Effective intracortical microstimulation parameters applied to the primary motor cortex for evoking forelimb movements to stable spatial end-points

Running Head: Effective stimulus parameters for evoking movement

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ABSTRACT

High frequency, long-duration intracortical microstimulation (HFLD-ICMS) applied to motor cortex has become recognized as a useful and informative method for corticomotor mapping by evoking natural-appearing movements of the limb to consistent stable end-point positions. An important feature of these movements is that stimulation of a specific site in motor cortex evokes movement to the same spatial end-point regardless of the starting position of the limb. The goal of this study was to delineate effective stimulus parameters for evoking forelimb movements to stable spatial end-points from HFLD-ICMS applied to primary motor cortex (M1) in awake monkeys. We investigated stimulation of M1 as combinations of frequency (30 - 400 Hz), amplitude (30 - 200 µA) and duration (0.5 - 2 s) while concurrently recording electromyographic (EMG) activity from 24 forelimb muscles and movement kinematics using a motion capture system. Our results suggest a range of parameters (80 - 140 Hz, 80 - 140 µA and 1000 ms train duration) that are both effective and safe for evoking forelimb translocation with subsequent stabilization at a spatial end-point. The mean time for stimulation to elicit successful movement of the forelimb to a stable spatial end-point was 475.8 ± 170.9 ms. The median successful frequency and amplitude were 110 Hz and 110 µA, respectively. Attenuated parameters resulted in inconsistent, truncated or undetectable movements, while intensified parameters yielded no change to movement end-points and increased potential for large-scale physiological spread and adverse focal motor effects. Establishing cortical stimulation parameters yielding consistent forelimb movements to stable spatial end-points forms the basis for a systematic and comprehensive mapping of M1 in terms of evoked movements and associated muscle synergies. Additionally, the
results increase our understanding of how the central nervous system may encode movement.

Keywords: motor control, primary motor cortex, primate, stimulation, EMG, forelimb.
INTRODUCTION

Corticomotor connectivity between neurons in M1 and muscles has been mapped previously using various forms of electrophysiological methods, tracer studies, and histological approaches (Cheney and Fetz 1985; Donoghue et al. 1992; Penfield and Boldrey 1937; Rathelot and Strick 2006; Woolsey et al. 1952). While early clinical electrophysiological approaches utilized surface stimulation of the cortex to evoke grossly observable responses (Penfield and Boldrey 1937; Woolsey et al. 1952), the introduction of intracortical microstimulation (ICMS) has permitted more refined mapping of cortical output to muscles (Asanuma and Sakata 1967). Short trains of high-frequency ICMS, often 10 cathodal pulses at 330 Hz, have been used to evoke twitch-like responses for cortical mapping and other purposes (Asanuma et al. 1976; Strick and Preston 1978). Such parameters have been used frequently, as consistent parameters have allowed for comparison of results between laboratories and studies. More recently this method of ICMS has been modified by applying ICMS trains with longer durations (500 ms) that more closely match the time scale of voluntary movements (Ethier et al. 2006; Graziano et al. 2005; Graziano et al. 2002). These HFLD-ICMS trains produce movements of the limbs characterized by having a common spatial end-point for a particular cortical site regardless of the starting position of the limb.

In the current study, our aim was to identify effective parameters for HFLD-ICMS applied to the monkey forelimb representation in M1 that yield translocation and subsequent stabilization of the forelimb at discrete spatial end-points. We applied stimulation as combinations of frequency (30 - 400 Hz), amplitude (30 - 200 uA) and duration (0.5 - 2 s) while concurrently recording the electromyographic (EMG) output to 24 forelimb muscles as well as the stimulus-evoked limb kinematics using a Vicon
motion capture system. Our results suggest a range of stimulus parameters for M1 forelimb representation that are both safe and effective for evoking movement and subsequent stabilization of the forelimb at a spatial end-point in an awake monkey. These parameters are 80 - 140 Hz, 80 - 140 µA and 1000 ms. The median successful frequency and stimulus intensity for all successful trials were 110 Hz and 110 µA, respectively, and the mean time for stimulation to elicit successful movement of the forelimb to a stable spatial end-point was 475.8 ± 170.9 msec. Attenuated parameters resulted in inconsistent, truncated or undetectable movements, while intensified parameters yielded no additional movement end-point data and increased the potential for large-scale physiological spread and adverse focal motor effects. Establishing stimulation parameters that yield consistent forelimb movements to stable spatial end-points formed the basis for a systematic and complete mapping of forelimb movement representations in primary motor cortex (Van Acker III et al. 2011). The results add to our understanding of how HFLD-ICMS applied to motor cortex affects motor output, and could aid in the advancement of neuroprosthetic devices.
EXPERIMENTAL PROCEDURES

