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Distributed Stimulation Increases Force Elicited With Functional Electrical Stimulation

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Abstract

Objective—The maximum muscle forces that can be evoked using functional electrical stimulation (FES) are relatively modest. The reason for this weakness is not fully understood but could be partly related to the widespread distribution of motor nerve branches within muscle. As such, a single stimulating electrode (as is conventionally used) may be incapable of activating the entire array of motor axons supplying a muscle. Therefore, the objective of this study was to determine whether stimulating a muscle with more than one source of current could boost force above that achievable with a single source.

Approach—We compared the maximum isometric forces that could be evoked in anterior deltoid of anesthetized monkeys using one or two intramuscular electrodes. We also evaluated whether temporally interleaved stimulation between two electrodes might reduce fatigue during prolonged activity compared to synchronized stimulation through two electrodes.

Main Results—We found that dual electrode stimulation consistently produced greater force (~50% greater on average) than maximal stimulation with single electrodes. No differences, however, were found in the fatigue responses using interleaved versus synchronized stimulation.

Significance—It seems reasonable to consider using multi-electrode stimulation to augment the force-generating capacity of muscles and thereby increase the utility of FES systems.

INTRODUCTION

Functional electrical stimulation (FES) involves artificial activation of muscles to restore some measure of motor function in paralyzed individuals. One vexing problem undermining the utility of FES is that the maximum forces exerted with stimulation are relatively weak. Indeed, even in healthy subjects, the largest forces that can be evoked with electrical stimulation are inexorably less than that produced during maximum voluntary contraction (Milner et al. 1969; Marsh et al. 1981; Kramer et al. 1984; Enoka and Fuglevand 1991; Koh and Herzog 1995; Vanderthommen and Duchateau 2007; Maffiuletti et al. 2014). Many

factors likely contribute to this weakness in individuals with spinal cord injuries, including muscle atrophy and muscle denervation. As a consequence, most FES systems applied to high-level tetraplegics, for example, require an external frame to support the arm because muscle stimulation alone is insufficient to elevate the limb against gravity (Hoshimiya et al. 1989; Nathan 1989; Schill et al. 2011; Memberg et al. 2014).

In addition, it seems possible that single site stimulation (as typically used in implanted FES applications) may simply be insufficient to fully activate most muscles, particularly larger muscles controlling more proximal joints like the shoulder. A widely held view is that if isometric force saturates with escalating stimulating current delivered by a single electrode, then the maximal force capacity of the muscle has likely been achieved (e.g. Merton 1954; Bigland-Ritchie et al. 1979; Rutherford et al. 1986). However, the highly distributed branching of motor nerves in human muscle (Sunderland and Hughes 1946; Amarali et al. 2007; Mu and Sanders 2010; Won et al. 2011; 2012; 2015) combined with the steep decay in the electric field strength with distance from a stimulating electrode (McIntyre and Grill 2002; Rattay 2004) may preclude activation of the entire array of motor axons within a muscle with a single intramuscular electrode (Memberg et al. 2014).

Therefore, the aim of this study was to determine whether stimulating a muscle using intramuscular electrodes but with more than one source of current could boost force above that achievable with a single source. An additional potential benefit of such distributed stimulation is that some degree of load sharing among the muscle fibers activated by different electrodes might help minimize fatigue during prolonged activity (Mortimer 1981; Yoshida and Horch 1993; Wise et al. 2001; McDonnall et al. 2004; Nguyen et al. 2011; Sayenko et al. 2014; Lou et al. 2017; Wiest et al. 2017). Because of the relatively prolonged force response to each stimulus pulse, it seems feasible that a target force could be maintained by alternating stimuli among the electrodes using lower rates than would be needed by a single electrode. In turn, lower rates of muscle fiber activation might lessen the overall degree of muscle fatigue. Therefore, a secondary aim of this study was to determine whether temporally interleaved stimulation between stimulation sites might reduce fatigue compared to synchronous stimulation.

METHODS

Subjects

Two adult male rhesus monkeys (*Macaca mulatta*), ages 11 and 13 years, were included in this study in accordance with IACUC guidelines and approved by the University of Arizona institutional review board. Subjects were anesthetized during the experiments (see details below). We initially attempted these experiments in awake healthy human subjects but the discomfort (Merton 1954; Edwards 1984, Vanderthommen and Duchateau 2007; Maffiuletti et al. 2014) and inadvertent co-contraction (Marsden et al. 1983) associated with intense stimulation precluded valid assessments. Because macaques have relatively large skeletal muscles and an upper limb anatomy similar to that of humans (Cheng & Scott 2000), they provide a reasonable experimental model for testing FES. Furthermore, because we were interested in the practical implementation of FES operating within intact joint systems, we opted not to surgically isolate the test muscle, the anterior deltoid. The anterior deltoid was

selected for study because it is a key contributor to arm elevation particularly for reaching movements (Soechting & Flanders 1997; Gronley et al. 2000). In macaques, the proportion of the anterior deltoid composed of slow twitch oxidative fibers is about 33% (Singh et al. 2002) whereas it is about 50% in humans (Tesch and Karlsson 1985). As such, the anterior deltoid of the macaque may be slightly faster and somewhat less resistant to fatigue than in humans. To minimize the number of animals tested, we carried out repeated testing of the two subjects across multiple sessions. The data reported here were obtained from 28 sessions, 15 for monkey S and 13 for monkey R. A minimum of seven days recovery were allowed between testing sessions for both monkeys.

