

**Forelimb movements and muscle responses evoked by microstimulation of cervical spinal
cord in sedated monkeys**

Chet T. Moritz¹; Timothy H. Lucas^{1,2}; Steve I. Perlmuter¹; Eberhard E. Fetz^{1*}

1. Physiology & Biophysics and Washington National Primate Research Center, and
2. Neurological Surgery, University of Washington, Seattle, WA, USA

*Address for correspondence
Eberhard E. Fetz, Ph. D.
Department of Physiology & Biophysics
Box 357290
University of Washington School of Medicine
Seattle, WA, 98195-7290
E-Mail: fetz@u.washington.edu
Phone: (206) 543-4839

Running Head: Cervical spinal stimulation

ABSTRACT

Documenting the forelimb responses evoked by stimulating sites in primate cervical spinal cord is significant for understanding spinal circuitry and for potential neuroprosthetic applications involving hand and arm. We examined the forelimb movements and electromyographic (EMG) muscle responses evoked by intraspinal microstimulation in three *M. Nemestrina* monkeys sedated with ketamine. Trains of 3 stimulus pulses (10-80 μ A) at 300 Hz were delivered at sites in regularly spaced tracks from C6 to T1. Hand and/or arm movements were evoked at 76% of the 745 sites stimulated. Specifically, movements were evoked in digits (76% of effective sites), wrist (15% of sites), elbow (26%) and shoulder (17%). To document the muscle activity evoked by a stimulus current just capable of eliciting consistent joint rotation, stimulus-triggered averages of rectified EMG were calculated at each site where a movement was observed. Typically, many muscles were coactivated at threshold currents needed to evoke movements. Out of the 13-15 muscles recorded per animal, only one muscle was active at 14% of the effective sites, and two to six muscles were coactivated at 47% of sites. Thus, intraspinal stimulation at threshold currents adequate for evoking movement typically coactivated multiple muscles, including antagonists. Histologic reconstruction of stimulation sites indicated that responses were elicited from the dorsal and ventral horn and from fiber tracts in the white matter, with little somatotopic organization for movement or muscle activation. The absence of a clear somatotopic map of output sites is probably due to stimulation of complex mixtures of fibers and cells.

Keywords: intraspinal microstimulation, primate, forelimb responses, neuroprosthetics, spinal circuitry

INTRODUCTION

Somatotopic maps of the output effects evoked by stimulation aid our understanding of the organization of motor areas of the nervous system and also identify potential targets for neuroprosthetic stimulation. In contrast to the many studies mapping the output effects evoked by stimulating motor cortex, there is no comparable investigation of output sites in primate cervical spinal cord. Such data would be important for neuroprosthetic applications involving intra-spinal stimulation to evoke hand and arm movements and could also elucidate the spinal circuits mediating control of forelimb muscles.

The effects of intra-spinal stimulation have been investigated in lumbar cord of cat, rat and frog; the evoked responses often include complex hindlimb movements and muscle synergies. For example, microstimulation at specific sites in the frog lumbar cord activates multiple leg muscles and results in sets of stereotyped force fields that have been suggested to represent movement primitives (Giszter et al. 2000; Giszter et al. 1993). Simultaneous stimulation of two such sites often results in a near-linear summation of these force-fields (Mussa-Ivaldi et al. 1994). Such force fields have also been documented for the lumbar cord of spinalized rats (Tresch and Bizzi 1999), although linear summation of stimulation effects was less often observed in the cat (Aoyagi et al. 2004; Grill and Lemay 2002).

Stimulation in the cat lumbar spinal cord elicits modular and functional movements and muscle activity (Lemay and Grill 2004; Mushahwar and Horch 2000). The force fields elicited by stimulating in the lumbar cord of anesthetized or decerebrate cats fall into four distinct groups (Lemay and Grill 2004). Relatively few electrodes can be used to achieve complex movements such as stepping. For example, stimulating at a particular site in the cat lumbar spinal cord activates multiple muscles in a synergy needed for weight support, while stimulation at a second site activates the muscles needed to swing the limb forward (Mushahwar et al. 2002). Lumbar

spinal stimulation evokes functional movements in awake, anesthetized, decerebrate and spinal cats, although the output effects from a given site can differ among these states (Lemay and Grill 2004; Mushahwar et al. 2004; Mushahwar et al. 2002).

While the lumbar spinal cord has been stimulated in several species, the responses evoked by stimulating cervical spinal cord in the primate remain to be documented. Of particular interest are the specificity of the effects in hand and arm, and the possibility of somatotopic organization. Lumbar stimulation in the cat often activates leg muscles whose motor pools lie near the electrodes (Mushahwar et al. 2000; Mushahwar et al. 2002; Mushahwar and Horch 1997). The arrangement of motor pools in the primate cervical cord (Jenny and Inukai 1983) suggests that stimulation effects might be similarly localized. Besides activating motoneurons directly, spinal stimulation can also activate interneurons and axons, including descending, intraspinal and afferent fibers (Mushahwar et al. 2003; Tresch and Bizzi 1999), suggesting that spinal stimulation could evoke diverse responses unrelated to the proximity of the stimulation sites to motoneuron pools.

Documenting the output effects of cervical spinal stimulation would be relevant for neuroprosthetic applications aimed at activating the limbs of individuals with motor impairments. Observations in the lumbar cord suggest that functional muscle synergies and coordinated movements might be evocable by stimulating through a single well-placed electrode in cervical spinal cord, as opposed to patterned stimulation through multiple electrodes placed in each of the relevant muscles. Accordingly, we sought to determine the types of movements and muscle response patterns that could be evoked by intraspinal microstimulation in the primate cervical spinal cord.

METHODS

Spinal stimulation was delivered to three sedated *Macaca nemestrina* monkeys (2 male, 1 female; weight 8.8 ± 5.1 kg; 10.7 ± 6.9 y/o; mean \pm SD). The experiments were approved by the IACUC at the University of Washington, and all procedures conformed to the National Institutes of Health “Guide for the Care and Use of Laboratory Animals”.

Spinal microstimulation (10-80 μ A) was delivered via tungsten electrodes positioned at regularly spaced sites in dorso-ventral tracks. Evoked movements were observed and electromyographic activity (EMGs) were recorded from 13-15 muscles in each animal and used to compute stimulus-triggered averages (StTA) of muscle activity.

Surgical implants

Implant surgeries were performed using sterile techniques while the animals were anesthetized using 1-1.5% sevoflurane in 50:50 O₂:N₂O. Dexamethasone was administered preoperatively (2 mg/kg IM or PO), and antibiotics (cephalexin, 25 mg/kg PO) and analgesics (ketoprofen, 5 mg/kg PO; buprenorphine, 0.015 mg/kg IM) were given postoperatively.

Following a hemilaminectomy of the lower cervical vertebrae, a chamber to hold the microdrive was cemented in place (Perlmutter et al. 1998). The right laminae and dorsal spinous processes of the lower cervical vertebrae were removed (Animal F: C3-C7; Animal G: C5-C7; Animal H: C4-C7). Bone screws were placed in the lateral masses to provide an anchor for the acrylic which cemented the stainless steel chamber in place over the laminectomy. Skin and underlying soft tissue were then sutured around the chamber.

EMG electrodes were surgically implanted in 16 arm and hand muscles, identified by anatomical features and by movements evoked by trains of low-intensity stimulation. Bipolar, multi-stranded stainless steel wires were sutured into each muscle, and wires were routed subcutaneously to a connector on the animal's back. A jacket worn by the monkeys prevented access to the back connector between recording sessions.

Of the 16 muscles implanted with pairs of wires, high quality recordings were obtained throughout the experiment from 15 muscles in animals F & G, and 13 muscles in animal H. A standard set of nine muscles was recorded in all three animals: biceps (BI) and triceps (TRI) crossing the elbow, wrist and finger flexors - flexor carpi ulnaris (FCU), flexor digitorum superficialis (FDS) and palmaris longus (PL), wrist and finger extensors - extensor carpi ulnaris (ECU), extensor digitorum-4,5 (ED-4,5), and abductor pollicis longus (APL), and an intrinsic hand muscle - first dorsal interosseus (FDI). Additional muscles recorded in 2 of the 3 animals included deltoid (DEL) and brachioradialis (BR) in animals F & H, extensor digitorum communis (EDC), extensor digitorum-2,3 (ED-2,3), flexor carpi radialis (FCR), and abductor pollicis brevis (APB) in animals F & G, and extensor carpi radialis (ECR) in animals G & H. Finally, pectoralis majoris was recorded in animal G, and abductor digiti minimi (ADM) in animal H.

Stimulation procedure

Prior to data collection animals were sedated with intramuscular injections of ketamine or telazol (10 mg/kg) and atropine to reduce salivation. Additional doses of ketamine were given as needed to eliminate spontaneous movements during the recording sessions. Animals were positioned prone on a padded table. The right shoulder was abducted 45°, the elbow flexed to 90° and the wrist supported by a raised pad such that the hands and fingers were hanging freely. The animals' body temperature was maintained with a heating blanket.

Stimuli were delivered using single tungsten microelectrodes (impedance $\approx 1\text{ M}\Omega$ at 1 kHz; Frederick Haer, Bowdionham, ME) positioned using a X-Y adaptor mounted on the spinal chamber. Electrode depth was controlled with a hydraulic microdrive (Frederick Haer). Stimuli consisted of 3 biphasic pulses with 0.2 ms square-wave durations at 300 Hz. Stimulus trains were separated by 1 s.

Penetrations were made in a 1 mm grid on the dorsal surface of the spinal cord (see Figure 1 – “Frontal Plane”). Electrodes were advanced ventrally until unit activity was recorded. Subsequently, stimulation was delivered in 10 μ A increments from 10-80 μ A until a consistent movement was evoked with every stimulus train. The lowest stimulation current that evoked a visible and consistent joint displacement was defined as movement threshold. Stimulation was subsequently delivered at 1.2 X movement threshold. EMG data were recorded for at least ten stimulus trains (54 ± 36 ; mean \pm SD) at threshold and 1.2 X threshold, in order to obtain data for stimulus-triggered averages (StTAs). Differential EMG activity was band-pass filtered 30 Hz – 2.5 kHz, amplified 200-1000 X, and digitized at 5 kHz.

The electrode was then advanced 200 μ m and the process repeated. A track was considered complete and the electrode was retracted when units could no longer be recorded, or when movements and stimulus thresholds became stereotyped, indicating that the electrode had reached the ventral funiculus.