Behavioral Task

Data were collected from two male rhesus macaques (*Macaca mulatta*; ~9 kg each, ages 12 and 4) performing a reach and prehension task that required co-activation of proximal and distal forelimb muscles, while also yielding discrete spatial locations to cue stimulus trains. Training procedures and the behavioral task have been described in detail previously (Belhaj-Saif et al. 1998; McKiernan et al. 1998). Briefly, the monkey was seated in a custom primate chair within a sound-attenuating chamber with his left arm comfortably restrained while his right arm retained freedom of movement. To discourage the monkey from interfering with reflective motion capture spheres attached to his arm, a custom face mask barrier was installed on the chair that contained a small hole through which the monkey could feed himself. In order to receive a food reward the monkey initiated the task with his right hand by depressing a homeplate lever located at waist height directly in front of him for one second duration, triggering the release of a food pellet into a food well located at shoulder height requiring full extension of the arm. Following the retrieval and consumption of the food reward, the monkey returned his hand to the homeplate for subsequent trials. Performance was guided by audio and visual cues.

Surgical Procedures

Following training, an MRI-compatible stainless steel chamber allowing 30 mm diameter of dura exposure, and exploration of the underlying cortical area, was
implanted stereotaxically over the primary motor cortex of the left hemisphere of each
monkey using procedures described in detail previously (Park et al. 2001). Briefly, the
chamber was anchored to the skull using titanium screws and dental acrylic, and was
centered over the hand area of M1 in the left hemisphere. In addition, threaded titanium
nuts were attached over the occipital aspect of the skull using titanium screws and
dental acrylic. These nuts provided a point of attachment for a flexible head restraint
system during data collection (McKiernan et al. 1998, 2000). The chambers were
centered at anterior 17.7 mm, lateral 27.2 mm and 26° to the sagittal plane for Monkey
X, and anterior 16 mm, lateral 22 mm 30° to the sagittal plane for Monkey A.

Twenty-four muscles of the right shoulder and forelimb were each implanted with
two multi-stranded stainless steel wires (Cooner Wire, AS632). Both monkeys were
implanted under sterile conditions using a cranial subcutaneous implant technique
described previously (Park et al. 2000). Briefly, all wires were stripped of ~2-3 mm of
insulation and tunneled subcutaneously from a cortical connector (Amphenol,
Wallingford, CT) to each muscle, where the wires were inserted into the muscles a
distance of ~2-3 cm, with a ~5 mm separation of the two wires in each muscle. The
cortical connector module was secured with dental acrylic near the cortical recording
chamber. We tested the placement accuracy of each electrode pair by observing
appropriate muscle twitches that resulted from applying short stimulus trains (Grass SD9
Stimulator). EMG activity was recorded from five shoulder muscles: pectoralis major
(PEC), anterior deltoid (ADE), posterior deltoid (PDE), teres major (TMAJ) and
latissimus dorsi (LAT); seven elbow muscles: biceps short head (BIS), biceps long head
(BIL), brachialis (BRA), brachioradialis (BR), triceps long head (TLON), triceps lateral
head (TLAT) and dorsoepitrochlearis (DE); five wrist muscles: extensor carpi radialis
(ECR), extensor carpi ulnaris (ECU), flexor carpi radialis (FCR), flexor carpi ulnaris
(FCU) and palmaris longus (PL); five digit muscles: extensor digitorum communis (EDC), extensor digitorum 2 and 3 (ED23) extensor digitorum 4 and 5 (ED45), flexor digitorum superficialis (FDS) and flexor digitorum profundus (FDP); and two intrinsic hand muscles: abductor pollicis brevis (APB) and first dorsal interosseus (FDI).

All surgeries were performed under deep general anesthesia and aseptic conditions. Prior to each implant surgery, the monkey was administered ketamine (10mg/kg IM), atropine (0.04 mg/kg IM), and medetomidine (0.05 mg/kg IM) for transportation purposes, and subsequently isoflurane gas for the duration of the surgery. Each monkey received prophylactic antibiotic (penicillin, 6000 U/kg SC) 10 hours pre-surgery, 1 hour directly following surgery, and 3 days following surgery. Postoperatively, the monkeys were given analgesics (buprenorphine, 0.01 mg/kg IM and carprofen, 5 mg/kg SC). All surgeries were performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care using full sterile procedures. All procedures conformed to the Guide for the Care and Use of Laboratory Animals, published by the United States Department of Health and Human Services and the National Institutes of Health.