Subject preparation

Prior to each experimental session, monkeys were sedated in their home cage with Ketamine HCL (10 – 15 mg/kg IM) and transported to the procedure room. Atropine (0.04 mg/kg IM) was given to reduce hyper-salivation common with Ketamine sedation. Carprofen (2.2 mg/kg SQ) was also given to reduce inflammation associated with endotracheal intubation. A 22-gauge intravenous catheter was placed in the saphenous or cephalic vein to deliver lactated Ringers (5–10 ml/kg/hr) to maintain hydration. Anesthesia was induced with isoflurane (1.5 – 2% in 100% oxygen, ~ 1 L/min) via mask insufflation. Following induction, an endotracheal tube was inserted to maintain airway patency and deliver anesthesia (1 – 2% isoflurane in 100% oxygen, ~1 L/min).

Monkeys were then placed into a modified infant car seat in a seated position (Fig. 1). A neonatal cervical collar was used to maintain the head in an upright position. The cervical collar was fixed in place to the car seat with cable ties passed through holes drilled in the car seat and through slots in the back of the collar. Straps situated midway between the neck and shoulder and across the torso secured the animal to the chair. The right (test) arm hung in a pendant position, free of obstruction.

Monkeys were instrumented with an esophageal thermometer to measure core temperature and a SpO2 monitor placed on one of the digits of the left hand. Heart rate, respiratory rate, electrocardiogram, end-tidal CO2, and non-invasive blood pressure (cuff over radial artery) signals were monitored throughout the experiment. Core temperature of ~ 36.7 ° C was maintained via a forced warm air blanket and bubble wrap placed over the torso. Physiologic parameters were noted every 5 – 10 minutes.

Force measurements

Once the animal was positioned in the chair, a custom-built force transducer was attached to the pendant arm by a strap that encircled the wrist and was used to measure shoulder flexion force. The transducer position was adjusted to pull the limb slightly in extension and thereby maintain a resting tension of ~ 2 N. The force transducer signal was amplified ($\times 1000$, Transbridge, World Precision Instruments, Sarasota FL, USA) and digitally sampled (1000 Hz, Spike2, Cambridge Electronics Design, Cambridge England).

Electrical stimulation

Hair was removed from the skin overlying the right deltoid and surrounding areas. The skin surface was then cleaned with sterile alcohol and gauze. Intramuscular cathode electrodes (tungsten, 250 μm shaft diameter, 1 – 5 μm tip diameter, 2 – 4 mm of insulation removed from the tip) were then inserted through the skin and into the anterior deltoid along the proximal-distal axis of the muscle. Individual electrodes were placed ~ 2 cm apart. In preliminary experiments using up to four intramuscular electrodes, the preponderance of force summation was achieved with two electrodes. As such, two independent intramuscular electrodes were used for the experiments reported here. Surface electrodes (1.5 cm diameter) placed over the clavicle and acromion process served as the return electrodes.

A programmable multi-channel stimulator (STG4008 MultiChannel Systems, Reutlinger, Germany) generated current-regulated rectangular pulses (0.5 ms duration). Given a compliance voltage of 160 V, and an approximate electrode/skin impedance (at 1000 Hz) of 8 k Ω , we estimated the maximal controlled current available for these experiments to be ~ 20 mA.

Initially, 1 Hz pulses (2 mA amplitude) were delivered to each electrode while the depth position of the electrode was manually adjusted in small increments until the largest twitch forces were elicited. The electrode was then left in that position for the remainder of the experiment. A more comprehensive mapping of the entire anterior deltoid to determine the optimal sites for stimulation was not undertaken primarily for the sake of expediency, as we tried to minimize the time monkeys were under anesthesia. The threshold current needed to elicit a detectable force response was then determined for each electrode. One-second trains of pulses (35 Hz, 4 s inter-train intervals) were delivered in 0.1 mA steps up until a force response was detected on the transducer. The magnitude of the current associated with the first detected response was deemed the threshold current.

Force-frequency relationship

In some of the sessions (10 for monkey R, 12 for monkey S), we characterized the relation between stimulus frequency and isometric force for the anterior deltoid. One-second trains of stimuli (0.5 ms, 6 mA) were delivered at 10, 20, 30, 40, 50, 60, 80, and 100 Hz with 4 s between trains. This was done using one electrode only. As far as we are aware, no previous studies have characterized the force-frequency relationship in limb muscles of non-human primates. Therefore, this procedure helped us to determine stimulation frequencies needed to evoke maximum force. Furthermore, because there is growing use of non-human primates as model systems for FES and motor neuroprosthetics (e.g. Moritz et al. 2008; Pohlmeier et al. 2009; Ethier et al. 2013; Capogrosso et al. 2016; Ethier et al. 2017), such force-frequency data could be useful for identifying suitable stimulation frequencies for specific applications.

Force–current relationship

The relationship between current-pulse amplitude and evoked force was assessed using 1-s trains of pulses delivered at 35 Hz first to one electrode then to the other. Trains were delivered repeatedly with pulse amplitude incremented in 1 mA steps from 1 mA to 10 – 20 mA with 4-s delay between trains. During this procedure, we observed the evoked actions of

the limb for any signs (e.g. shoulder adduction, abduction) indicating that neighboring muscles were being recruited at higher stimulus intensities. If activation of other muscles was detected, stimulation was halted. On the few occasions when this did occur, the most common added action was shoulder abduction, suggesting activation of the middle head of the deltoid. In these cases, the position of the electrode was changed which usually involved removing the electrode and reinserting at different location farther away from the muscle that was unintentionally recruited. After a few minutes of rest, the procedure for evaluating the relationship between current-pulse amplitude and force was repeated for the new electrode position. The entire procedure was then repeated for the second electrode. For each electrode, the minimum current above which no clear increment in force was observed over the entire range of currents tested was identified and referred to as the 'maximum' current.