Stimulus-triggered average of EMG

For each site where a movement was evoked, StTA of rectified EMG of all recorded arm muscle EMGs were constructed using custom-written software in Matlab 7 (The Mathworks, Natick, MA). StTAs were aligned with the time of the first stimulus, and included data from -100 to +500 ms around this time. A muscle was considered active when the average rectified EMG reached a peak ≥ 5 SD of the baseline (values in the interval -100 to 0 ms), and had a total duration of at least 3 ms.

To help estimate the onset latency of evoked responses amidst the stimulus artifacts, the artifact associated with the first stimulus pulse was used as a template and subtracted from the second and third artifacts. This method partially or completely eliminated the later artifacts. In addition, the response onset time was defined as the beginning of the first period that the StTA remained above 2 SD of the baseline for ≥ 1 ms continuously; this reduced the possibility of

detecting brief stimulus artifacts (which were ≤ 0.4 ms). Similarly, offset was defined as the end of the last period that the StTA remained above 2 SD of the baseline for ≥ 1 ms.

Statistics

Regression analyses were performed to search for possible trends in movement threshold and muscle activation patterns as a function of the location of the stimulus sites within the spinal cord. For each animal, variables were independently regressed against the rostro-caudal, medio-lateral, and dorso-ventral coordinates of the stimulation sites. Pearson's chi-squared statistic was also used to compare the distribution of co-active muscles to a binomial distribution based on the average probability that any one muscle was active at a given stimulation site (0.2742).

Correlations were performed to determine whether there was a higher likelihood of evoking similar movements at adjacent stimulation sites. Each movement was assigned a number from 1-15, and the frequency of observing a given pair of movements at adjacent stimulation sites in the rostro-caudal, medio-lateral, and dorsal-ventral directions was determined. If the same movements were observed at adjacent sites, then the data would fall perfectly on a diagonal, and generate high linear regression coefficients.

To document the degree to which certain muscle groups were preferentially co-activated we performed a cluster analysis. For the nine muscles recorded in all animals, a binary matrix was constructed, with each stimulation site represented by a row, and each muscle a column. All 566 stimulation sites where a movement was evoked were used in the cluster analysis. Muscles activated at each site by stimulation at movement threshold were assigned a value of 1, and non-active muscles a value of 0. Each stimulation site was then plotted as a point in a 9-dimensional muscle space, with co-ordinate of 1 for the active muscles and 0 for inactive muscles. The Euclidean distance between each stimulation site was computed, and a hierarchical cluster tree was formed using the average linkage method (Poliakov and Schieber 1999). In combining groups using this method, the Euclidean distances of all members of an existing group were

averaged to produce a single value for that group. These group values were then used to determine the Euclidean distance between newly joined groups. The results of the cluster analysis are presented in a dendrogram and similarity matrix. Very similar results were obtained using the squared Euclidean distance or the single linkage methods (Johnson and Wichern 1992).

Histological procedures

Toward the conclusion of each experiment small electrolytic lesions were made at several sites within the spinal cord by passing DC current of 30 μ A for 30 s through the stimulating electrode. Animals were euthanized with pentobarbital sodium (50 mg/kg iv) and perfused with 10% Formalin. The excised spinal cord was fixed in egg yolk to preserve the rootlets before being cut into 50 μ m sections and stained with cresyl violet.

The histological slices were photographed and the positions of the lesions, grey matter, and pia were digitized. Digitized histology data were corrected for 10% shrinkage in all dimensions. Several slices in monkey H were obviously compressed in the dorso-ventral axes, and these slices were re-scaled to the dorso-ventral dimensions of the average of all non-compressed slices from the same animal.

The figures plotting motoneuron pools in Jenny and Inukai (1983) were also digitized to approximate their location on the histology slices. Motoneurons were estimated to lie on the sections in the same relative positions by using a percentage of the horizontal and vertical distance from the central canal to grey matter border. Motoneuron pools are reconstructed in Figures 1 and 9.

RESULTS

Evoked movements

Intraspinal microstimulation at currents up to 80 μ A evoked hand or arm movements at 76% of the 745 tested stimulation sites (totaled for all three animals). Figure 1 shows the locations from which movements were evoked within the spinal cord of two monkeys. Movements were evoked throughout the spinal cord, including the dorsal and ventral grey matter and from surrounding fiber tracts.

Stimulation most commonly evoked movements of the digits, but also produced wrist and arm movements. Specifically, movements were evoked in the fingers or thumb at 76% of effective sites, in the wrist at 15% of sites, and at the elbow and shoulder at 26% and 17% of sites, respectively. Figure 2 shows the proportion of movements for all three animals combined. Thumb flexion was most commonly observed, followed closely by finger flexion. The only shoulder movement observed was adduction. Remaining shoulder movements were not observed, nor expected, given that most stimulation sites were in spinal segments C7 and caudal.

Figure 3(A,B) shows the locations where specific movements were evoked in the 3 monkeys. These plots show little systematic topographic organization of the evoked movements relative to anatomic features. For example, many different movements were elicited at different depths within a single electrode penetration. Further, stimulation at comparable anatomic sites in different animals showed little similarity in the movements evoked. Surprisingly, very little organization was evident in the rostro-caudal direction. One exception was observed in animal H, where movements about the elbow were restricted to stimulation in the C7 and C8 spinal segments.

Figure 3C plots the occurrence of movements at adjacent sites in depth, medio-lateral and rostro-caudal penetrations. The same or similar movements were often evoked at adjacent depth increments of 200 μ m along a single electrode penetration ($r^2 = 0.485$, $p < 0.001$). Less

movement similarity was observed at equivalent depths in penetrations separated by 1 mm medio-laterally ($r^2 = 0.165$, $p < 0.001$), and none was observed at equivalent depths rostro-caudally ($r^2 = 0.005$, $p = 0.415$).

Stimulus current thresholds needed to evoke movement were distributed nearly uniformly over the range from 10-80 μA tested (Figure 4). The threshold stimulus intensity to evoke a movement showed no relation with the location within the spinal cord, in either the rostro-caudal, medio-lateral, or dorso-ventral directions ($r^2 \leq 0.053$).

At about one-third of effective stimulation sites (36%), movements at multiple joints were simultaneously evoked at a threshold current just large enough to produce any consistent movement. Figure 5A shows that combinations of the fingers and thumb were the most common when multiple movements were simultaneously evoked at threshold. With only three exceptions, this combination involved flexion of both the fingers and thumb in a gripping movement. For all sites associated with simultaneous movements, pairs of flexor movements occurred with a similar proportion (45%) as combinations of flexor and extensor movements (53%). Pairs of extensor movements were quite rare (2% of sites).

At 20% of sites additional movements appeared when stimulation was increased to 1.2 X threshold for any consistent movement. These newly evoked movements are illustrated in Figure 5B, where the right axes displays the original threshold movement, and the left axes the newly evoked movement. Combinations of the fingers and thumb were common, as were combinations of the thumb and elbow. Pairs of flexor movements were evoked at 29% of sites, whereas pairs of extensor movements only appeared at 5% of sites. Flexor movements at threshold joined by extensor movements at 1.2 X threshold occurred at a rate similar to extensor movements joined by flexor movements (35% and 31%, respectively).

Muscle responses

To document the muscle activity evoked by stimulation currents just capable of eliciting a movement, stimulus-triggered averages (StTAs) of rectified EMG were compiled for each site where a movement was observed. Figure 6 shows the StTAs for a site in rostral C7 where stimuli evoked a gripping movement of the fingers and thumb. Three of the recorded muscles were activated by this 20 μ A stimulation current, including not only agonists of the gripping movement (FDS and APB) but also an antagonist (ECU). 11 muscles were not activated in this case, but the trace for FDI is shown to illustrate stimulus artifacts and baseline activity.

Responses were commonly evoked in multiple muscles at threshold intensities required to evoke movements. Of the 13-15 muscles recorded in each animal, a single muscle was activated in isolation at only 14% of effective sites, whereas two to six muscles were activated at 47% of sites, and more than six muscles were activated at 39% of sites (Figure 7). This bi-modal distribution was significantly different from the binomial distribution expected if muscle were each independently activated at chance levels ($p < 0.001$). Post-stimulus effects occurred in finger flexor muscles at 66% of sites from which movements were evoked, and finger extensors were activated at 50% of all sites. Finger flexor and extensor muscles were co-activated at 44% of all sites. The number of muscles simultaneously activated was not related to the location of stimulus site within the spinal cord ($r^2 \leq 0.045$).

Figure 8 documents the timing of stimulus-evoked muscle activity, which was similar among animals and therefore pooled. The timing of muscle activity is shown separately for sites where threshold stimulation co-activated ≤ 4 muscles and > 4 muscles, based on the bi-modal distribution in Figure 7. EMG activity at sites with > 4 co-activated muscles began earlier (9.0 vs. 13.6 ms; $p < 0.001$) and had a longer duration (22.7 vs. 18.4 ms; $p < 0.001$) compared with sites where fewer muscles were co-activated. There was no correlation between stimulation location within the spinal cord and response onset time ($r^2 \leq 0.018$) or duration ($r^2 \leq 0.002$). The

timing of muscle activity at 1.2 X threshold stimulation was nearly identical to that evoked by threshold stimulation (mean onset: 9.60 ± 8.84 ms, mean duration 23.28 ± 13.30 ms).

Figure 9 shows the location of the spinal sites that activated each of six muscles in the three monkeys. Muscles were activated from a wide range of sites, including motoneuron pools in the ventral horn, the dorsal horn, and fiber tracts. As with the movements, little topographic organization was observed for the sites that activated muscles. In addition, the sites for particular muscles varied among animals. There was no relation between the location of stimulation sites and the number of flexor or extensor muscles activated ($r^2 \leq 0.105$), nor for the number of arm or hand muscles activated ($r^2 \leq 0.020$).

To test for possible muscle synergies, a cluster analysis was performed on the activity of the nine muscles recorded in all animals. Figure 10A shows the resulting dendrogram, representing each muscle by a vertical line rising from the abscissa. Horizontal lines join vertical lines at the ordinate value representing the distance between muscles in the 9-dimensional muscle space. As additional muscles are added to an existing group, a horizontal line joins the newly added muscle to the group at a value representing the distance from the new muscle to the existing group average. Therefore, muscles more frequently co-activated by spinal stimulation will be joined by a horizontal line with a smaller cluster distance and located lower along the ordinate.