Recording Forelimb Kinematics

Limb kinematics were monitored and recorded using a Vicon (Oxford, UK) motion capture system. For the purposes of this study, a virtual sphere located on the dorsal tubercle of the radius was calculated and used to evaluate limb kinematics as well as the spatial end-point resulting from HFLD-ICMS applied to each cortical site. The 3-dimensional coordinates of this virtual sphere were calculated using Point Cluster Technique described previously (Andriacchi et al. 1998; Senesh and Wolf 2009). Briefly,
a cluster of reflective spheres were attached to the forearm of each monkey. The three-
dimensional coordinates of the spheres in each cluster, collected at a sampling rate of
100 Hz using 4 Vicon MX3+ cameras with reconstruction done offline using Vicon Nexus
software, were used to triangulate the coordinates of the virtual sphere located on the
dorsal tubercle of the radius, which was identified during static trials at the start of each
recording session. The dorsal tubercle of the radius was used as a surrogate for end-
point position of the hand in space. Utilizing subsets of the numerous wrist markers to
calculate the spatial position of the reference point (dorsal tubercle of the radius)
reduced the need for the dorsal tubercle of the radius to be within view of any particular
set of Vicon cameras in order to be monitored.

**HFLD-ICMS Protocol**

One-thousand, six-hundred and twenty-two HFLD-ICMS trains were applied to
19 distinct cortical sites within the right forelimb representation of M1 in the left
hemisphere (Figure 1) of two rhesus macaques performing a reach and prehension task
over the course of the study. Stimulation was applied through glass insulated platinum-
iridium electrodes suitable for single unit recording (FHC Inc., Bowdoin, ME), with typical
impedances of 0.7 - 1.5 MΩ at the start of each recording session. We positioned the
electrodes using an X-Y positioner secured to the chamber for the duration of each
recording session, and advanced the electrode into the brain using a manual hydraulic
microdrive (FHC Corp.) until the electrode tip was located in cortical lamina V, or
approximately 1.5 mm below the surface of the brain. Location of layer V was
determined by depth, audio cues (Grass AM8 Audio Monitor), visual cues (oscilloscope
display of large spike waveforms), and finally by poststimulus effects in stimulus
triggered averages (StTAs) of EMG activity computed at an intensity of 15μA and a stimulus frequency of 10 Hz (Park et al. 2001). HFLD-ICMS was applied when the monkey's hand was at one of three task positions (homeplate, food well or mouth), and the location that was furthest from the stimulus-evoked spatial end-point was chosen once a test stimulation trial elicited a detectable movement. Because the position at the food well was on the periphery of the monkey's workspace, this location was used most frequently for stimulation onset.

At each cortical site we applied systematic combinations of frequency (30 - 400 Hz), amplitude (30 - 200 μA) and duration (0.5 - 2 s) in order to delineate parameters that yielded forelimb movement with subsequent stabilization. Individual stimuli for each stimulus train were symmetrical cathodal biphasic pulses: initial negative pulse 0.2 ms in duration followed by a positive pulse 0.2 ms in duration. Stimulus train duration of one second was revealed early in the study to be an optimal epoch for assessing the adequacy of the stimulus intensity and frequency parameters. A 500 ms stimulus train duration often resulted in movements that were truncated relative to spatial end-points achieved with longer durations and was therefore too short to reliably determine a spatial end-point for many elicited movements. Movements to spatial end-points were always achieved with stimulus durations of less than 1000 ms, and therefore longer durations were unnecessary and increased the risk of adverse focal motor effects. Finally, once the forelimb reached the spatial end-point, the time remaining in the 1000 ms train was used to ensure that the forelimb was locked in the final posture for the duration of stimulation, and that stimulation was not evoking a truncated movement or a movement to an unstable end-point.

While we initially explored frequencies ranging from 200 - 400 Hz as well as intensities ranging from 150 - 200 μA, we concluded that these parameter ranges were
unnecessarily high for achieving a stable stimulus-evoked end-point, and that these parameters also increased the potential for adverse focal motor effects. Therefore each parameter set collected for data analysis in this study was a combination within a frequency range of 50 - 170 Hz, a stimulus intensity range of 30 - 150 μA and a one-second train duration. Five trials were conducted for each HFLD-ICMS parameter set. Occasionally an additional set of five stimulation trials were repeated at the same cortical site later in the study session to allow comparison of movement end-points between the beginning and end of the stimulation recording session.

Determining successful stimulus parameters for evoked movements

The kinematics of HFLD-ICMS-evoked wrist movement (marker on the dorsal tubercle of the radius) were used to assess successful spatial end-points achieved through stimulation of M1. HFLD-ICMS-evoked movement trials were deemed successful if the stimulus parameters applied to the M1 site were sufficient to translocate the monkey's forelimb to a spatial location distinct from its location at the initiation of stimulation and sufficient to maintain the limb at the stimulus-evoked location for the duration of stimulation. Attenuated parameters resulted in incomplete, truncated, or undetectable movements of the forelimb, often resulting in the monkey overcoming the stimulated movement and returning to the voluntary movement task. Intensifying parameters beyond those deemed successful at each cortical site also yielded similar end-points. However, intensified parameters increased the potential for adverse focal motor effects, possibly due to extensive physiological spread along horizontal cortical collaterals exacerbated by excessive stimulus frequency. Therefore, our goal was to
delineate the safe and effective range of parameters that yielded movement and subsequent stabilization of the forelimb for the duration of stimulation.

