Properties of primary motor cortex output to hindlimb muscles in the macaque monkey

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ABSTRACT

The cortical control of forelimb motor function has been extensively studied, especially in the primate. In contrast, cortical control of the hindlimb has been relatively neglected. This study assessed the output properties of the M1 (primary motor cortex) hindlimb representation in terms of the sign, latency, magnitude, and distribution of effects in stimulus-triggered averages of EMG activity recorded from 19 muscles, including hip, knee, ankle, digit and intrinsic foot muscles, during a push-pull task in comparison to previously reported data on the forelimb. Stimulus-triggered averages (15, 30 and 60 μA at 15 Hz) of EMG activity were computed at 317 putative layer V sites in two rhesus macaques. Poststimulus facilitation (PStF) was equally distributed between distal and proximal muscles while suppression (PStS) was more common in distal muscles than proximal muscles (51%/49% respectively for PStF, 72%/28% respectively for PStS) at 30 μA. Mean PStF and PStS onset latency generally increased the more distal the joint of a muscle’s action. Most significantly, the average magnitude of hindlimb poststimulus effects was considerably weaker than the average magnitude of effects from forelimb M1. In addition, forelimb PStF magnitude increased consistently from proximal to distal joints while hindlimb PStF magnitude was similar at all joints except the intrinsic foot muscles, which had a magnitude about double that of all other muscles. The results suggest a greater monosynaptic input to forelimb compared to hindlimb motoneurons as well as a more direct synaptic linkage for the intrinsic foot muscles compared to the other hindlimb muscles.

Keywords: Motor cortex, hindlimb, EMG, stimulus-triggered averaging
INTRODUCTION

The cortical control of the hindlimb in primates has been relatively neglected compared to the extensive studies of the forelimb. Forelimb motor cortex has been investigated using a variety of anatomical and electrophysiological techniques including unit recording, spike- and stimulus-triggered averaging (StTA) of EMG activity, high frequency intracortical microstimulation (ICMS), intracellular recording and retrograde tracing (Asanuma et al., 1978; Asanuma and Rosén, 1972; Baker et al., 1998; Churchland et al., 2012; Dancause et al., 2005; Graziano et al., 2005; Luppino et al., 1991; McKiernan et al., 1998; Park et al., 2001, 2004; Plautz et al., 2000; Schieber and Rivlis, 2005). These studies have demonstrated important findings concerning the somatotopic organization of forelimb M1 cortex, anatomical projections to and from forelimb M1, neuron density, output effects of M1 on forelimb muscles, activity relationships of neurons to various parameters of movement and map reorganization in response to use and injury.

By comparison, there have been far fewer studies of the cortical control of the hindlimb in non-human primates. Monosynaptic linkages have been observed between corticospinal neurons and motoneurons in both the forelimb and the hindlimb (Asanuma et al., 1979; Clough et al., 1968; Edgley et al., 1997; He et al., 1993, 1995; Jankowska et al., 1975; Lemon, 1990; Muir and Porter, 1973; Phillips and Porter, 1964; Preston et al., 1967; Preston and Whitlock, 1961, 1963; Shapovalov, 1975; Shapovalov and Kurchavyi, 1974). In fact, physiological evidence establishing the existence of monosynaptic linkages from cortex to motoneurons actually came from work on the hindlimb by Bernhard et al. (1953). Bernhard and Bohm (1954) then introduced the term corticomotoneuronal system to refer to the fast conducting corticospinal neurons that make monosynaptic connections with motoneurons. Nevertheless, in contrast to the numerous single unit and electrode array recording studies of forelimb cortex, there have been only three unit recording studies of hindlimb M1 neurons in non-human primates (Neafsey, 1980; Sahrmann et al., 1984; Ma and He, 2010). Retrograde tracer and ICMS studies in macaque monkeys have confirmed the location of hindlimb M1 medial to forelimb M1 in the precentral gyrus and extending into the bank of the central sulcus and the bank of the medial wall of the hemisphere (Hatanaka et al., 2001; He et al., 1993; Luppino et al., 1991; Wise and Tanji, 1981). Hindlimb studies in the cat have shown modulation of cortical neurons during treadmill locomotion and intense cortical activity related to trajectory modification (Widajewicz et al., 1994, Drew et al., 2002). In humans, facilitation and suppression effects in leg muscles have been demonstrated using transcranial magnetic stimulation (TMS) to evoke EMG potentials (Bawa et al., 2002; Brouwer and Ashby, 1990, 1992; Perez et al., 2004; Thomas and Gorassini, 2005).
Recently, we developed a method for chronically implanting EMG electrodes in large numbers of hindlimb muscles (Hudson et al., 2010). In the present study, we applied this method together with stimulus-triggered averaging (StTA) of electromyographic (EMG) activity recorded from 19 muscles of the hindlimb while the monkey performed a push-pull task, to investigate the sign, latency and magnitude of poststimulus output effects based on a systematic exploration of hindlimb M1 cortex. The methods we used are very similar to those of a previous study from our laboratory on the forelimb (Park et al., 2004) allowing direct comparison of hindlimb and forelimb M1 output properties. Our results show that short latency facilitation of hindlimb EMG activity is present in stimulus-triggered averages, however, the magnitude of effects is substantially weaker than forelimb effects suggesting a much weaker synaptic linkage.