Force Summation

We then sought to compare the force exerted during 'maximal' stimulation delivered by each electrode individually to that generated by simultaneous stimulation through both electrodes. For this procedure, we stimulated using the maximum current identified for each electrode. Stimulus trains were 2-s in duration. Because we were also interested to estimate the overall maximum force that could be evoked in anterior deltoid, for these procedures we stimulated at 60 Hz. In preliminary experiments, 60 Hz was the most common frequency associated with the initial plateau in the force-frequency relation. 'Maximal' stimulation was delivered first to one electrode, then to the other, followed by two trials in which maximal current was delivered simultaneously to both electrodes. This was then followed by stimulation to the second electrode by itself and finally to the first electrode by itself. The duration between stimulus trains for this procedure was 10 s.

Fatigue

As mentioned in the Introduction, a possible advantage of using multiple electrodes is that force might be better maintained during prolonged activity by interleaving stimulation to each of the electrodes. In this way, the stimulus frequency delivered to any one electrode (and the muscle fibers it activates) can be lessened, while the net frequency delivered to the muscle is maintained at a higher level enabling larger target forces to be achieved. This in turn might help minimize fatigue that is associated with high-frequency stimulation of muscle fibers (Naess and Storm-Mathisen 1955; Jones et al. 1979; Metzger and Fitts 1986). To test this idea, we recorded the force evoked by repetitive stimulation of anterior deltoid using 2-s trains, with a 50% duty cycle, for 4 minutes. Stimuli were delivered to both electrodes using one of three distinct patterns outlined below. Only one pattern was tested during a given session.

In some sessions, stimuli were delivered at 35 Hz synchronously to both electrodes. Thirty-five Hz was selected because at this rate, force was relatively well fused yet did not produce full tetanic tension. In other sessions, stimuli were delivered at 17.5 Hz but with stimuli interleaved between the electrodes (i.e., 180 degrees out of phase with one another) such that the net stimulus frequency was 35 Hz. And to partially evaluate the effect of interleaved versus synchronous stimulation but using the same stimulus frequency, in a third set of sessions stimuli were delivered in an interleaved fashion at 35 Hz per electrode. Prior to each

fatigue run, stimulus intensity (current amplitude) was adjusted individually on each electrode such that the forces produced by each electrode were similar to one another and that the total initial force (due to the combined actions of both electrodes) detected at the wrist was approximately 2 N above the resting level and was same across all sessions. Finally, to assess recovery following the fatigue protocols, we recorded force responses to 2-s stimulus trains (using the same stimulus patterns for that session) at 1, 2, 4, 8 and 16 minutes following the end of the fatigue protocol.

Data analysis

For each train of stimuli, force was averaged over two 0.5 s windows registered to the beginning and end of the peak force. The larger of the two averages was taken as the force measurement. For force summation experiments, in each session the mean force for the 2 trials involving dual-electrode stimulation was normalized as a percentage of the mean force evoked during the 4 trials of single-electrode stimulation. A one-sample t-test was performed on normalized dual-electrode forces (using a hypothesized mean of 100%) to determine whether force exerted with dual-electrode stimulation was greater than that evoked by single-electrode stimulation.

For each fatigue trial, we made three measurements: endurance index (ratio of force of last train to that of the first train), normalized force area (summation of all force responses during the trial divided by the sum of all force responses had no fatigue occurred, i.e. all force response the same as the first), and slowing index (ratio of the half-relaxation times of last train to that of the first train). Half-relaxation time (1/2 RT) was calculated as the time required for force to decay from the peak value measured immediately following the last stimulus pulse in a train to 50% of that value. A one-way ANOVA was performed on each these fatigue measures with stimulation pattern (35-Hz synchronous, 17.5 interleaved, and 35-Hz interleaved) as the factor. Data are reported as means $\pm$ standard deviation (SD) with $P < 0.05$ considered significant.

RESULTS

Both animals underwent several sessions of general anesthesia, each lasting about 2 hours. No adverse events occurred in any of the sessions. The animals recovered within ~ 45 minutes following cessation of anesthesia, exhibiting fully coordinated movements, eating and drinking. The animals remained healthy throughout the entire testing period (~ 4 months) and showed no long-term effects of repeated anesthesia. As such, this method can be used to repeatedly and safely evaluate aspects of FES delivered with percutaneous or chronically implanted electrodes in macaque monkeys.

Examples of anterior deltoid force responses to varying stimulus frequencies delivered through a single electrode are shown in Figure 2A. Force increased progressively with increased frequencies up to about 60 Hz beyond which there was little additional force increase. Unfused ripple can be observed at frequencies up through ~ 30 Hz with the contraction becoming almost completely fused at 40 Hz.

A similar profile was observed for all force-frequency trials carried out on the two monkeys across 22 sessions (10 for monkey R, 12 for monkey S). Figure 2B shows mean (SD) force versus frequency relationships for the two monkeys separately. The responses were very similar across the two subjects with force increasing steeply from 10 – 30 Hz, increasing modestly from 40 – 60 Hz, and not further increasing at frequencies greater than 60 Hz.

Force– current relationship

Figure 3A shows an example trial of isometric force responses to progressively increasing pulse amplitudes using 35 Hz trains delivered through one of two electrodes. Note that for stimulus currents from 2 to 5 mA, force appeared to saturate (highlighted by red line above force traces). However, above 6 mA, force increased markedly for increments in current up to 15 mA when force again appeared to saturate. Because we did not stimulate above 16 mA in this experiment, we could not be certain whether another escalation in force would have occurred at higher stimulus intensities. There was no detectable activation of neighboring muscles even for the highest currents delivered. For purposes of these experiments, the minimal current associated with ‘maximal’ force (dashed horizontal line) was deemed to be 15 mA for this trial.