The specific success criteria we used required, firstly, that stimulus-evoked movement velocity reach a level of ≥ 40% of the average maximum stimulus-induced velocity within the first 500 ms of stimulation onset to indicate adequate stimulus-induced movement. The average maximum stimulus-induced velocity was calculated from a subset of approximately 25% of the data for which we determined success based upon individual trial velocity data. An iterative classification process was used because we first needed to determine qualitatively the parameters that resulted in a stable posture, and subsequently to create an algorithm that matched closely our visual interpretation. This was important particularly due to the fact that there were multiple points within the evoked movement velocity profile for which the algorithm required modeling to fit movements to stable end-points. Therefore, we first determined qualitatively which evoked movements were stable, then generated an algorithm that distinguished these movements as stable, and finally retested to ensure the algorithm was accurately grouping the movements as successful or unsuccessful as determined qualitatively. This iterative process allowed for final analysis of all trials using one algorithm with consistent velocity thresholds.

The second criterion was that, on the slowing phase of movement, the velocity had to cross below a threshold of 372.1 mm/s, or 25% of the average maximum stimulus-induced velocity of 1488.1 mm/s based on data from both monkeys. Finally, once this lower threshold was crossed, the velocity of the movement had to remain below this 25% threshold for the remainder of the applied stimulus train to ensure a stable spatial end-point had been achieved (Figure 2). A 25% threshold was used to allow for oscillations of the wrist about a single endpoint which occurred often once the
limb reached the spatial end-point. This threshold was low enough to separate the
oscillations from voluntary movements superimposed on stimulus driven movements
within the epoch of stimulation. Time to spatial end-point stabilization for successful trials
was measured at the point where the velocity of the stimulus-evoked movement crossed
the threshold of 25% toward achieving a stable end-point position. These criteria
provided an objective and reproducible method of identifying successful trials that
closely matched our subjective visual assessment.

Stimulus Triggered Averages

Stimulus triggered averages (StTAs) of EMG activity (Park et al. 2001) were
acquired for all implanted muscles from stimuli applied throughout all phases of the
reach and prehension task. StTAs were used to help confirm positioning of the electrode
in lamina V and to determine cortico-muscle connectivity of corticospinal cells directly
surrounding the electrode tip. StTAs were obtained at 10 Hz and at current intensities
that matched those used for each HFLD-ICMS train applied at the same site. In addition,
muscle facilitation maps (Figures 1A and 1B) were based on StTAs collected at 15 and
30 µA with stimulus sites at 1 mm intervals on the surface of the precentral gyrus and
0.5 mm vertical intervals down the bank of the central sulcus. EMG activity was filtered
from 30 Hz to 1 kHz, digitized at 4 kHz and full-wave rectified. Individual stimuli for the
StTAs were symmetrical biphasic pulses: initial negative pulse 0.2 ms in duration
followed directly by a positive pulse 0.2 ms in duration. StTAs were based on a minimum
of 1,000 trigger events. Averages were compiled using a 60 ms epoch, of which 20 ms
prior to the trigger was considered baseline. StTAs were identified as having a significant
PStE if the peak or trough of the effect exceeded ±5 SD of the baseline for a period of ≥ 0.75 ms as described previously for moderate-to-large effects (Park et al. 2004).

Measurement of EMG cross-talk

We evaluated cross-talk between EMG electrodes by constructing EMG-triggered averages. This procedure used the motor unit potentials from one muscle as triggers for compiling averages of rectified EMG activity of all other muscles. Criteria established previously (Buys et al. 1986) were used to eliminate effects that might have been affected by cross-talk. To be accepted as a valid PStE, the ratio of PStF between the test and trigger muscles must have exceeded the ratio of their cross-talk peaks by a factor of two or more. Based on this criterion, FDI in monkey X was removed from the analysis.
RESULTS