Synergist muscles or muscles crossing the same joint often fall into distinct groups. Specifically, extrinsic finger flexor muscles (FCU, FDS, PL) are distinct from finger extensor muscles (ECU, ED_{4,5}). Further, an intrinsic hand muscle (FDI) is separate from all other muscles. The distance between groups, however, is often smaller than the distance separating synergist muscles within a cluster, indicating that antagonists were often co-activated by spinal stimulation. In the two animals in which it was recorded, shoulder muscle (DEL) clustered as expected with the BI-TRI group, at a cluster distance of 0.25, before merging with APL.

Figure 10B shows the corresponding similarity matrix, which illustrates the Euclidean distance between any two muscles, regardless of their membership in a cluster. In addition to showing related muscles as darker squares on their intersecting coordinates, the similarity matrix reveals the least related muscles as light or white squares. For example, biceps and ECU were seldom co-activated, as were triceps and FDI or FDI and ECU.

DISCUSSION

The major findings of this study are that (1) forearm movements are evoked from the majority of stimulation sites throughout the primate cervical spinal cord at relatively low stimulus currents; (2) multiple forelimb muscles, including antagonists, are commonly co-activated by threshold intraspinal stimulation; (3) most sites elicit flexor movements, with a dominant representation of the fingers and thumb; and (4) the stimulation sites show little somatotopic organization for the movements evoked or the muscles activated. These results can be compared to the hindlimb responses evoked by intraspinal stimulation in lumbar cord of other species and to the forelimb effects evoked from intracortical stimulation in primates.

Modes of activation

In contrast to many other studies using longer trains of stimuli, we chose to document the output effects evoked from brief trains (3 pulses at 300 Hz), in order to minimize the amount of additional activity generated by temporal summation of post-synaptic effects. The brief muscle twitches represent relatively direct outputs evoked from particular sites. Most studies of spinal stimulation used longer trains of stimuli (200-1000 ms) at 25-70 Hz in order to evoke prolonged muscle force or movement (Giszter et al. 1993; Lemay and Grill 2004; Mushahwar et al. 2002; Saigal et al. 2004; Tresch and Bizzi 1999). While longer stimulus trains in the primate cervical cord (e.g., 50 cycles at 300 Hz) produce prolonged movements such as those shown in Figure 6A, we did not systematically investigate the effects of long trains in this study. The choice of three pulses rather than one was designed to reduce the current necessary to evoke a response in our sedated preparation, thereby reducing current spread and the number of neural processes that would be stimulated by a single high-current stimulus. Single stimuli have been used previously to map the output effects and to determine muscle activation thresholds of ISMS in cats (Mushahwar et al. 2002; Mushahwar and Horch 1998). We felt that three stimuli struck the

appropriate balance to document focal effects from specific sites: this limited the temporal summation produced by longer trains and limited spatial summation necessary with single shocks. Clearly, the relation between the effects evoked by different train parameters is an important issue, but remains to be adequately documented in another study.

The observation that multiple muscles are simultaneously activated at most (86%) of the stimulation sites that evoked movements agrees with results from lumbar intraspinal microstimulation (ISMS) in the frog (Giszter et al. 1993) and cat (Lemay and Grill 2004; Mushahwar et al. 2004). Lumbar ISMS in the cat often evoked simultaneous activation of multiple muscles, and occasionally activated all four muscles from which EMG was recorded (Lemay and Grill 2004). In addition, muscles across multiple joints could be co-activated by a single stimulating electrode, allowing stimulation of only two electrodes to alternately support the weight of the hindquarters and swing the hindlimb forward (Mushahwar et al. 2002).

Microstimulation in primate motor cortex or red nucleus also co-facilitates muscles at most stimulation sites. Single-pulse intracortical microstimulation (S-ICMS) in hand and arm area of motor cortex during reaching movements produces co-facilitation of two or more muscles at 73% of stimulation sites (Park et al. 2004). The same proportion of rubral sites (73%) produce co-facilitation of multiple muscles (Belhaj-Saif et al. 1998).

The latencies of muscle activation (Figure 8) suggest that in most cases our spinal stimulation did not directly activate fast motoneurons or even last-order interneurons. The earliest possible activation time for effects mediated by direct stimulation of motoneurons is estimated as 2.6 ms, with an additional 1.1 ms required for activation through a single interneuron (Gustafsson and Jankowska 1976; Jankowska and Roberts 1972; Perlmutter et al. 1998). Only 3.2% of the evoked muscle activity in this study begins before 3.5 ms, with the vast majority (72%) of muscle activity beginning between 3.5 and 11 ms. It is possible that very slow

motoneurons or motoneuron axons were directly activated, but there was no trend toward shorter latencies in the ventral grey matter, as might be expected if motoneurons were directly activated. The use of three stimulus pulses to evoke effects via temporal summation is an additional factor. Nonetheless, response latencies from the present study are similar to latencies of post-spike effects on EMG activity evoked from single-pulse stimulation in the cervical spinal cord of behaving monkeys (Perlmutter et al. 1998; Figure 4B).

Spinal stimulation likely excites motoneurons indirectly by activating a sufficient number of their inputs, for example propriospinal, corticospinal or afferent fibers. Fibers have lower activation thresholds compared to cell bodies, and are thus recruited at lower stimulus currents (Gustafsson and Jankowska 1976; Ranck 1975). For example, spinal stimulation activates afferent axons at lower stimulus intensities than motoneuron cell bodies, and this activity is propagated from the stimulus site to all terminals of the afferent, thereby affecting motoneurons of multiple muscles (Mushahwar et al. 2003). Propriospinal and local interneurons are also likely to play a key role in mediating effects of spinal stimulation. The effects evoked by lumbar stimulation in the rat and frog are relatively unchanged by chronic deafferentation and spinalization, with sufficient time to allow afferent and corticospinal fibers to degenerate (Giszter et al. 1993; Tresch and Bizzi 1999). Regardless of which fibers are activated, the resultant wide distribution of stimulus effects via collaterals of low-threshold fibers would help explain the lack of somatotopic organization we observed.

Stimulation in the cat lumbar cord tends to activate muscles of nearby motoneuron pools (Mushahwar et al. 2000; Mushahwar et al. 2002; Mushahwar and Horch 1997). Although the motoneuron pools in the primate cervical cord appear similarly organized and minimally overlapping (Jenny and Inukai 1983), we found that stimulation in the primate cervical cord activates muscles with little relation to the proximity of motor pools. This may be explained by

the differences between the fiber systems in the primate cervical motor system and the cat lumbar cord. For example, the monkey cervical cord contains a greater number of cortico-spinal terminals compared to the cat lumbar cord (Porter and Lemon 1993).

Movements evoked and muscles activated

Indirect mediation via fibers explains why cervical spinal stimulation throughout the grey matter activates muscles and evokes movements. The predominance of flexor movements and flexor muscle activity evoked by spinal stimulation in the present study is consistent with the large proportion of spinal interneurons affecting flexor muscles. Cervical “pre-motor” interneurons exert postspike effects twice as often in flexor muscles compared to extensors (Perlmutter et al. 1998). These spinal interneurons, however, generally have small muscle fields, with the majority of neurons affecting only one muscle.

In contrast the responses to intraspinal stimulation probably also reflect the output effects evoked via corticospinal fibers. The predominance of thumb flexor movements, as well as simultaneous finger and thumb flexion, evoked by spinal stimulation is consistent with activation of corticospinal fibers. A large number of ICMS sites in motor cortex evoke movements of the thumb alone (Asanuma and Rosen 1972), or combinations of thumb and finger flexion in the monkey (Kwan et al. 1978). In general, the relatively frequent appearance of hand movements evoked by spinal stimulation (48% of all movements) is generally similar to the proportion of effects from ICMS. Stimulation of motor cortex facilitates distal muscles at 60% of sites (Park et al. 2004), and stimulation of red nucleus at 58% of sites (Belhaj-Saif et al. 1998), further suggesting that spinal stimulation may activate descending axons. Corticomotoneuronal synapses are widely distributed (Lawrence et al. 1985) and the magnitude of EPSPs evoked in motoneurons from cortical fibers are similar to those evoked from Ia afferents (Clough et al. 1968).

We found less relationship between stimulus location and the type of movement than reported in other animals. Lemay and Grill (2004) report that stimulation of the cat lumbar spinal cord elicits mostly flexor activation at shallow depths (Lamina I-VII), and extensor activation in the motor pools of Lamina IX. In another study, however, both flexor and extensor movements were elicited from sites in the intermediate grey matter or ventral horn (Mushahwar et al. 2004; Mushahwar et al. 2002; Mushahwar and Horch 1997). Notably, stimulation of the rat lumbar cord after spinal transaction elicits movements primarily from the dorsal and intermediate lamina, and relatively few movements from the motor pools in the ventral horn (Tresch and Bizzi 1999).

Undoubtedly the state of the preparation, including the anesthetic used, affects the output of spinal stimulation. Flexor responses are almost exclusively observed when spinal stimulation is delivered to a spinalized rat (Tresch and Bizzi 1999), or spinalized or decerebrate cat (Mushahwar et al. 2004). The slight predominance of flexor movements in the present study (65% of all movements) is unlikely to be caused by the Ketamine anesthesia used. Ketamine acts as a non-competitive NMDA antagonist that does not affect the monosynaptic reflex (Brockmeyer and Kendig 1995; Kendig 2002), and selectively depresses only polysynaptic reflexes in conjunction with pentobarbitone (Lodge and Anis 1984).

Distribution of sites generating output effects

The lack of obvious relation between output effects and location of spinal sites might be attributable to several sources of variance. Accurate estimation of electrode depth within the spinal cord is difficult due to dimpling of the dorsal surface during electrode penetration and variability in the position of the first active neuron, used to estimate entry into the dorsal horn grey matter (Perlmutter et al. 1998). Nonetheless, even within a single electrode penetration

general organization was not observed, suggesting that the outcome of the study was not explainable by the variance in estimating stimulus depth.

Electrode penetrations were made using a 1-mm grid on the dorsal surface of the spinal cord, but not all intersections of this grid could be sampled in each animal. It might be thought that more complete sampling of this 1-mm grid, or finer resolution could reveal an organization of evoked movements and/or muscle activity. This seems unlikely, considering the widely divergent and overlapping regions of activity in the present data set. For example, the wide distribution of thumb flexion sites throughout the spinal cord seems unlikely to be altered by a finer stimulus grid.

Another variable is stimulus current, which ranged from 10-80 μA in order to evoke hand and arm movements. The radius of current spread away from the stimulating electrode is estimated at 57-200 μm for each 10 μA stimulus, and 500-1000 μm for each 80 μA stimulus (Cheney and Fetz 1985; Mushahwar and Horch 1997; Ranck 1975). While stimulation likely activates a large volume of tissue at higher stimulus currents, the 1-mm grid in combination with the maximal radius of current spread (0.5-1.0 mm) was selected in an attempt to explore activation of the entire cervical spinal cord.