Figure 3B shows the force responses to incrementing current pulses obtained in the same experiment as for Figure 3A but for the other electrode (electrode 2) that was placed about 2 cm distal to the one used in Figure 3A (electrode 1). The pattern of force increases to incrementing current delivered through electrode 2 was roughly similar to that for electrode 1. An intermediate plateau was observed for currents from 3 to 5 mA followed by a ~ linear increase in force with current up to about 14 – 15 mA at which point force appeared to saturate. The stimulus current needed to elicit 50% of ‘maximal’ force was only about 3 mA for electrode 2 (Figure 3B) whereas it was about 10 mA for electrode 1 (Figure 3A). The most striking difference, however, between the responses elicited with the two electrodes was the absolute magnitude of the forces. The ‘maximal’ force evoked by electrode 1 (13.5 N) was twice as large as that for electrode 2 (6.7 N). That disparity, in itself, indicates that the force evoked by electrode 2 was not maximal despite the presence of the plateau in force at the highest currents tested with that electrode.

False plateaus

The average current at which force responses were first detected was 0.9 ± 0.4 mA. The average current associated with maximal force was 11.8 ± 3.4 mA. It should be noted, however, that in about half of the sessions (mainly the initial sessions), we did not always allow current to reach the maximum capacity of the stimulator (~20 mA). Often we halted the current steps once we observed what appeared to be a plateau in the force responses. We later noticed the presence of intermediate plateaus, wherein force would appear to saturate for two or more 1-mA increments in current (see Figs. 3A, 3B). Consequently, it is possible that when we halted current steps before reaching the maximum current capacity, we had actually encountered an intermediate or ‘false’ plateau.

To quantify the incidence of such false plateaus, we calculated the change in force associated with each increment in current for all of the current-force sequences. Figure 4A

shows an example of the relation between force and current for one sequence while Figure 4B shows the associated change in force for each current step. Based on plots like that shown in Figure 4B, we then determined the number of cases for which the change in force fell below 5% for two or more steps in current and which was then followed by increases in force above 5%. Figure 4B shows one such case of a false plateau (highlighted in red) for which little change in force was detected for increments in current from 2 – 5 mA but was then followed by clear increases in force at higher current levels. In total, 42% of the current-force sequences recorded had one or more such false plateaus. We believe that this percentage would have been even higher had we tested up through the maximum current capacity in all trials. The physiological and practical significance of such false plateaus will be addressed in the Discussion.

Force Summation

Figure 5A shows example force responses to 60-Hz trains of stimuli using ‘maximal’ current pulses delivered first to one electrode, then to the other electrode, and then to both electrodes together (first three trials). The order of stimulation was then reversed for the last three trials. Sixty-Hz stimulation was used in these experiments in an attempt to elicit the overall maximum force capacity of the anterior deltoid. The recordings shown in Figure 5A were taken from the same experimental session as that used for Figure 3. For the first trial, ‘maximal’ stimulation to electrode 1 (E1) produced a force of 12.8 N while ‘maximal’ stimulation to electrode 2 (E2) produced only 7.0 N of force. Simultaneous ‘maximal’ stimulation to both electrodes, however, produced a force of 16.5 N. A reduction in the evoked forces for the last three trials suggests some development of fatigue.

The average force for the two trials using dual-electrode stimulation in the experiment shown in Figure 5A was 15.2 N whereas the average force across the four trials using single electrode stimulation was 8.6 N. In this case, dual-electrode stimulation produced 177% of the force generated by single electrode stimulation.

Figure 5B shows the mean (SD) and individual values of isometric force resulting from dual-electrode stimulation, measured as a percentage of single electrode ‘maximal’ force, across all 28 sessions for the two subjects. In every case, dual-electrode stimulation produced greater force than single-electrode stimulation. On average, dual-electrode force produced 147.0 ± 22.3 % of single-electrode force ($P < 0.001$ compared to 100%). These results strongly indicate that using two (or more) sites of intramuscular stimulation enables activation of a greater volume of muscle than that which can be readily achieved with single site stimulation.

Fatigue

Another possible advantage of multiple-site stimulation is that interleaving stimulation between electrodes might enable a given target force to be maintained for longer durations by using lower stimulus frequencies at each electrode than if stimulation were delivered synchronously to all the electrodes. Figure 6 shows example recordings made in three separate sessions from one monkey in response to repetitive 2-s trains of pulses using: A) synchronous stimulation at 35 Hz/electrode, B) interleaved stimulation at 17.5 Hz/electrode,

and C) interleaved stimulation at 35 Hz/electrode. The insets show one-second segments of force and stimulus pulses at the outset of the tenth train from the end of each fatigue protocol to help illustrate differences in the stimulus patterns across sessions.

In all three sessions, the initial force was slightly greater than 2 N. For synchronous 35-Hz stimulation (Fig. 6A), after an initial relatively stable period of force production, force declined progressively over the remaining stimulus trains. The force produced by the last train was 36% of that produced by the first train. The force profile associated with interleaved 17.5 Hz stimulation (Fig. 6B) was very similar to that produced with synchronous stimulation at 35 Hz (Fig. 6A) with the last train producing 34% of the force of the initial train. Stimulation using interleaved stimulation at 35 Hz (Fig. 6C) had a slightly different profile with force appearing to drop a little more quickly at the outset of stimulation and with the last train producing 29% of the force of the initial train.

Figures 7A and 7B show the averaged force profiles for the three fatigue tasks for monkey R and monkey S, respectively. The averages in Figure 7A included 4 trials of synchronous 35-Hz stimulation, 4 trials of interleaved 17.5-Hz stimulation, and 5 trials of 35-Hz interleaved stimulation. The averages in Fig. 7B included 5 trials for each pattern of stimulation. The overall average current amplitudes used for the three tasks were: 2.0 ± 1.1 mA, 4.8 ± 2.6 mA, and 2.9 ± 1.2 mA for the 35-Hz synchronous, 17.5-Hz interleaved, and 35-Hz interleaved protocols, respectively. The higher currents for the 17.5 Hz stimulation were needed to offset the weaker forces produced with lower stimulus rates in order to achieve the target force.