Success rate for parameter pairings

The ratio of successful HFLD-ICMS-evoked movement trials to total trials was calculated for each frequency and current intensity parameter pairing. A successful trial was defined as one in which HFLD-ICMS produced translocation of the arm to a new end-point position and which remained stable for the duration of stimulation (see Methods). Figure 3 illustrates the success rate for each parameter pairing color coded as a ratio of successful trials to total trials for each pairing. Total trials attempted are noted in the center of each parameter pairing. The results reveal a clear boundary of the lower range of stimulus parameters that yield successful forelimb movements to stable spatial end-points from HFLD-ICMS applied to M1 cortex. At a given site, once the threshold of successful stimulus parameter pairings was found, increasing stimulus parameters at that site resulted in the same spatial end-point, although increased the risk for adverse focal motor effects. Due to this risk, a clear upper boundary was not aggressively sought. Successful stimulus parameters primarily fell within the frequency range of 80 - 140 Hz coupled with a stimulus intensity range of 80 - 140 µA. For successful HFLD-ICMS-evoked movements of the forelimb, the mean time from stimulus onset to the arm reaching a spatial end-point position was 475.8 ± 170.9 ms, and the range was 110 - 940 ms. The mean successful frequency, calculated using a weighted average based upon the ratio of successful to total trials for each parameter pairing, was 117.6 ± 23.2 Hz and the mean successful stimulus intensity was 108.3 ± 24.4 µA. Additionally, the median successful frequency and stimulus intensity for all trials were 110 Hz and 110 µA, respectively. Attenuated parameters resulted in inconsistent, truncated, or
undetectable movements of the forelimb, often resulting in the monkey overcoming the stimulated movement and returning to the volitional movement task. Intensified parameters beyond the optimal range identified above yielded no additional benefit while increasing the potential for physiological spread and adverse focal motor effects, and therefore were avoided.

Success rate for parameter pairings for an individual site

Figure 4 illustrates stimulus parameter pairings that led to successful spatial end-points at an individual cortical site. In this example, 158 stimulation trials were applied to cortical site six of monkey X (Figure 1B). The range of stimulus parameters that evoked successful spatial end-points were relatively consistent across M1 cortical sites. For each cortical site at which stimulation was applied in this study, a locus of successful stimulus parameter pairings was evident. The range of parameters that yielded the highest success rate for the cortical site illustrated in Figure 4 were 80 - 140 Hz and 100 - 140 µA, with a mean time to spatial end-point stabilization of 556.9 ± 49.9 ms. The mean successful frequency and amplitude at this site were 127.5 ± 18.5 Hz and 109.7 ± 25.9 µA respectively, and the median successful frequency and amplitude were 120 Hz and 120 µA respectively.

Effect of stimulus parameters on peak movement velocity

To characterize the relationship between stimulus parameters and movement velocity of the forelimb during stimulation, we first applied a multiple regression analysis to all successful parameter pairings at all sites for both monkeys. Figure 5 illustrates a
density plot of stimulus parameter pairings color coded as the peak stimulus-induced velocity for each parameter pairing for both monkeys averaged over the number of trials listed in the center of each square. A stimulation duration of one second was applied for each parameter pairing, and only trials that yielded successful spatial end-points were included. Combining all data, Pearson linear correlation analysis assessing peak velocity relative to stimulus frequency (r = 0.1054, p = 0.4070) or to stimulus intensity (r = 0.1906, p = 0.1313) did not yield statistically significant relationships, although there is a clear tendency evident from Figure 5 for velocity to increase in going from the lower left corner of the plot to the upper right corner. However, illustrated in Figure 6, data collected from the single cortical site 5 of Monkey X (Figure 1B), for which multiple regression was used to calculate the effect of the combination of stimulus frequency and intensity on velocity, yielded a significant correlation between stimulation parameters and velocity (R² = 0.81871, p = <0.0001). This suggested that significant variance between stimulus parameters and velocity may have been obscured or washed out by combining data from all cortical sites and both monkeys.

To further investigate whether the monkey or cortical site affected variations in velocity, we used an ANOVA to look at the effect of frequency, stimulus intensity and cortical site on the stimulus-evoked movement velocity. A 4-way ANOVA showed significant effects (p<0.05) for each independent variable of frequency, stimulus intensity and cortical site. We further investigated this using a Tukey’s multiple group comparison with a Boneferonni correction, in which case we found that the significant differences observed did not come from monkeys, specifically, rather they arose from the cortical sites. These data are consistent with prior long duration microstimulation
studies involving oculomotor and forelimb movements, revealing that higher stimulus frequency and intensity at a given site results in increased movement velocity.