The lack of obvious somatotopic organization of output effects evoked from spinal sites contrasts with the greater organization of cortical sites. In primate motor cortex microstimulation of sites in a column typically evokes the same joint movement, often related to the input to the cells in the column (Park et al. 2004; Rosen and Asanuma 1972). In spinal cord successive sites in a dorsoventral track can evoke a variety of responses. While motor cortex displays a somatotopically organized map of the body, stimulation effects from spinal cord are loosely related to the segmental representation. These differences in the degree of organization can be explained by the fact that stimulation effects are largely evoked via fibers of passage. In

cerebral cortex afferent and efferent fibers are aligned with the column, so their stimulation exerts the most powerful output effects on the local layer V cells. In spinal cord the relatively heterogeneous intermixture of fibers from periphery, descending tracts and intraspinal connections results in comparably diverse threshold effects.

Implications for neuroprosthetics

The fact that a wide range of synergistic hand and arm movements can be evoked from stimulation throughout the cervical spinal cord suggest that spinal stimulation may be a viable target for neuroprosthetics aiming to restore movements after spinal cord injury or stroke. Single stimulation sites activate multiple muscles including both synergists and antagonists. Simultaneous activation of antagonist muscles about the wrist, for example, may be useful for stabilizing the wrist while grasping objects (Illert and Kummel 1999). Compared to direct muscle stimulation, activation of functional muscle synergies by single stimulating electrodes could significantly reduce the number of implanted electrodes as well as the number of independent control signals needed from a neuroprosthetic system. Conversely, the limited ability to activate individual muscles may compromise the specificity of movements that can be produced by spinal stimulation. Spinal microwires are subject to less mechanical fatigue than wires implanted in muscles, and also require lower stimulus currents, which would extend the battery life of autonomous systems. Finally, there is evidence that spinal stimulation results in more natural, graded recruitment of motor units compared to muscle or nerve stimulation (Mushahwar and Horch 1998).

On the other hand, intraspinal stimulation presents certain challenges for prosthetic applications. Further study is required to determine the effects of longer stimulus trains and spinal cord lesions on ISMS outputs. ISMS effects depend significantly on the stimulus duration and intensity (Prochazka et al. 2002). In addition, ISMS outputs may change substantially after

spinal cord lesions, especially if activation of descending fibers accounts for a significant portion of the present findings. Similarly, set-dependent changes in spinal circuitry or reflex excitability may influence evoked responses, for example during different phases of volitional movements.

Efficacious electrode placement in the cervical spinal cord of primates may be more challenging than in the lumbar cord of the cat. The lack of somatotopy observed in the present study, combined with the differences among animals, suggests that the location of therapeutically useful electrodes will need to be determined by stimulation effects during placement.

Fortunately, stimulation sites evoking coordinated movements are fairly common in the cervical spinal cord, with grasping movements of the digits evoked from many of these sites. Finally, the variation of effects evoked from comparable sites in different monkeys suggests that output effects in the human probably cannot be reliably predicted from anatomical landmarks, but need to be empirically tested.

ACKNOWLEDGEMENTS

The authors thank C Kirby and J Fadok for expert animal care and assistance, L Shupe and S Votaw for programming assistance, and V Mushahwar for helpful discussions.

GRANTS

This work was supported by NIH grants NS12542 and RR00166 to EE Fetz, NS40867 to SI Perlmutter, and a Kirschstein NRSA to CT Moritz.

REFERENCES

- Aoyagi Y, Mushahwar VK, Stein RB, and Prochazka A. Movements elicited by electrical stimulation of muscles, nerves, intermediate spinal cord, and spinal roots in anesthetized and decerebrate cats. *IEEE Trans Neural Syst Rehabil Eng* 12: 1-11, 2004.
- Asanuma H and Rosen I. Topographical organization of cortical efferent zones projecting to distal forelimb muscles in the monkey. *Exp Brain Res* 14: 243-256, 1972.
- Belhaj-Saif A, Karrer JH, and Cheney PD. Distribution and characteristics of poststimulus effects in proximal and distal forelimb muscles from red nucleus in the monkey. *J Neurophysiol* 79: 1777-1789, 1998.
- Brockmeyer DM and Kendig JJ. Selective effects of ketamine on amino acid-mediated pathways in neonatal rat spinal cord. *Br J Anaesth* 74: 79-84, 1995.
- Cheney PD and Fetz EE. Comparable patterns of muscle facilitation evoked by individual corticomotoneuronal (CM) cells and by single intracortical microstimuli in primates: evidence for functional groups of CM cells. *J Neurophysiol* 53: 786-804, 1985.
- Clough JFM, Kernell D, and Phillips CG. The distribution of monosynaptic excitation from the pyramidal tract and from primary spindle afferents to motoneurons of the baboon's hand and forearm. *J Physiol* 198: 145-166, 1968.
- Giszter SF, Loeb E, Mussa-Ivaldi FA, and Bizzi E. Repeatable spatial maps of a few force and joint torque patterns elicited by microstimulation applied throughout the lumbar spinal cord of the spinal frog. *Human Movement Science* 19: 597-626, 2000.
- Giszter SF, Mussa-Ivaldi FA, and Bizzi E. Convergent force fields organized in the frog's spinal cord. *J Neurosci* 13: 467-491, 1993.
- Grill WM and Lemay MA. Hindlimb motor responses evoked by dual-electrode intraspinal microstimulation in the cat. *IFESS*, 2002.

Gustafsson B and Jankowska E. Direct and indirect activation of nerve cells by electrical pulses applied extracellularly. *J Physiol* 258: 33-61, 1976.

Illert M and Kummel H. Reflex pathways from large muscle spindle afferents and recurrent axon collaterals to motoneurons of wrist and digit muscles: a comparison in cats, monkeys and humans. *Exp Brain Res* 128: 13-19, 1999.

Jankowska E and Roberts WJ. Synaptic actions of single interneurons mediating reciprocal Ia inhibition of motoneurons. *J Physiol* 222: 623-642, 1972.

Jenny AB and Inukai J. Principles of motor organization of the monkey cervical spinal cord. *J Neurosci* 3: 567-575, 1983.

Johnson RA and Wichern DW. *Applied Multivariate Statistical Analysis*. Englewood Cliffs, NJ: Prentice Hall, 1992.

Kendig JJ. In vitro networks: subcortical mechanisms of anaesthetic action. *Br J Anaesth* 89: 91-101, 2002.

Kwan HC, MacKay WA, Murphy JT, and Wong YC. Spatial organization of precentral cortex in awake primates. II. Motor outputs. *J Neurophysiol* 41: 1120-1131, 1978.

Lawrence DG, Porter R, and Redman SJ. Corticomotoneuronal synapses in the monkey: light microscopic localization upon motoneurons of intrinsic muscles of the hand. *J Comp Neurol* 232: 499-510, 1985.

Lemay MA and Grill WM. Modularity of motor output evoked by intraspinal microstimulation in cats. *J Neurophysiol* 91: 502-514, 2004.

Lodge D and Anis NA. Effects of ketamine and three other anaesthetics on spinal reflexes and inhibitions in the cat. *Br J Anaesth* 56: 1143-1151, 1984.

Mushahwar VK, Aoyagi Y, Stein RB, and Prochazka A. Movements generated by intraspinal microstimulation in the intermediate gray matter of the anesthetized, decerebrate, and spinal cat. *Can J Physiol Pharmacol* 82: 702-714, 2004.

Mushahwar VK, Collins DF, and Prochazka A. Spinal cord microstimulation generates functional limb movements in chronically implanted cats. *Exp Neurol* 163: 422-429, 2000.

Mushahwar VK, Gillard DM, Gauthier MJ, and Prochazka A. Intraspinal micro stimulation generates locomotor-like and feedback-controlled movements. *IEEE Trans Neural Syst Rehabil Eng* 10: 68-81, 2002.

Mushahwar VK and Horch KW. Proposed specifications for a lumbar spinal cord electrode array for control of lower extremities in paraplegia. *IEEE Trans Rehabil Eng* 5: 237-243, 1997.

Mushahwar VK and Horch KW. Selective activation and graded recruitment of functional muscle groups through spinal cord stimulation. *Ann N Y Acad Sci* 860: 531-535, 1998.

Mushahwar VK and Horch KW. Selective activation of muscle groups in the feline hindlimb through electrical microstimulation of the ventral lumbo-sacral spinal cord. *IEEE Trans Rehabil Eng* 8: 11-21, 2000.

Mushahwar VK, Prochazka A, Ellaway PH, Guevremont L, and Gaunt RA. Microstimulation in CNS excites axons before neuronal cell bodies. Program No. 276.6. *2003 Abstract Viewer/Itinerary Planner*, Washington, DC. Society for Neuroscience, 2003.

Mussa-Ivaldi FA, Giszter SF, and Bizzi E. Linear combinations of primitives in vertebrate motor control. *Proc Natl Acad Sci U S A* 91: 7534-7538, 1994.

Park MC, Belhaj-Saif A, and Cheney PD. Properties of primary motor cortex output to forelimb muscles in rhesus macaques. *J Neurophysiol* 92: 2968-2984, 2004.

Perlmutter SI, Maier MA, and Fetz EE. Activity of spinal interneurons and their effects on forearm muscles during voluntary wrist movements in the monkey. *J Neurophysiol* 80: 2475-2494, 1998.

Poliakov AV and Schieber MH. Limited functional grouping of neurons in the motor cortex hand area during individuated finger movements: A cluster analysis. *J Neurophysiol* 82: 3488-3505, 1999.

Porter R and Hore J. Time course of minimal corticomotoneuronal excitatory postsynaptic potentials in lumbar motoneurons of the monkey. *J Neurophysiol* 32: 443-451, 1969.

Porter R and Lemon RN. *Corticospinal function and voluntary movement*: Oxford University Press, 1993.

Prochazka A, Mushahwar VK, and Yakovenko S. Activation and coordination of spinal motoneuron pools after spinal cord injury. In: *Neural Repair and Recovery*, edited by McKerracher L, Douchet G and Rossignol S. Amsterdam: Elsevier, 2002, p. 109-124.

Ranck JB, Jr. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res* 98: 417-440, 1975.