For monkey R (Fig. 7A), the pattern of force loss over time was very similar across the three fatigue protocols. The main difference was a slightly steeper force loss at the outset of the 35-Hz interleaved stimulation protocol, similar to that shown for the example recording in Figure 5C. Likewise, for monkey S (Fig. 7B), the patterns of force loss were similar across stimulation protocols and were also similar to that of monkey R (Fig. 7A). However, for monkey S, the initial steep force loss with 35-Hz interleaved stimulation was sustained throughout the entire protocol.

One-way ANOVAs run on endurance index and normalized force area for monkey R yielded no significant effects (Table 1) of stimulation pattern. For monkey S, while there was no significant effect of stimulation pattern on endurance index, there was a significant effect ($p = 0.04$) on normalized force area. Post-hoc analysis indicated that normalized force area for 35-Hz interleaved stimulation was significantly less than that of either 35-Hz synchronous or 17.5-Hz interleaved stimulation. Such a tendency for greater fatigue with interleaved 35-Hz stimulation might be due to the higher *net* frequency delivered (70 Hz) to muscle fibers that happened to be activated by both electrodes. Such high-frequency stimulation can promote more rapid fatigue than stimulation at lower (and more natural) frequencies (Naess and Storm-Mathisen 1955; Jones et al. 1979; Metzger and Fitts 1986; McDonnall et al. 2004).

In addition to force loss, sustained activity can lead to slowing in muscle contraction and relaxation. For all stimulus patterns and for both monkeys, one-half relaxation time ($\frac{1}{2}$ RT) doubled over the course of the fatigue trials (Table 1). There was no significant effect,

however, of stimulus pattern on $\frac{1}{2}$ RT for either monkey individually or with data from both monkeys combined.

Following the fatiguing contractions, single brief (2 s) trains of stimuli were delivered at various intervals to evaluate recovery from fatigue. Figures 7C and 7D show the mean force (normalized to that of the first train at the outset of each fatigue protocol) during the recovery period for each of the three fatigue protocols for monkeys R and S, respectively. For both animals, force recovered steeply with one minute of rest, then continued to recover more slowly out to 4 minutes of rest up to 60 – 80% of the pre-fatigue force. Interestingly, for both animals, force then progressively diminished beyond 4 minutes out to 16 minutes (longest duration tested). Such delayed fatigue has been observed previously in fast twitch motor units in cat (Jami et al. 1983; Bevan et al. 1993), in rat motor units (Lannergren et al. 1989), and in whole human muscle (Edwards et al. 1977; Fuglevand et al. 1993). A two-way ANOVA using the combined data from the two monkeys with stimulus pattern and recovery time as factors yielded a significant effect of recovery time ($P < 0.001$) but no effect of stimulus pattern ($P = 0.07$) and no significant interactions. Post-hoc analysis indicated that force was significantly ($P < 0.001$) greater at all recovery times compared to that immediately after the fatigue test (i.e. at time 0), and that the force at 16 min recovery was significantly ($P < 0.001$) less than that at 2 and 4 minutes of recovery.

Evoked Movements with Multi-electrode Stimulation

As a simple test of the efficacy of multi-electrode stimulation to evoke movements, in one monkey we delivered stimuli synchronously to two intramuscular electrodes inserted into the anterior deltoid and recorded the evoked displacement of the hand using electromagnetic position sensors (Liberty, Polhemus) placed on the hand and shoulder (Figure 8A). Stimuli consisted of constant amplitude pulse trains (4 mA, 0.5 ms pulse duration, 3-s trains, 30 Hz). The limb hung pendant at the side of the monkey before each trial. Dual electrode stimulation evoked strong flexion at the shoulder causing the hand to briskly rise (peak speed ~ 250 cm/s) above the shoulder before settling and holding for ~ 2 -s just below shoulder height (Fig. 8B). After each trial, the hand was repositioned to approximately the same starting position. The time between successive trials was about 3 s. The evoked trajectory was quite similar across 10 repeated trials (Fig. 8C) and was almost entirely in the sagittal plane. We then strapped a 100 g load in the hand of the monkey and delivered the same stimulus sequence 3 times (Fig 8D). While the extent of arm movement was not as great as for the unloaded case (as expected for uncompensated stimulus strength), the arm reliably lifted and held the load against gravity. On numerous occasions (not shown), single electrode stimulation using high currents evoked only modest movements, often advancing the hand only a few centimeters forward of the starting position.

DISCUSSION

Here we have shown that stimulation of muscle with more than one intramuscular electrode clearly produces greater forces than can be readily produced with single electrodes. This occurred even when force responses to single electrodes appeared to saturate – traditionally considered a sign that the maximum force capacity of the muscle was achieved by the

stimulation. It seems reasonable, therefore, to consider using multi-electrode stimulation to augment the force-generating capacity of muscles (particularly large proximal muscles) to increase the utility of chronically implanted FES systems. Indeed, we showed in a single case that such multi-electrode stimulation of one muscle (anterior deltoid) was sufficient to briskly and repeatedly elevate the arm against gravity, an action that, as far as we are aware, has not previously been realized using FES alone.

Limitations

A limitation of the present study was that the maximum output capacity of the stimulator used was about 20 mA/channel. It should be pointed out, however, that the maximum current capacity of the most advanced implanted FES systems presently used in human patients is 20 mA (Memberg et al. 2014). Nevertheless, it is possible that had greater currents been delivered through single electrodes in the present experiments, greater forces might have been evoked and thereby diminished the relative advantage of using dual-electrode stimulation. However, with increased current intensity delivered at a single site, the radius of the effective current field could exceed the boundaries of the targeted muscle and begin to activate neighboring muscles while leaving distant portions of the target muscle un-activated. As such, perhaps an additional benefit of multi-electrode stimulation is that the activating current fields can be more readily sculpted to the oblong shape of most muscles.