HFLD-ICMS effect on circuitry not affected directly by stimulation

Successful spatial end-points as calculated in this study were unattainable at a number of sites in M1. HFLD-ICMS applied to M1 cortical sites that yielded either solely proximal or solely distal muscle facilitation, as determined with StTA mapping, were more likely than proximal-distal co-facilitation (PDC) sites (Figure 1) to yield stimulus evoked movements with superimposed voluntary activity. For example, if we applied stimulation to an area assessed by StTA to yield distal forelimb facilitation, the likelihood of achieving a spatial end-point of the wrist marker was reduced in comparison to sites in or close to PDC sites. This appeared to be due to the monkey’s ability to retain voluntary control of the joint associated with the muscles that were not affected directly by stimulation. At such purely distal-representation sites, the monkey often preserved the ability to move his shoulder and/or elbow, while his distal muscles were fully stimulus-activated and apparently locked in the stimulus-evoked posture. As a consequence, the monkey was able to move the virtual sphere located on the wrist continuously during stimulation by moving his shoulder and/or elbow joints. The result was an unsuccessful trial. Ability to move the proximal muscles under voluntary control while the distal muscles appeared to be locked into a stimulus driven posture was observed at 15/45 (33.3%) and 10/31 (32.3%) distal-only cortical sites (determined via StTA mapping) for Monkeys A and X, respectively, using parameters that normally would produce a successful trial. Conversely, stimulation at 15/35 (42.9%) and 15/37 (40.5%) proximal-only cortical sites for Monkeys A and X, respectively, resulted in a locked posture of
proximal muscles while leaving distal muscles accessible to voluntary control. These
results include data from all cortical sites explored throughout the entire mapped M1 in
both monkeys. However, not every proximal- or distal-only site based on StTA effects
responded in this manner, as some yielded both proximal and distal muscle activation
when HFLD-ICMS was applied. Broadened activation of muscles with repetitive
stimulation is likely due to physiological spread through neural networks that connect
proximal and distal muscles for coordinated multi-joint limb movements.
DISCUSSION

The goal of this study was to determine effective HFLD-ICMS parameters applied to M1 that yield translocation and subsequent stabilization of the forelimb at a fixed end-point within the monkey’s work space. The use of HFLD-ICMS to study motor output has gained traction as an additional technique to include with other electrical stimulation methods such as TMS, RS-ICMS and StTA in studying cortical encoding of movement (Ethier et al. 2006; Graziano et al. 2005; Graziano et al. 2002; Tehovnik and Lee 1993; Thier and Andersen 1998). Delineating stimulation parameters that translocate the limb and subsequently stabilize it at a spatial end-point will provide a foundation for selection of appropriate parameters for systematic mapping of M1 output in terms of HFLD-ICMS-induced movements. We would like to point out that, due to the large number of stimulation combinations applied to each cortical site in this study, a limited number of cortical sites were investigated. While the findings were relatively consistent across sites, and the successful parameters were used effectively in a subsequent study for the systematic mapping of M1, appropriate caution should be maintained when interpreting the results of this study.

Mechanism of stimulus-evoked movement

In this study we determined the effective HFLD-ICMS parameters that, when applied to M1 cortex, would supplant volitional movement of the forelimb with movement induced by stimulation. This replacement of volitional movement with stimulated movement requires elimination or interruption of the subject’s voluntary efferent output to target muscles of the forelimb, and replacement of the eliminated volitional commands.
with those imposed by stimulus-evoked activity (Griffin et al. 2011). One mechanism by which volitional cortical commands may be eliminated is by antidromic interruption and blockage of natural afferent input to corticospinal neurons by antidromically generated action potentials in the axons of neurons supplying afferent input to cortical output neurons. With natural synaptic input eliminated, corticospinal neurons would fire at a rate determined solely by stimulation.

This phenomenon of replacement, or “hijacking,” has been shown to occur in M1 with high frequency microstimulation (Griffin et al. 2011) as well as in subcortical areas such as the subthalamic nucleus with deep brain stimulation (Garcia et al. 2005). Interestingly, frequency parameters that have been empirically adopted to therapeutically treat diseases such as Parkinson’s Disease using deep brain stimulation (DBS) are in the range of 120 - 180 Hz (Garcia et al. 2005). The similarity in effective frequency ranges used in DBS and the current study likely reflects a common mechanism underlying achievement of the desired behavioral outcome in which neural activity of target cells is replaced with artificial stimulus-evoked activity. Hijacking of corticospinal cell activity by electrical stimulation likely occurs in direct response to application of stimulus frequencies that exceed the natural movement-related firing rates of cortical neurons and afferent inputs (Griffin et al. 2011). The stimulus rates found to be optimal in the present study (80-140 Hz, median 110 Hz) are similar to the expected average maximum firing rates of cortical cells (Capaday et al. 2011) and, therefore, would be expected to be effective in blocking and replacing natural movement-related activity. However, in order to evoke complete movements using stimulation, it is necessary to recruit a sufficient number of corticospinal cells to activate a muscle synergy capable of generating the joint forces needed to move the limb to a new position. Stimulus intensity clearly plays a major role in the number of corticospinal
neurons that are activated. The stimulus intensity range found to be effective in
producing HFLD-ICMS-evoked translocation of the limb to a new stable end-point
position was 80–140 µA with a median intensity of 110 µA. The following equation can
be used to calculate the spread of effective stimulus current within the cortex