Rosen I and Asanuma H. Peripheral afferent inputs to the forelimb area of the monkey motor cortex: input-output relations. *Exp Brain Res* 14: 257-273, 1972.

Saigal R, Renzi C, and Mushahwar VK. Intraspinal microstimulation generates functional movements after spinal-cord injury. *IEEE Trans Neural Syst Rehabil Eng* 12: 430-440, 2004.

Tresch MC and Bizzi E. Responses to spinal microstimulation in the chronically spinalized rat and their relationship to spinal systems activated by low threshold cutaneous stimulation. *Exp Brain Res* 129: 401-416, 1999.

Figure Legends

Figure 1: Location of spinal stimulation sites where movements of the hand or arm were evoked for three monkeys (H, F & G). For each animal, a 3-dimensionsal histological reconstruction is shown in the central panel, along with corresponding 2-dimensional views. Open circles denote the central canal, grey lines denote grey matter border, and black lines denote pia. Tested stimulation sites in the third animal (G) were sparser, and are shown in Figures 3 and 9. Inset depicts approximate location of motoneurons for six example muscles from Jenny & Inukai (1983).

Figure 2: Number of sites where each type of movement was evoked by spinal stimulation in all three animals combined. Movements occurred both singly and in combination at threshold stimulus currents, and plot includes each specific instance of a given movement.

Figure 3: (A & B) Location of sites whose stimulation evoked specific movements. Open circles denote the central canal, grey lines denote grey matter border, and black lines denote pia. Left column shows 3-dimensional rendering for animals F and H. Right column shows movements collapsed on representative histology slices for all three animals (H, F, and G). Grey tracks denote all sites where stimulation was delivered. Overlaying tracks within each example slice are offset laterally for visibility. For simplicity, only a single movement evoked at threshold is shown at each site. **(C)** Histograms showing the type and number of occurrences of movements at adjacent stimulation sites. The colors on the “site 1” axis denote movements from the legend in part A. The movements denoted on the “site 2” axis begin with shoulder flexion (SF) at left, and progressing to thumb flexion (TF) at right in the same order as in A. Movements were evoked at adjacent stimulation sites in depth (200 μ m) within a single electrode penetration (left) or at equivalent depths in adjacent penetrations in the medio-lateral (center) or rostro-caudal (right) directions (1 mm). For example, the dark blue bar in the back corner of each figure represents thumb flexion occurring at adjacent stimulation sites in each direction. Data clustered along the

diagonal indicate that the same or similar movements were evoked at adjacent stimulation sites, as for the left figure. Scattered data indicate dissimilar movements, as for the right figure.

Figure 4: Distribution of threshold stimulus currents required to elicit a hand or arm movement for all three animals combined. Stimulation was increased in 10 μ A steps from 10-80 μ A. "No response" designates sites with no visible movement for stimulus currents \leq 80 μ A.

Figure 5: The number of sites where combinations of movements were observed. *A:* Two movements occurred simultaneously at threshold stimulus currents at 36% of sites. The majority of these movement combinations were flexions of the finger and thumb together. *B:* At 20% of sites, an additional movement appeared during suprathreshold stimulation (1.2 x Threshold) in addition to the movement already present at threshold stimulation (1.0 x Threshold).

Figure 6: Example of hand grasp movement and muscle responses evoked by stimulating at 20 μ A in lamina VII of rostral C7 (monkey F). *A:* Photos of hand grasping involving both finger and thumb flexion. The stimulation used for the experiment (3 pulses at 300 Hz) resulted in brief twitches of the hand. The photographs illustrate the movement evoked by longer stimulus trains (50 pulses at 300 Hz). Values above each photo give time relative to stimulus onset. *B:* Stimulus-triggered averages of EMGs from four muscles, three of which were activated from this site. Arrows indicate stimulation pulses. The left column shows unrectified EMG, the right column shows averages constructed from rectified EMG and used for analysis (negative values result from artifact subtraction – see methods). In the right column, dashed lines denote 2 SD of background EMG, and red marks denote onset and offset of EMG response.

Figure 7: The number of muscles simultaneously activated by spinal stimulation at individual sites for all animals combined. Of the 13-15 muscles recorded in each animal, spinal stimulation activated multiple muscles much more often than a single muscle alone.

Figure 8: Timing of EMG responses in stimulus-triggered averages for all animals combined. Histograms show onset, offset, and duration of each muscle response evoked by stimulation that

was threshold for hand or arm movements. The timing of muscle activity at sites with ≤ 4 muscles co-activated by threshold stimulation is superimposed on [not summed with] the data from sites with > 4 muscles co-activated.

Figure 9: Locations where stimulation activated six specific muscles in each animal (H, F, and G). Red circles denote sites where each muscle was active, all stimulation sites are shown by grey tracks. Locations of motoneurons for each muscle (black circles) are approximated from Jenny & Inukai (1983). Both rostral ('r') and caudal ('c') sections of each spinal segment are shown.

Figure 10: *A:* Dendrogram from a cluster analysis of the nine muscles common to all animals (see methods). Wrist flexors (FCU, FDS, PL) and extensors (ECU, ED-4,5) fall into distinct clusters, as does FDI, an intrinsic hand muscle. *B:* Similarity matrix showing the distance between each muscle regardless of clustering. Dark regions indicate muscles that were frequently co-activated, and light regions muscles that were seldom co-activated by stimulation.