Another limitation of the present study is that the test subjects were monkeys, not humans, and we only tested two subjects. As such, we had no ready way to determine the magnitude of the evoked forces relative to the maximum voluntary force capacity, as is commonly done in healthy human studies. On the other hand, because we were interested to examine the maximum force capacity produced by electrical stimulation, the pain associated with the high stimulation currents makes it practically intolerable for intact human subjects and also provokes complex nociceptive reflexes leading to inadvertent co-activation of other muscles that could compromise force measurements (Marsden et al. 1983; Martin et al. 2008). Therefore, we used general anaesthetics in macaque monkeys to enable fully reversible, functional paralysis while eliminating pain perception associated with high stimulation currents. In addition, we did not detect overt signs of spinal reflexes triggered by the stimulation, viz. co-activation of other muscles or sustained contraction of the target muscle following cessation of stimulation (Lagerquist et al. 2009). We cannot, however, rule out activation of brief reflexes (such as Hreflexes) under isoflurane anaesthesia (Leis et al. 1996). Another important feature of this method was that the animal was maintained in an upright, seated position, akin to its natural posture enabling relatively unrestricted motion of the upper limb. As such, this method would seem to be one that could be used to repeatedly and safely evaluate aspects of FES delivered with percutaneous or chronically implanted electrodes in macaque monkeys.

We used only two animals in keeping with ethical concerns to reduce the number of non-human primates tested in experiments. Each animal was tested during multiple sessions and the data from each session were more or less treated as independent samples from the set of all possible responses to stimulation of a given muscle. As such, some caution is warranted in extrapolating the results beyond the individual monkeys. However, the consistency of

responses within monkeys and similarity of responses across the two monkeys provides some confidence that the results are likely generally representative.

The fatigue tests used in the present investigation involved target forces that were relatively weak (perhaps 10 – 15% of maximum capacity). While such low forces might seem too weak to be functionally practical, most natural muscle activity in healthy subjects rarely exceeds 15% of maximum voluntary capacity (Klein et al. 2010). Therefore, force levels in this range are critical to the performance of activities of daily living. In a spinal-cord-injured individual, however, typical daily activities (e.g. lifting a coffee cup) would likely require substantially higher percentages of force capacities because of muscle atrophy. Nevertheless, if fatigue is evident at these low force levels (as shown in the present study) then this will significantly impact the utility of FES systems. Consequently, understanding the nature of fatigue in response to electrical stimulation even at low forces will be important for advancing the effectiveness of FES. While such heightened fatigue with FES has been ascribed to reverse recruitment (i.e., selective recruitment of high-threshold, fatiguable motor units, Kubiak et al. 1987; Trimble and Enoka 1991), a number of investigations have shown that orderly activation is more likely than reverse with electrical stimulation (Knaflitz et al. 1990; Feiereisen et al. 1997; Thomas et al. 2002; Farina et al. 2004).

False Plateaus

A widely used method to elicit putative maximal responses of human skeletal muscle using electrical stimulation is to progressively increment stimulus intensity delivered to a single electrode until a plateau is observed in the evoked responses. Stimulus strength is then typically increased by 20 – 50% above this level to ensure stimuli are ‘supramaximal’ (Merton 1954; Bigland-Ritchie et al. 1979; Rutherford et al. 1986; Stein et al. 1992; Fuglevand et al. 1993; Bilodeau 2006; Parise et al 2001; Lagerquist et al 2009; Seigler et al. 2016; Millet et al 2011, Maffiuletti et al 1994). In some instances, however, this approach may lead to stimulation at submaximal levels because of the presence of false plateaus as often encountered in the present study. Indeed, emergence from a false plateau often required stimulus strengths 250% higher than that just needed to reach the plateau. We assume that such false plateaus were mainly due to the activation of a subset of nerve branches close to the stimulating electrode and that substantially higher currents were then needed to activate more distant nerve branches. The validity of this assumption, however, warrants investigation.

Interestingly, in an important previous study, Crago et al. (1980) did not observe clear-cut plateaus in the current-force relationship of denervated soleus in the anesthetized cat. Some reduction in the slope of the current-force relationship was occasionally seen at intermediate intensities but not to the degree observed in the present investigation (see Figs. 3 and 4). This discrepancy could be due to differences in the masses (and associated volumes) of the two muscles tested (~ 3 g for cat soleus, Nelson 1968, ~7.5 g for macaque anterior deltoid, Cheng and Scott 2000). Notably, however, Crago et al. (1980) often showed saturation of force output at the highest intramuscular stimulus intensities tested that were below the maximum force capacity of the muscle (measured in response to whole nerve stimulation). Those results are consistent with the overall observations of the present study.

Fatigue

Somewhat contrary to our expectations, interleaving stimulus pulses to two intramuscular electrodes at relatively low rates to each electrode (17.5 Hz) did not improve force maintenance during prolonged stimulation compared to synchronous stimulation at a higher rate (35 Hz). Both methods led to about a 70% decline in force, a 50% reduction in force area, and about a doubling in relaxation time over 4 minutes of intermittent stimulation. There would seem to be at least two theoretical reasons why lower frequencies of stimulation, by itself, should tend to lessen fatigue during prolonged activity for tasks that involve an initial submaximal target force (as was used here). First, because of the force-frequency relation, the forces exerted by the activated muscle fibers using lower stimulus frequencies will be less than that occurring with higher frequencies. Therefore, in order to achieve the initial target force, more muscle fibers will need to be recruited (using higher stimulus currents), and consequently, the load will be more broadly shared across a greater number of muscle fibers. Conversely, with higher frequencies of stimulation, the load burden is carried by fewer muscle fibers, all of which must contract more intensely in order to achieve the initial target force. This latter situation would seem likely to promote more rapid fatigue.

