damage as possible to the nerve. The device should be biocompatible and cause neural interface that can both stimulate and record from a large parallel representation in the peripheral nerve, one must use a sensory feedback information. Because this information has a the muscle fibers of many different muscles simultaneously back. Therefore the restoration of efficient muscle activity neurons innervating each muscle and providing sensory feed- motor system often arises from the interplay of the hundreds of changes in motor performance. Graceful motion in the intact integration of sensory feedback information to correct for ear characteristics of the force produced by the muscles require of the maximum twitch force, the computed overlap was always large.

Calculated values of the subthreshold interaction zones are plotted in Fig. 10A against the summed responses to stimulation with a small, nonzero interstimulus interval (s1, see METHODS). Again, if the individual twitches were small, the subthreshold interaction zone could be small or large (with values of several hundred percent), depending on the relative position of the electrodes in a fascicle. However, when the twitches were a larger fraction of their maximum values, then the interaction zone was relatively smaller.

**DISCUSSION**

One of the goals of current research aimed at restoring efficient motor function in individuals with spinal cord injuries is to stimulate the distal muscles in as natural a manner as possible. Consequently, there needs to be a gradual recruitment of the muscle fibers preferably from oxidative to glycolytic for fatigue resistance and selective stimulation of multiple muscle fibers for the production of graded forces. Further, the nonlinear characteristics of the force produced by the muscles require integration of sensory feedback information to correct for changes in motor performance. Graceful motion in the intact motor system often arises from the interplay of the hundreds of neurons innervating each muscle and providing sensory feedback. Therefore the restoration of efficient muscle activity through artificial means will require that one be able to activate the muscle fibers of many different muscles simultaneously and selectively, while monitoring muscle performance using sensory feedback information. Because this information has a parallel representation in the peripheral nerve, one must use a neural interface that can both stimulate and record from a large number of sites. The device should be biocompatible and cause as little damage as possible to the nerve.

In this paper, we described an intrafascicular multielectrode array, the USEA, that provides substantial access to sensory fibers and motor neurons in a peripheral nerve. The unique architecture of the array presents an electrical interface to many individual nerve fibers distributed across the fascicles in the nerve. In this paper, we specifically addressed stimulation via the USEA but selective recording from sensory neurons is also possible using arrays of penetrating electrodes (Branner and Normann 2000).

In results, it was shown that motor fibers could be stimulated with almost all electrodes. The fact that several different muscles could selectively be stimulated suggests that the electrodes of the array were placed in fascicles throughout the nerve. There is always a possibility that the active tips of some electrodes are located outside of the nerve or between fascicles, but this should not pose a problem because there are 100 potential electrodes implanted. The recruitment curves of the USEA are by over a factor of 4 broader than for the cuff electrodes. This should allow for a more controllable stimulation of the different muscle groups. Stimulation currents were an order of magnitude smaller than those evoked with the cuff electrode. This results in more localized stimulation and consequently, the stimulation will be less likely to cause axonal damage, although this has to be tested in chronic experiments. For the selected electrodes, the mean current to produce a maximal muscle response was 31.6 ± 21.6 (SE) μA, which corresponds to a charge of 6.3 ± 4.3 nC/phase and a charge density of 126.4 ± 86.4 μC/cm². While these values are well below the margins for safe repetitive stimulation determined at 150 nC/phase by McCreery et al. (1992), they are not below the chemically reversible charge injection limit of 40 μC/cm² for platinum electrodes (Roblee and Rose 1990). While this does not pose a problem in acute experiments, the dissolution of the electrodes in chronic experiments could be avoided by changing the metal deposited on the electrode tips to activated iridium or platinum black which both have superior charge transfer properties.

One important result was that we could stimulate all the motor nerve fibers in one fascicle without any apparent spread to other fascicles. The clearest examples involved the nerves to the gastrocnemius muscle. Several electrodes mainly stimulated gastrocnemius (see Fig. 8B, for example) and presumably lay within a medial gastrocnemius fascicle. Although the response did level off, it did not reach the maximum force produced by stimulation of the cuff electrode. This would support the hypothesis that only one of the two fascicles containing gastrocnemius fibers was activated. There was also a small activation of the soleus muscle, which could be due to mechanical interactions between gastrocnemius and soleus or current spread to fibers innervating soleus. Other electrodes stimulated comparable numbers of gastrocnemius and soleus fibers (Fig. 8A) and presumably lay in a lateral gastrocnemius-soleus fascicle.

Again, the response in this case did not reach maximum force obtained by stimulation with the cuff electrode. Only in one experiment did these electrodes that activated gastrocnemius or soleus stimulate nerve fibers to plantar is (not shown). For none of the other electrodes was there a co-activation of gastrocnemius or soleus and plantaris or flexor hallucis longus (Fig. 8, C and D), which likely ran in another fascicle. The same is true for the dorsiflexors, which should run in still another fascicle(s) that eventually forms the common peroneal nerve. The degree of selectivity between muscles with nerves...
in a given fascicle presumably depended on how much mixing of fibers occurred between the point of stimulation and the point where the nerves separate.

Normally, motor units are activated asynchronously, which results in a smooth contraction. This occurs even when the individual motor units are activated at relatively low rates so that their contractile responses are unfused. Rack and Westbury (1969) cut and split the ventral roots of cat sciatic nerves into several parts. By interleaving stimuli to individual parts of the roots, they could also produce a relatively smooth contraction at low rates of stimulation. The obvious limitation of their technique was that the roots were cut and contained fibers from many different muscles. In the present study, we could provide better selectivity by stimulation with multiple electrodes that were located within individual fascicles of a peripheral nerve. Thus we felt we could test whether interleaved stimulation of several electrodes also produced smooth contractions at relatively low rates.