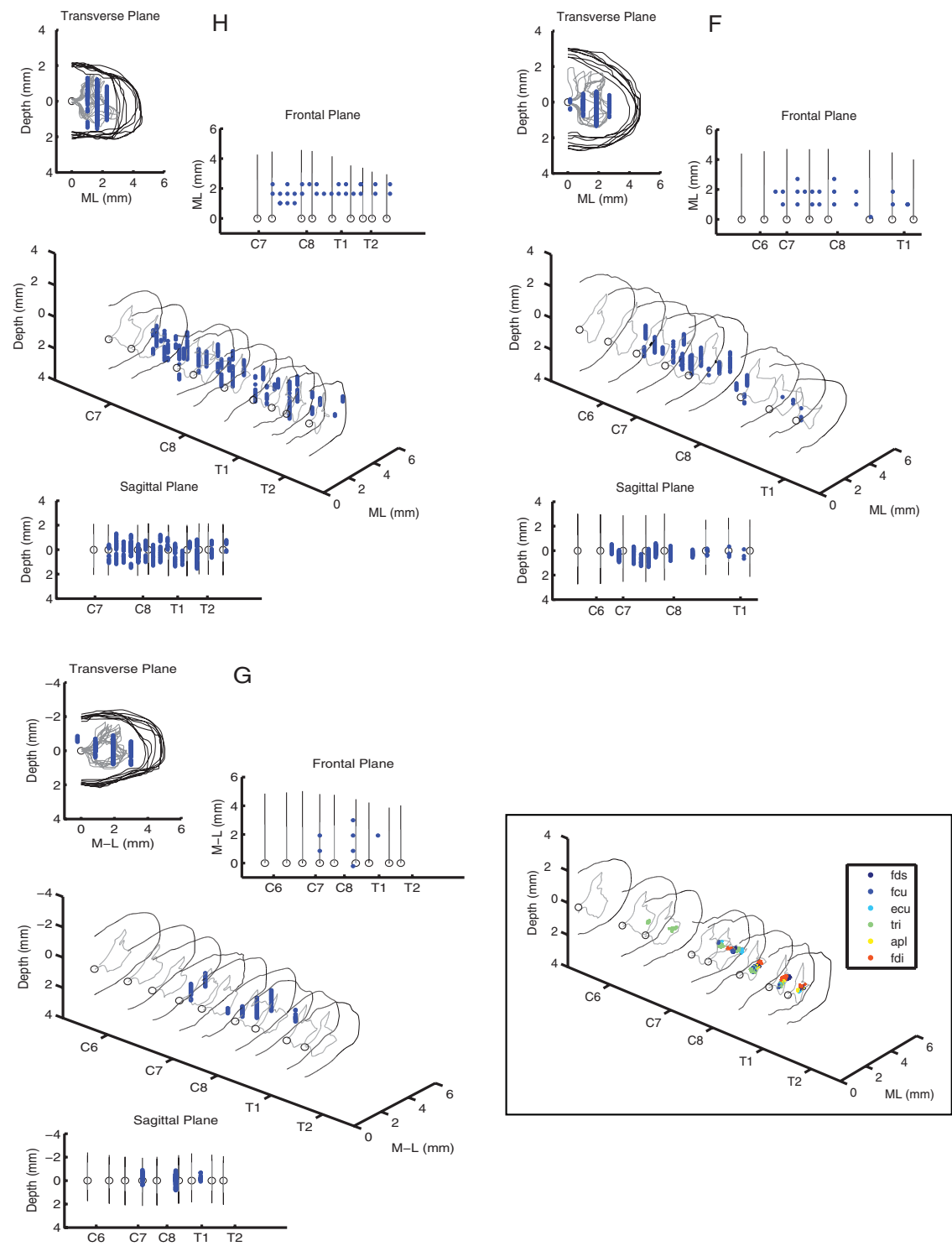


Figure 1

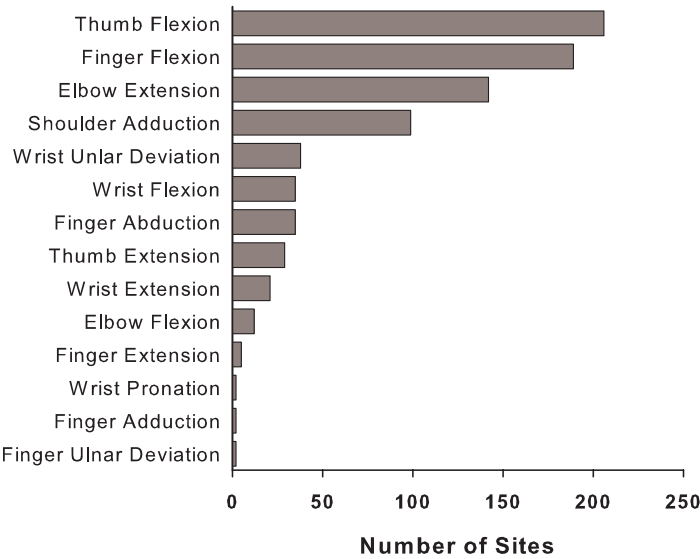


Figure 2

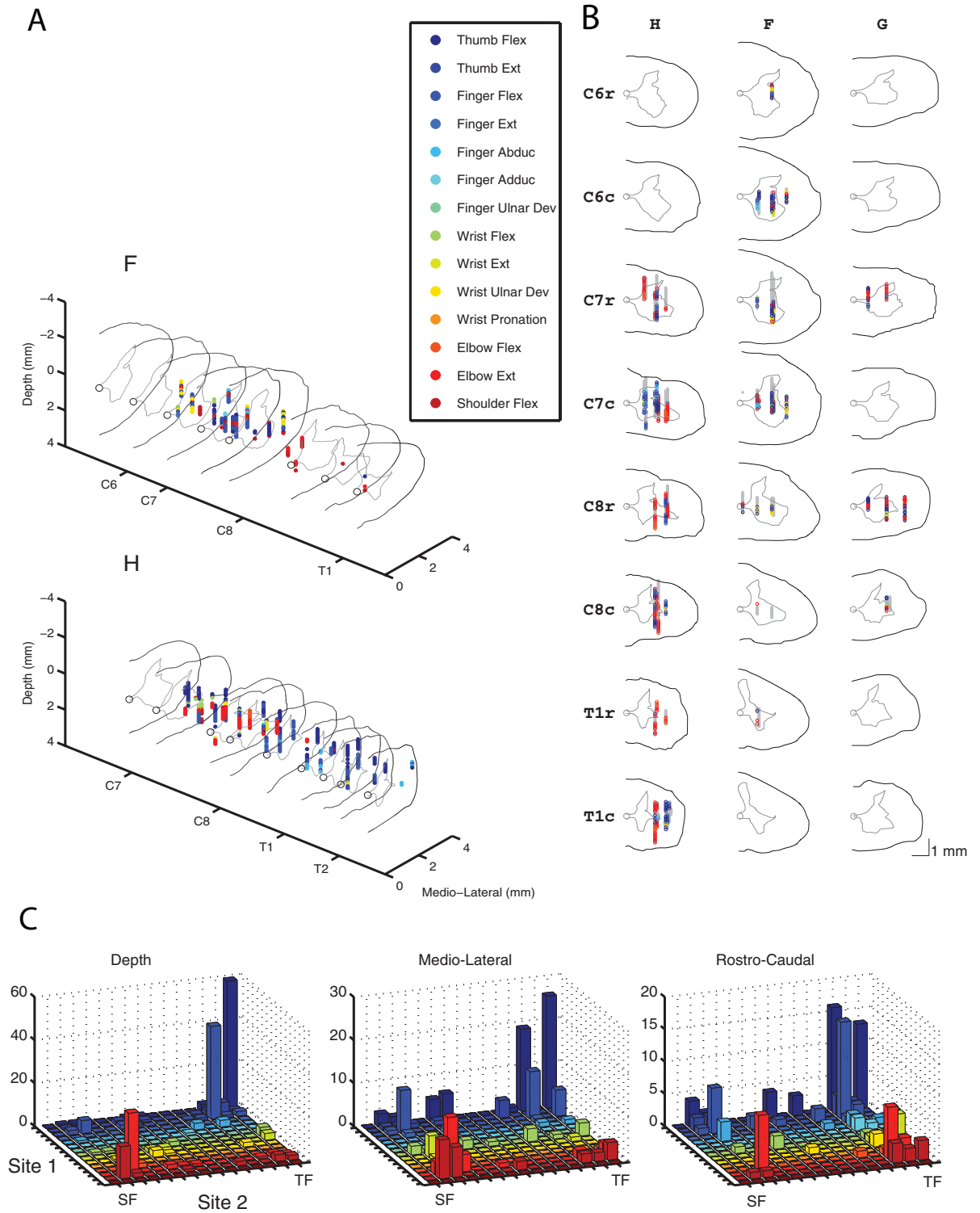


Figure 3

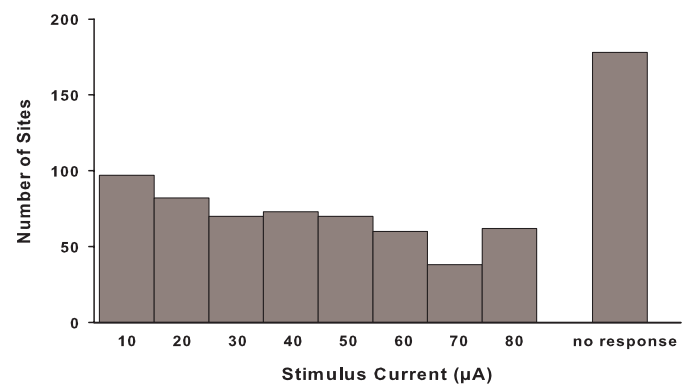


Figure 4

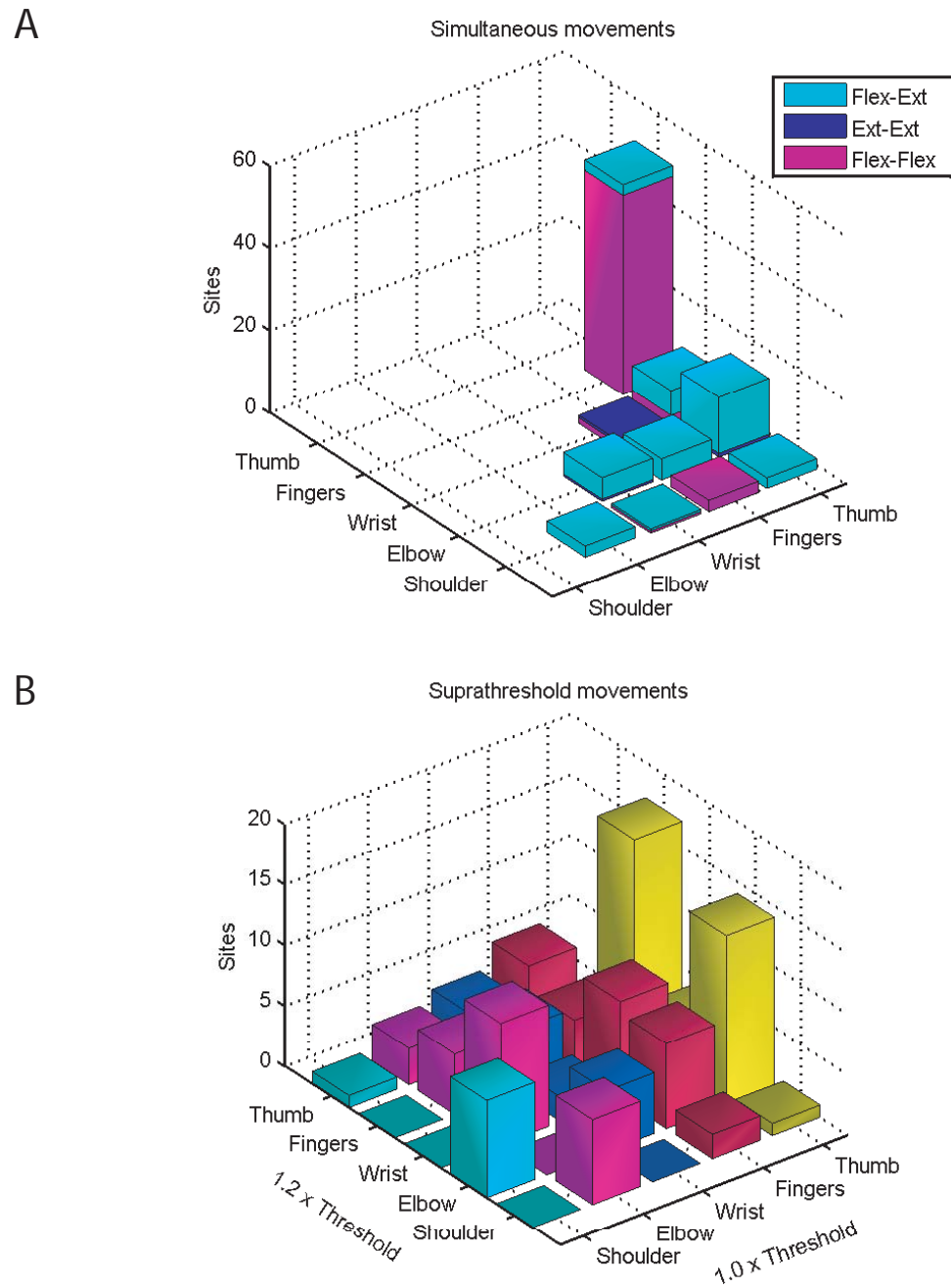
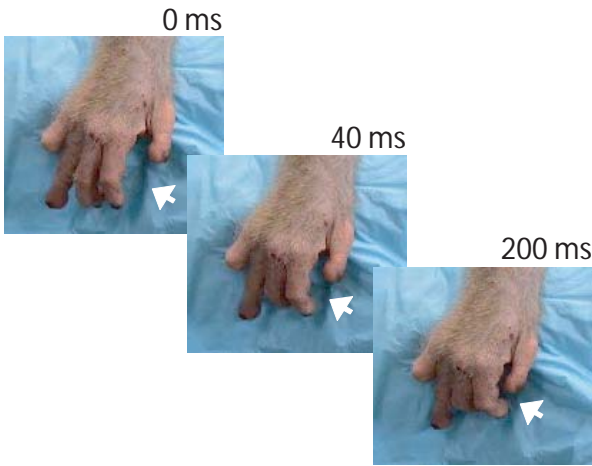