Second, at the extreme, high stimulation frequencies can lead to impairment of excitation-contraction coupling and cause relatively rapid fatigue (Naess and Storm-Mathisen 1955; Jones et al. 1979; Metzger and Fitts 1986, McDonnall et al. 2004). The slightly greater fatigue in one monkey to 35-Hz interleaved stimulation might have occurred due to a high net frequency of 70 Hz imposed on the subset of muscle fibers activated by both electrodes. While we did not quantify the degree of overlap in activation of muscle fibers by the two electrodes for the fatigue experiments, the results of the force summation experiments (Fig. 5B) suggests an upper limit of ~ 50% overlap in fibers activated by the two electrodes.

Previous studies that have used interleaved stimulation to assess fatigue have yielded results not entirely consistent with the present investigation. For example, in the noteworthy work by Yoshida and Horch (1993), fatigue responses in the cat gastrocnemius were studied in response to single- or dual-electrode intraneural stimulation. Stimulation at 60 Hz, either with a single electrode or synchronously delivered to two electrodes, led to substantially greater fatigue than with interleaved stimulation to two electrodes at 30 Hz. Unfortunately, no tests were performed using *synchronous* stimulation at 30 Hz. As such, it is unclear whether the enhanced fatigue resistance seen in the Yoshida and Horch (1993) study with 30-Hz interleaved stimulation was due to interleaving the stimulation *per se* or was simply due to the use of lower stimulus frequencies.

Similarly, McDonnall et al. (2004) compared fatigue responses in cat gastrocnemius to stimulation with single or four intraneural electrodes. Stimulation at 60 Hz synchronously to all four electrodes caused greater fatigue than with interleaved stimulation to four electrodes at 15 Hz. Importantly, they also showed that interleaved stimulation at such low frequencies (15 Hz) markedly attenuated the unfused ripple evident with single electrode stimulation at 15 Hz. Puzzlingly, however, the degree of fatigue was *greater* with interleaved stimulation at 15 Hz compared to that associated with single electrode stimulation at 15 Hz.

In a number of recent studies using human subjects, fatigue resistance during FES has been shown to be consistently and significantly enhanced using low frequency stimuli delivered in an interleaved way to multiple surface electrodes compared to higher frequency stimuli delivered to a single electrode (Popovi and Maleševi 2009; Maleševi et al. 2010; Nguyen et al. 2011; Popovi Maneski et al. 2013; Sayenko et al. 2014; Lou et al. 2016; Bergquist et al. 2016; Bergquist et al. 2017; Laubacher et al. 2017). These impressive results clearly demonstrate that distributed stimulation is more efficacious in maintaining muscle output than single site stimulation. Yet, it is not entirely clear from these studies as to whether interleaving of stimulation by itself helps to minimize fatigue or whether it is primarily the use of lower stimulus frequencies during interleaved stimulation. Nevertheless, interleaving stimulus pulses across multiple electrodes using low stimulus frequencies will help minimize the large force fluctuations that otherwise would occur using synchronized stimulation. This is an important benefit in terms of output stability for FES.

In the present study, we observed little difference in fatigue resistance using interleaved versus synchronous stimulation. Indeed, when using the same stimulus frequency (35 Hz), interleaved stimulation caused slightly greater fatigue than synchronous stimulation in one animal. Importantly, the lack of an effect on fatigue using interleaved stimulation at 17.5 Hz versus synchronous stimulation at 35 Hz is at variance with some of the previously mentioned studies. There are perhaps two factors that might account for this discrepancy. First, the relative difference in stimulation frequencies used here for interleaved vs. synchronous stimulation (17.5 Hz) was modest compared to that in other studies (30 – 45 Hz) (Yoshida and Horch, 1993; McDonnall et al. 2004; Nguyen et al. 2011). And second, as pointed out by Yoshida and Horch (1993) and Wiest et al. (2017), the benefits in terms of fatigue resistance using interleaved multi-electrode stimulation diminishes as the degree of overlap in muscle fibers activated by the electrodes increases. Here the degree of overlap may have been substantial in comparison to the previous investigations.

Force Augmentation with Multiple Electrodes

And finally, despite relatively wide recognition that single electrode stimulation is unlikely to activate all motor units within a muscle (e.g. Vanderthommen and Duchateau 2007; Memberg et al. 2014), there are few previous studies that have evaluated the force-generating capacity of multisite versus single-site intramuscular stimulation. In one study, Maffiuletti et al. (2014) found that the maximum evoked force in healthy human quadriceps using three surface electrodes was greater (44% of maximum voluntary contraction force) than that produced by a more conventional dual-electrode configuration for the quadriceps (36% of maximum voluntary contraction force). Part of the increased force was due to the higher tolerance in the subjects for larger current intensities using the three-electrode configuration possessing a larger overall electrode surface area than the conventional configuration. In the present investigation, where stimulation discomfort was not a complicating factor, we found dual-electrode stimulation produced about 50% greater force than single-electrode stimulation.

Practical Implications

Given the greater force output achieved with dual-electrode compared to single-electrode stimulation in the present study, it would seem appropriate to consider including multiple electrodes per muscle in chronically implanted FES systems. Furthermore, an advantage of using simultaneous stimulation with two (or more) electrodes is that distant muscle regions that are below threshold for activation by any one electrode can be brought above threshold due to summation of the otherwise sub-threshold electric fields (Mortimer 1981). Practical factors, however, must also be taken into account including the increased surgical complexity, hardware needs, and power requirements for a multi-electrode approach. Nerve cuff, rather than intramuscular electrodes, would seem a viable alternative (Sweeney et al. 1990; Tyler and Durand, 2002; Schiefer et al. 2013) but these also have some drawbacks including lack of muscle selectivity and possibility of nerve damage (Grill and Mortimer, 2000). Perhaps the most efficacious approach at present would be to use a combination of electrode designs (single intramuscular electrodes for small muscles, multiple intramuscular electrodes for large muscles, and cuff electrodes where applicable), similar to that recently used in implanted upper limb (Memberg et al. 2014) and lower limb neuroprostheses (Guiraud et al. 2014).