The results were confounded by several nonlinearities that will require more detailed examination. If stimuli were applied simultaneously to two electrodes, the response was often many times as larger than the response to either stimulus alone or to the sum of the two separate stimuli (Fig. 8A). This was attributed to the presence of a subthreshold interaction zone of neurons that receive a subthreshold depolarization when either electrode was stimulated alone. When currents were passed through the two electrodes together, action potentials and twitch contractions were produced in a substantial number of these originally subthreshold motor units.

If the two stimuli were applied with a brief interstimulus interval (e.g., 0.5 or 1 ms), the muscle forces summed less than linearly. We attributed this to an overlap in the group of fibers stimulated. If a subset of fibers was activated by the first stimulus, these would be refractory to a second stimulus delivered after this brief interstimulus interval. This was confirmed (Fig. 8B) by applying a second stimulus to the same electrode at these brief intervals. The muscle forces evoked by two stimuli separated by 0.5 and 1 ms were not significantly different from the forces produced by one stimulus. Note that we used biphasic stimuli so that the depolarization produced by one phase of the first stimulus in subthreshold neurons was largely cancelled by the second phase of that stimulus before the second stimulus was applied.

Further, if two stimuli were applied at longer intervals (more than 3 ms), a more than linear summation resulted. This was attributed to a potentiation in the contractile response, where the same motor neurons were stimulated twice because they were located in the overlap zone of the two electrodes. This was again confirmed by sequential stimulation via the same electrode. Such sequential stimulation at these intervals produced more than twice the peak force and integrated force. This well known phenomenon (Burke et al. 1976; Stein and Parmiggiani 1979) has been associated with residual cytoplasmic calcium remaining in muscle fibers after the first stimulus which can enhance the contraction produced by a second stimulus (Melzer et al. 1986).

Although of interest in terms of physiological mechanisms, one could argue that these nonlinearities are not important for the stated goal of interleaving stimuli to several electrodes that selectively stimulate parts of a motor fascicle. The presence of a subthreshold interaction zone is not a problem because the use of biphasic stimuli, even at short intervals can reduce or eliminate the effects of this zone. However, overlap is of more concern. To the extent that the same neuron is being stimulated by several overlapping fields, it will be stimulated at much higher rates as stimulating currents are passed via each electrode. Although the response will be potentiated initially, it may fatigue over time. Overlay can often be reduced by using smaller stimuli (see Fig. 9A) but is inevitably present when larger stimuli are used that produce more than 20% of the maximum twitch. For example, if stimulating currents are passed via four electrodes, each of which produces 25% of the maximum twitch in a muscle when stimulated alone, the response to all four may only be 50%, rather than 100%, because of the overlap. Increasing the stimuli further to try to generate a maximal response will only exacerbate the problem of overlap. However, these redundant electrodes might be useful in chronic applications where failing electrodes could be replaced by electrodes with similar stimulation properties.

One final problem is that stimuli that produce 25–50% of the maximum twitch in a muscle typically lie on the steepest part of the recruitment curves (see Fig. 3). Thus even small movements of the electrode or slight fluctuations in the excitability of the nerve fibers may change considerably the percentage of force generated. The combination of fatigue due to overlap and variability due to electrode movement or threshold fluctuations may limit the practical application of interleaved stimulation of several electrodes in a fascicle in a behaving animal. However, the experimental results and the analysis developed in METHODS provide the means to determine the extent to which these nonlinearities are a problem in a given preparation. From this information, the effects can be reduced to some extent, although the usefulness of the technique needs to be investigated further in chronically implanted animals.

The USEA has been shown to manifest very good stimulation properties in acute experiments, and clearly it could serve as an excellent tool for basic neuroscience in acute experiments. However, its utility as a neuroprosthetic device is less clear. The first application that comes to mind is functional neuromuscular stimulation to restore motor function. One important question that is being addressed in ongoing experiments is whether it is possible to selectively activate slow oxidative versus fast glycolytic muscle fibers using the electrode array since this would enable fatigue-resistant stimulation.

This multielectrode, intrafascicular technology still has to be validated in chronic experiments where we can monitor long-term stimulation and recording stability, and long-term biocompatibility of the array in peripheral nerve. Clearly the implantation of a structure as complex as the USEA is an invasive process that can potentially cause significant damage to the nerve. However, we have shown in a previous study (Branner and Normann 2000) using the UEA that there does not appear to be any significant impact on the nerve’s performance after implantation in acute experiments. Movement of surrounding muscles could displace the array, cause damage to the nerve, and produce tethering forces in the lead wires that connect the array to the transtcutaneous connector. We therefore require a containment system for the array and strain relief for the wires. All these issues will be addressed in future studies.

In a chronic preparation, we could study the representation of kinematic information in populations of afferent neurons in

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cat sciatic nerve. The last step toward a neuroprosthesis would be to establish feedback control of limb rotation using the USEA. If these features can be demonstrated on a long-term basis, the use of high-electrode count, penetrating electrode arrays like the USEA to contact individual nerve fibers could form the foundation for a variety of neuroprosthetic applications such as: chronic pain relief, phrenic nerve pacing, vagal nerve stimulation, control of bladder voiding, and limb movements.

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