Figure 5

A



B

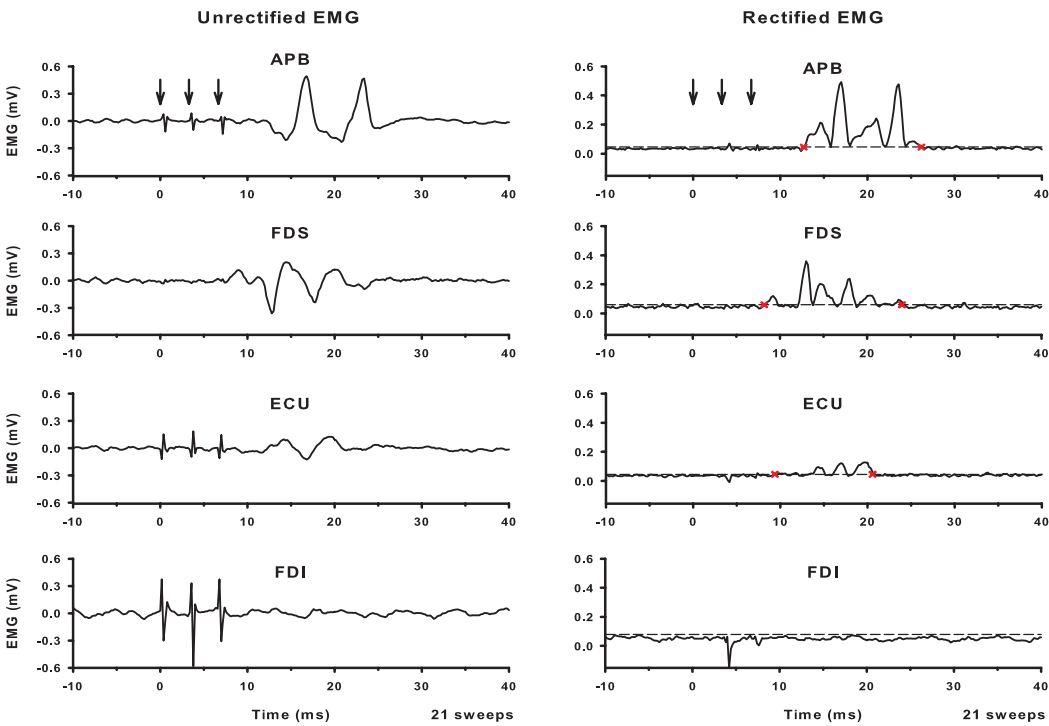


Figure 6

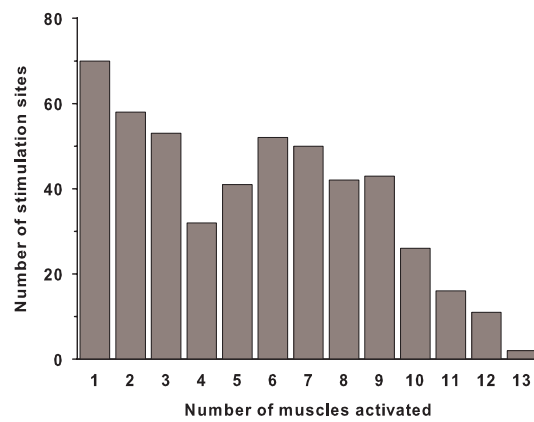


Figure 7

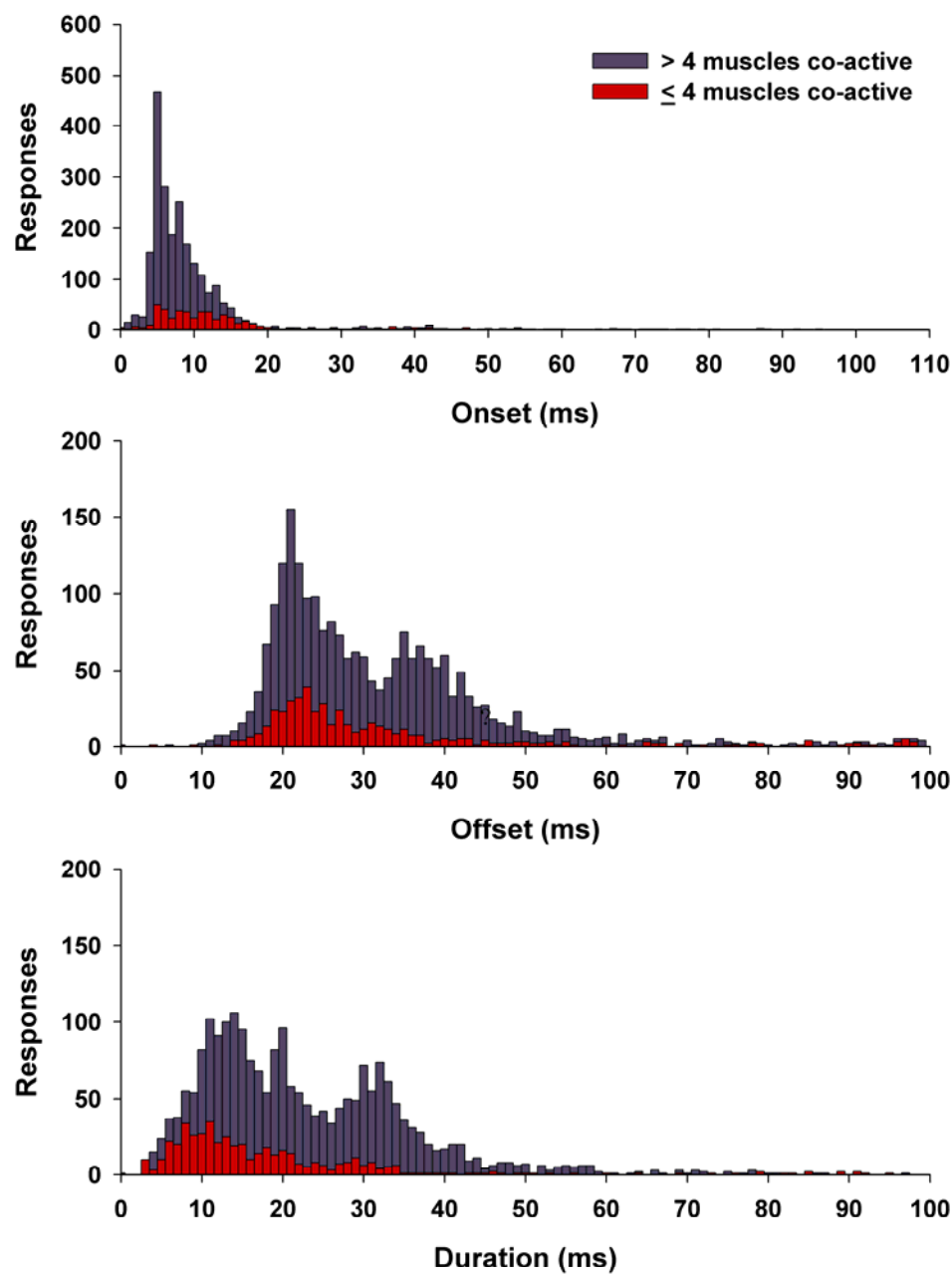


Figure 8

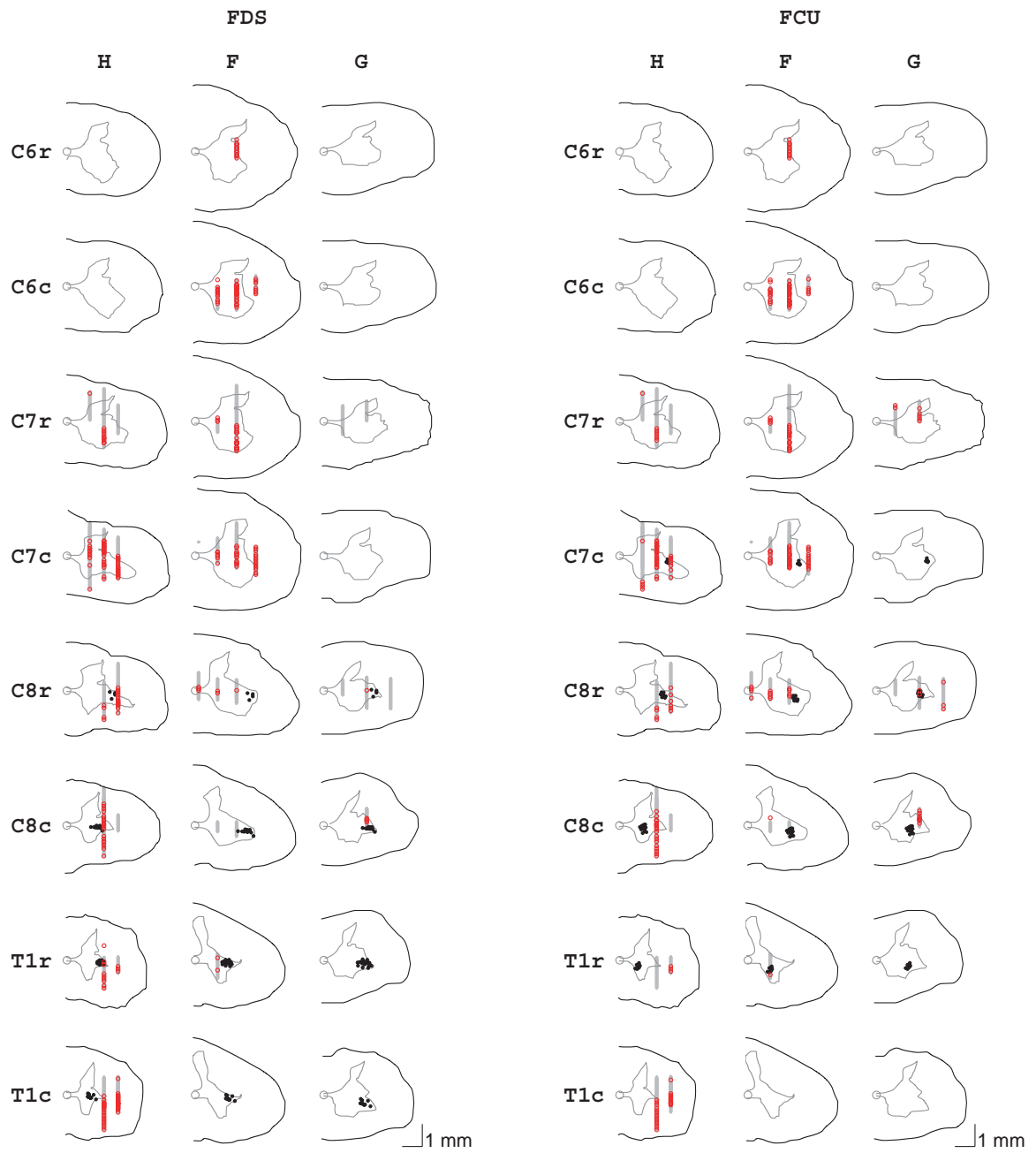