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Figure 1.

Experimental set up. Anesthetized monkeys were positioned and secured upright in a modified infant car seat. Two intramuscular electrodes were inserted percutaneously into the right anterior deltoid muscle. Current-regulated pulses were delivered independently through each electrode and the evoked isometric shoulder-flexion forces were recorded with a transducer attached to the wrist. Surface electrodes placed over the clavicle served as return electrodes for each stimulation channel.

Figure 2.

(A) Example set of isometric force responses to 1-s trains of stimulus pulses (0.5 ms, 6 mA) at increasing frequencies from 10 – 100 Hz delivered through one electrode. (B) Mean (SD) force as a function of stimulus frequency for monkey R (10 sessions) and monkey S (12 sessions).

Figure 3.

Examples of force response to 1-s trains of stimulus pulses (0.5 ms, 35 Hz) with increasing current amplitudes from 1 – 16 mA delivered to (A) electrode 1 (E1) and (B) to electrode 2 (E2). Red horizontal lines indicate intermediate plateaus in force responses to increasing current. The dashed horizontal lines indicate force saturation for highest current levels tested.

Figure 4.

(A) Example force – stimulus current relationship for one electrode showing little increase in force for three, 1-mA step increments in current above 2 mA (red circles). (B) Percentage change in force for each increment in current depicted for the force – current relation shown in in panel A. Three consecutive increments in current (to 3, 4, and 5 mA – red circles) evoked less than a 5% increase in force (dashed horizontal line), and which were then followed by a greater than 5% increase in force (at 6 mA). Such a sequence was deemed a ‘false’ plateau.

Figure 5.

(A) Example force responses (top trace) to 'maximal' stimulation delivered at 60 Hz to electrode 1 (E1), electrode 2 (E2), or to both simultaneously. Forces produced in response to stimulation through single electrodes were exceeded with simultaneous stimulation through both electrodes. (B) Mean (SD) isometric force resulting from dual electrode stimulation expressed as a percentage of single electrode "maximal" force across all 28 sessions for the two monkeys. Individual values are shown separately for each session and for each monkey (open and closed symbols).

Figure 6.

Examples of isometric force responses generated by anterior deltoid of one monkey during four minutes of repetitive stimulation using two electrodes across three sessions involving: (A) synchronous stimulation at 35 Hz, (B) interleaved stimulation at 17.5 Hz, and (C) interleaved stimulation at 35 Hz. In all cases, stimulus trains were 2-s in duration with a 2-s inter-train interval. Insets show stimulus pulses and the initial portion of the force response for the 51st train in each sequence.

Figure 7.

Averaged force as a function of time for three fatigue tasks involving two electrodes: 35-Hz synchronized, 17.5 Hz interleaved, and 35-Hz interleaved in monkey R (A) and monkey S (B). Averaged force responses to 2-s trains of stimuli delivered at 1, 2, 4, 8, and 16 minutes after the three fatigue tasks in monkey R (C) and monkey S (D).

Figure 8.

Movements evoked by stimulation of anterior deltoid in anesthetized monkey using two intramuscular electrodes. (A) Image captured from video depicting arm being elevated in anesthetized monkey in response to stimulation (4 mA, 0.5 ms pulse duration, 3-s trains, 30 Hz) of anterior deltoid with two electrodes synchronously. The start position of the hand is indicated. Polhemus sensors were placed on the hand and shoulder to record the evoked movements. Image was retouched to remove extraneous physiological-monitoring equipment in the background. (B) Displacement of hand during a single trial. Each dot indicates sampled position of hand relative to shoulder (sampling interval ~ 8 ms) (C) evoked hand trajectory for 10 trials superimposed, (D) evoked trajectory for 3 trials with 100 g load in strapped in the hand.

Table 1

Mean (SD) values of endurance index, normalized force area, and slowing index (change in 1/2 relaxation time) in response to three types of fatigue protocols involving dual electrode stimulation.

	Monkey R					Monkey S					Both			
	35-Hz Synch.	17.5-Hz Interleaved	35-Hz Interleaved	P value	P value	35-Hz Synch.	17.5-Hz Interleaved	35-Hz Interleaved	p value	p value	35-Hz Synch.	17.5-Hz Interleaved	35-Hz Interleaved	P value
n	5	4	4	4		5	5	5			10	9	9	
Endurance Index	0.30 ± 0.11	0.30 ± 0.07	0.34 ± 0.10	0.78		0.35 ± 0.03	0.29 ± 0.06	0.20 ± 0.14	0.07		0.33 ± 0.08	0.29 ± 0.06	0.26 ± 0.14	0.15
Normalized Area	0.55 ± 0.13	0.56 ± 0.10	0.53 ± 0.11	0.93		0.55 ± 0.08	0.58 ± 0.07	0.40 ± 0.14	0.04*		0.55 ± 0.10	0.57 ± 0.08	0.46 ± 0.14	0.08
Normalized 1/2 relax. time	2.14 ± 0.14	2.07 ± 0.25	1.96 ± 0.13	0.37		2.10 ± 0.23	2.32 ± 0.42	1.95 ± 0.24	0.2		2.12 ± 0.18	2.21 ± 0.36	1.95 ± 0.19	0.12