\[ r = \sqrt{\frac{i}{k}} \]

where \( r \) is the radius of the stimulus-activated cortical volume, \( i \) is the stimulus intensity
and \( k \) is the current-distance constant. Based on a minimal excitability constant of 250
µA/mm² for the largest cortical neurons or an intermediate excitability of 1292 µA/mm²
(Cheney and Fetz 1985; Tehovnik et al. 2006), the area of directly activated cortical
tissue can be estimated to be 1.01 - 1.76 mm² or 0.20 - 0.34 mm², respectively. Based
on estimates of the density of corticospinal neurons (He et al. 1993) and a minimal
excitability constant, this would correspond to activation of 302 - 528 corticospinal
neurons. However, this does not take into account trans-synaptic activation via
physiological spread of stimulus current, which is could be substantial at the HFLD-ICMS
frequency ranges used in this study. Therefore, the true number of corticospinal cells
activated becomes very difficult to estimate with certainty (Cheney and Fetz 1985).

Effect of stimulus parameters on muscles not directly affected by stimulation

At what level does HFLD-ICMS interfere with the internal motor program
underlying voluntary movement? Does HFLD-ICMS hijack only the cortical output
mechanism responsible for movement execution, or does it also interfere with
“upstream” events involved with motor planning or other higher level functions?
Interference with higher level functions might occur through antidromic activation of
neurons in secondary cortical motor areas as well as thalamic afferent input to M1. If
higher level functions were interrupted by HFLD-ICMS, the effects on movement might extend beyond the muscles directly activated (hijacked) by stimulation, leaving the monkey unable to produce voluntary movements in unaffected parts of the stimulated limb or even more distant body parts. However, we found that muscles not directly affected by stimulation preserve voluntary activity. As a result, the monkey retains voluntary control over these unaffected muscles during stimulation, even for muscles acting on joints adjacent to those controlled by stimulation. For example when HFLD-ICMS applied to M1 hijacked wrist and digit muscles, the monkey’s ability to activate proximal muscles under voluntary control was preserved. Indeed, in such trials the monkey would often move the unaffected proximal joints in an apparent attempt to reposition the hand near an unclaimed food reward although the monkey was then unable to use its hand to grasp the food. The reverse was also observed with loss of voluntary control over proximal muscles due to hijacking but retention of voluntary control over wrist and digit muscles. These findings suggest that HFLD-ICMS acts primarily on the neural circuits responsible for movement execution rather than on upstream processes such as those involved with motivation and movement planning. Alternatively, stimulation antidromically affecting upstream circuits may primarily act upon somatotopically similar output circuits as those in M1 to which they project.

Stimulus parameters used in the current study compared to those used in previous studies

We found that applying stimulus parameters of 80 - 140 Hz, 80 - 140 μA and 1000 ms duration (using cathodal biphasic 0.2 ms pulses) were effective for evoking translocation of the forelimb to new stable end-point positions in an awake and behaving
monkey. These parameters have similarities to stimulus parameters used in previous studies to evoke prolonged limb movements or eye saccades, however, there are some significant differences. To elicit smooth and stable eye saccades from parietal cortex, parameters of 500 Hz, 100 - 200 µA, 100 ms, with biphasic pulses of 0.1 ms duration were found to be effective (Thier and Andersen 1998). To evoke limb movements in an anesthetized cat, parameters of 333 Hz, 10-100 µA, 500 ms, with 0.2 µs pulses were used (Ethier et al. 2006), although these movements did not necessarily meet the criteria applied in our study. For eliciting movements in the monkey forelimb, Graziano et al. (2002a) used 200 Hz, 100 µA and a stimulus train duration of 500 ms with biphasic 0.2 ms pulses. In anaesthetized rodents, parameters of 181-400 Hz, 20-75 µA and train durations of less than 40 ms using cathodal pulses were found to be effective ranges for eliciting movements (Young et al. 2011), although the goals of their study were somewhat different than ours and did not include evoking complete movements to stable end-points. The variability in stimulus parameters used between studies likely also reflects species differences and the use of anesthetizing agents. The stimulus duration used in the present study (1000 ms) was longer than those used in previous mapping studies due to the fact that the latency of stimulus onset to arrival of the forelimb at a stable spatial end-point had a wide range, with nearly half of the evoked spatial end-points exceeding stimulation durations used in previous studies. 