Figure 9

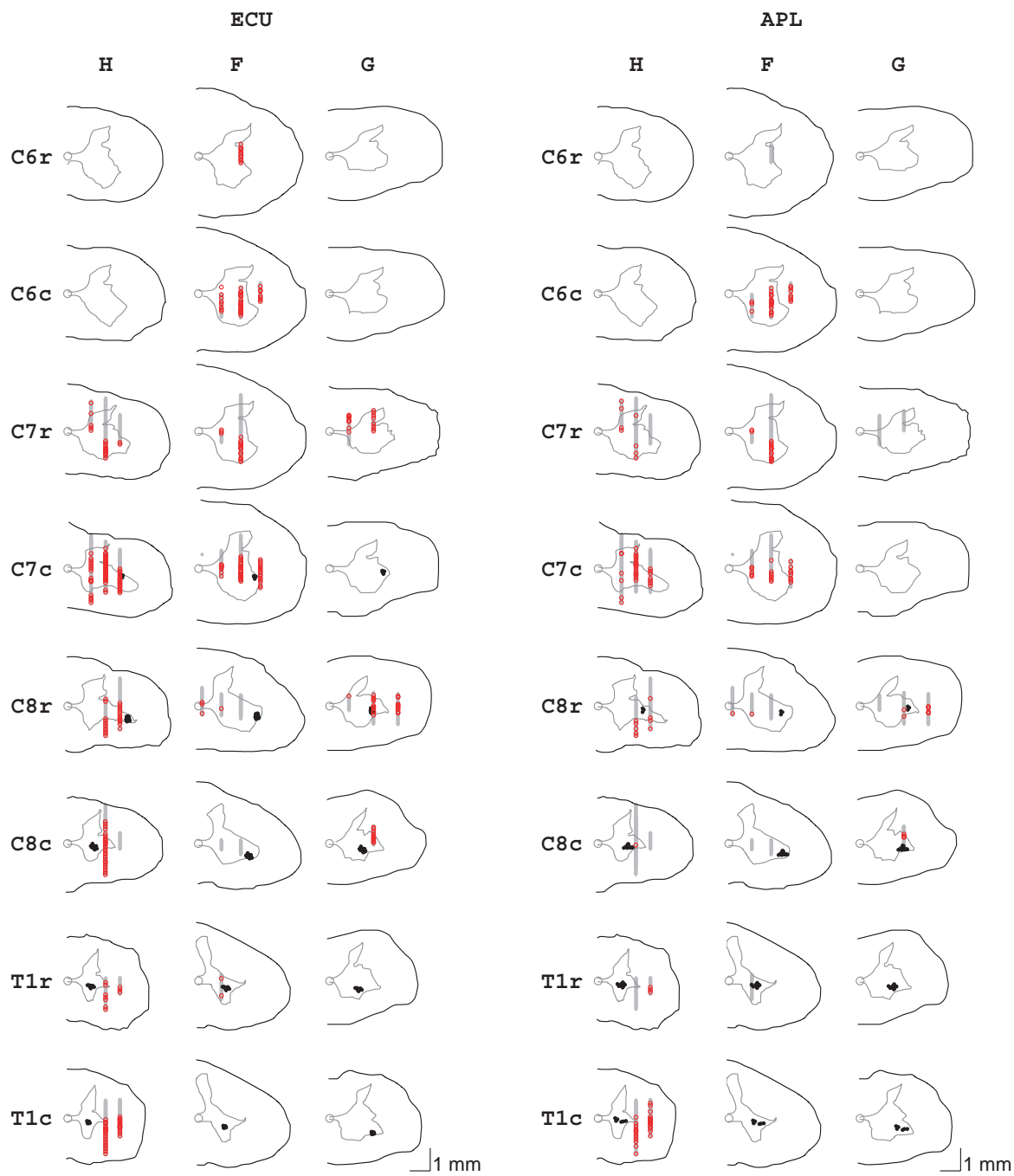


Figure 9 (con't)

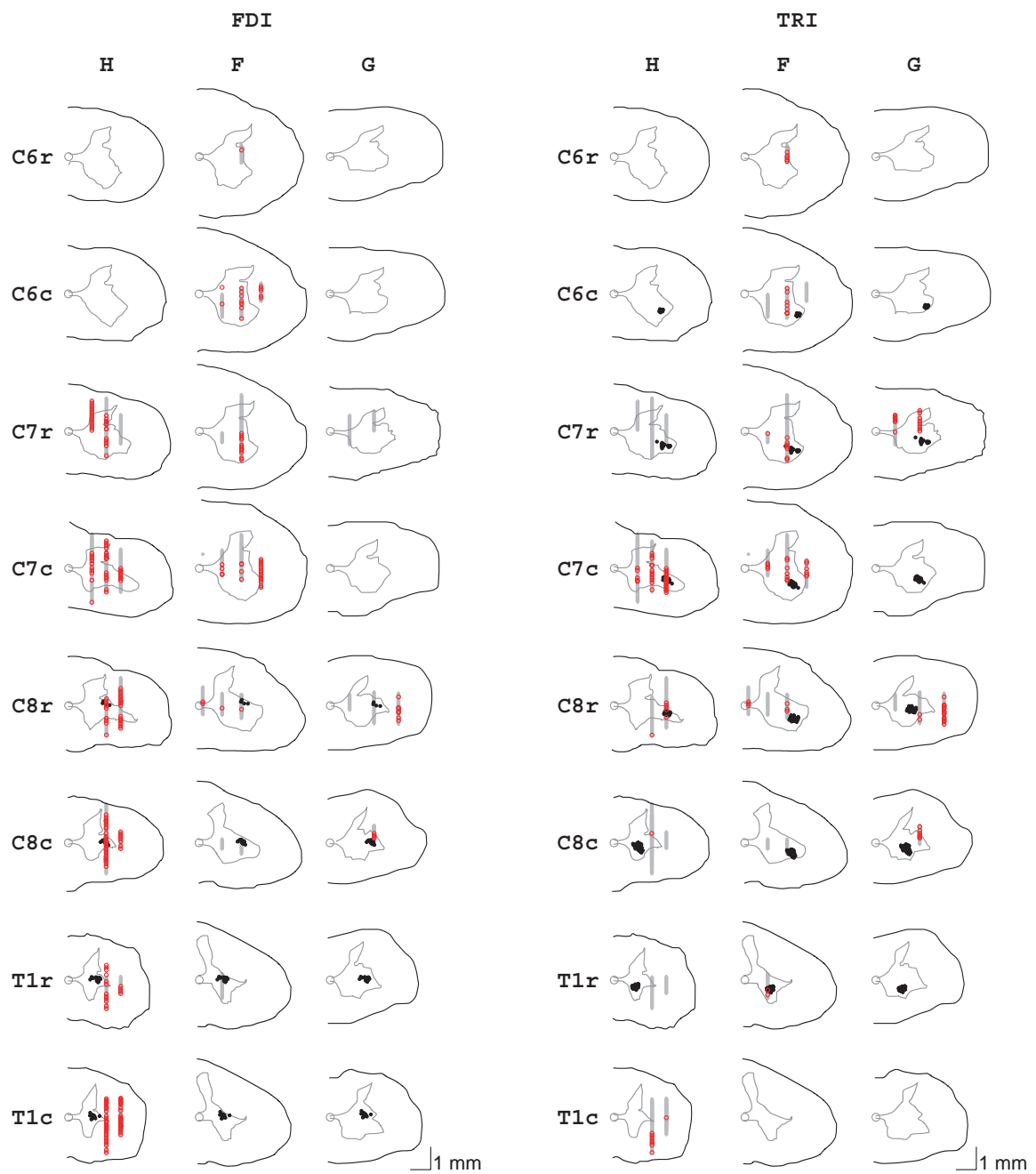


Figure 9 (con't)

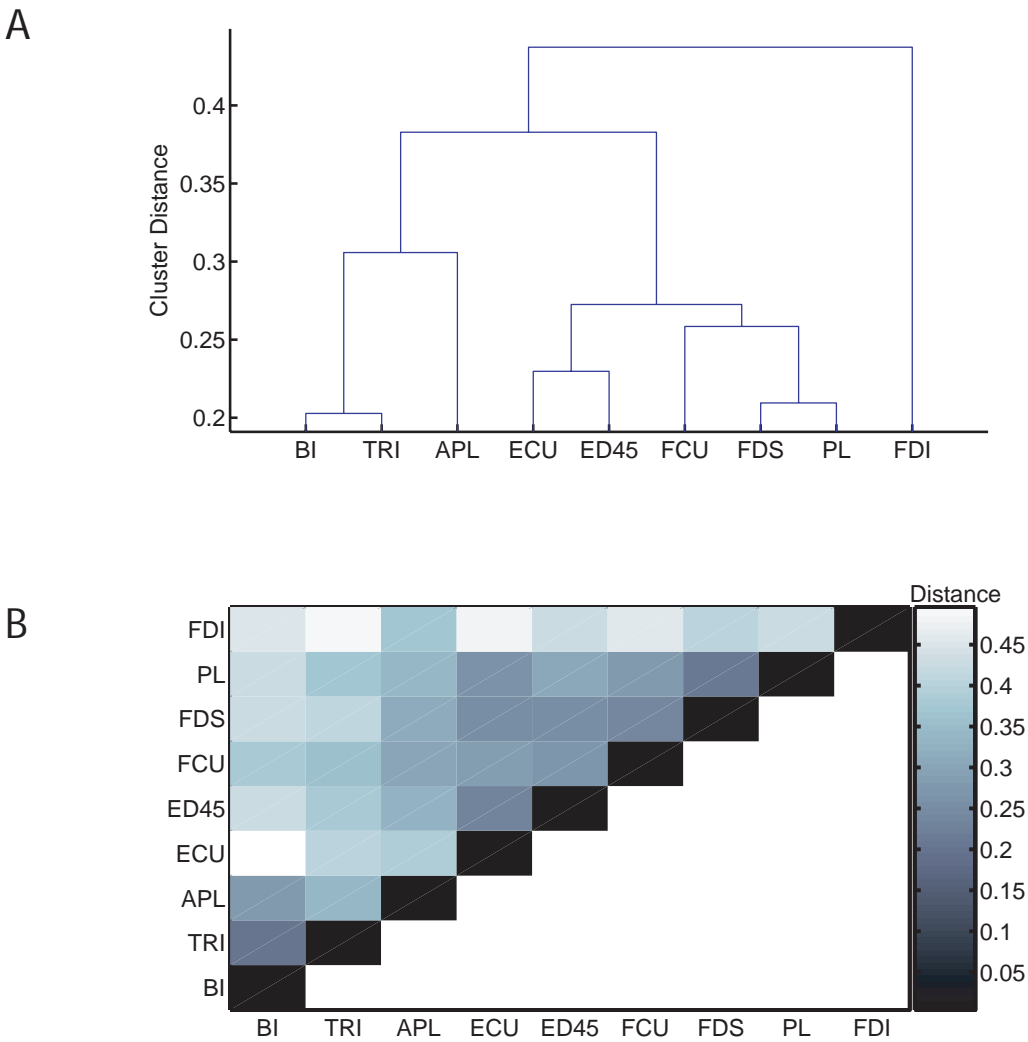


Figure 10