**Summary and Conclusions**

Our results suggest a range of stimulus parameters applied to M1 of an awake and behaving monkey that are both safe and effective for evoking translocation and subsequent stabilization of the stimulated limb at a spatial end-point: 80 - 140 Hz, 80 -
140 µA and 1000 ms, with a mean spatial end-point time of 475.8 ± 170.9 ms. The mean successful frequency, normalized for all trials recorded in the study, was 117.6 ± 23.2 Hz, and the mean successful intensity was 108.3 ± 24.4 µA. Additionally, for all trials, the median successful stimulus frequency was 110 Hz and the median stimulus intensity was 110 µA. Attenuated parameters resulted in inconsistent, truncated or undetectable movements, while intensified parameters increased the potential for large-scale physiological spread and adverse focal motor effects. Establishing cortical stimulation parameters that yield consistent stimulus-evoked end-points provides a foundation for the systematic and comprehensive mapping of movement space and associated muscle synergies in primary motor cortex. The results add to our understanding of how the central nervous system encodes movement, and could aid in advancing the application of neuroprosthetic devices.
ACKNOWLEDGMENTS

We thank Ian Edwards and Molly McVey for their technical assistance, as well as Annaria Barnds from University of Kansas Bioengineering department for assisting in statistical analysis. This work was supported by NIH Grants NS058129, NS064054, NIH Center Grant HD02528, the KUMC Biomedical Research Training Program and the Madison and Lila Self Graduate Fellowship.


FIGURE LEGENDS

Figure 1. Unfolded maps of Monkey A's (A) and Monkey X's (B) M1 forelimb representations showing the location of sites from which stimulus parameter data was collected. The color-coded muscle representation maps were derived from stimulus triggered averaging of EMG activity at 15 µA. Each unfolded map illustrates the M1 forelimb representation in the left hemisphere with a view of the dorsal surface of the precentral gyrus and the rostral wall of the central sulcus. Cortical site locations used for stimulus parameter analyses are indicated with numbered orange dots. Anterior, posterior, medial and lateral are indicated by the compass rose. Distal forearm facilitation in M1 is represented as blue, proximal forearm facilitation as red, and proximal-distal co-facilitation as purple. Each hash mark represents a distance of 1 mm.

Figure 2. Example of velocity profiles of the monkey's wrist (dorsal tubercle of the radius) in space during three separate trials using different parameters applied to the same cortical site. Stimulation is represented by vertical lines under "HFLD-ICMS", and stimulus duration is represented by gray background underlying velocity tracings. A successful trial is defined as stimulation resulting in translocation of the limb to a spatial end-point with subsequent stabilization for the duration of the stimulation (see Methods). The average maximum velocity was 1.49 m/s, while the 25% and 40% thresholds were 0.37 and 0.60 m/s respectively. The velocity profile represented by the solid line represents a successful trial, whereas those by the dashed lines represent unsuccessful trials.

Figure 3. Success rate for HFLD-ICMS pairings for both monkeys. Density plot of stimulus parameter pairings color-coded as a ratio of successful trials to total trials for each pairing. Stimulus train duration was one second for each parameter pairing. Total trials for each
parameter pairing are listed in the center of each square. On the ratio scale, 1.0 is equal to a success rate of 100% for that stimulus parameter combination. Abbreviation: NT, parameters not tested.

Figure 4. Success rate for HFLD-ICMS parameter pairings at one cortical site (site six in monkey X, Figure 1). Density plot of stimulus parameter pairings color-coded as a ratio of successful trials to total trials for each pairing. Stimulus train duration was one second for each parameter pairing. Total trials for each parameter pairing are listed in the center of each square. On the ratio scale, 1.0 is equal to a success rate of 100% for that stimulus parameter combination. Abbreviation: NT, parameters not tested.

Figure 5. Peak velocity for successful HFLD-ICMS parameter pairings for both monkeys. Density plot of stimulus parameter pairings color coded as the maximum velocity for each pairing averaged over the number of trials listed in the center of each square. Only successful trials were included. Stimulus train duration was one second for each parameter pairing.

Figure 6. Peak velocity for successful HFLD-ICMS parameter pairings at one cortical site. Data represented were collected from cortical site 5 of Monkey X (Figure 1B). Density plot of stimulus parameter pairings color coded as the maximum velocity for each pairing averaged over the number of trials listed in the center of each square. Only successful trials were included. Stimulus train duration was one second for each parameter pairing.
Voluntary Movement from Homeplate to Food Well

HFLD-ICMS

Resume Voluntary Movement

Stimulus (V)

Wrist velocity (m/s)

Time (s)

Successful trial; stable end-point
Unsuccessful trial; unstable end-point
Unsuccessful trial; no evoked movement

Average maximum velocity

40%
35%
Stimulus Frequency (Hz)

Stimulus Intensity /g11/g541/g36/g12

30 170 160 150 140 130 120 110 100 90 80 70 60 50 40

Peak Stimulus-evoked Wrist Velocity (m/s)