MATERIALS AND METHODS

Behavioral task

Data were collected from the left primary motor cortex of two male rhesus macaques (Macaca mulatta, ~10 kg, 6-7 years old). Both monkeys were trained to perform a hindlimb push-pull task engaging both proximal and distal muscles in reliable and stereotyped patterns of activation (Hudson et al., 2010). Within a sound-attenuating chamber, the monkey was seated in a custom primate chair with both arms and the left leg restrained. With the right foot, the monkey gripped a manipulandum (horizontal post) and extended the leg until the target zone was achieved. After a hold period of 500 ms in the target zone, the monkey flexed the leg pulling the manipulandum to a second target zone. An applesauce reward was given following a second hold period of 500 ms and the monkey repeated this cycle. Visual and auditory cues guided the behavioral task.

MRI

The monkey’s head was placed in an MRI-compatible stereotaxic apparatus and structural MRIs in the sagittal, coronal and horizontal planes were obtained using a Siemens Allegra 3T system. A 3-dimensional reconstruction of each monkey’s brain was produced using CARET software (Computerized Anatomical Reconstruction and Editing Tool Kit) and this was used to confirm placement of the cortical chamber.

Surgical procedures
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Upon completion of training, each monkey was implanted with a titanium cortical recording chamber (30 mm inside diameter) centered at anterior 13.5 mm, lateral 0 mm and 0° angle to the sagittal plane (Paxinos et al., 2000). Pairs of insulated, multi-stranded stainless steel wires (Cooner Wire, AS632) were implanted during an aseptic surgical procedure (Hudson et. al., 2010) to record EMG activity simultaneously from 19 muscles of the hindlimb. Briefly, pairs of wires were tunneled subcutaneously to their target muscles from either four external connector modules (ITT Canon) affixed to the upper arm with elastic medical adhesive tape (arm-mounted subcutaneous implant, monkey F) or an external circular connector (Amphenol) affixed to the skull using dental acrylic (cranial-mounted subcutaneous implant, monkey C). Each muscle was tested for proper placement of electrode pairs by stimulating through the electrodes with brief stimulus trains (biphasic pulse, 0.2 ms/phase, ~50 Hz) while observing the evoked movements. Wires were removed and reinserted if proper placement was not confirmed.

EMGs were recorded from four hip muscles: gluteus maximus (GMAX), adductor brevis (ADB), gracilis (GRA) and tensor fascia latae (TFL); six knee muscles: rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), biceps femoris (BFL), semimembranosus (SEM) and semitendinosus (SET); five ankle muscles: peroneus longus (PERL), medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL) and tibialis anterior (TA); two digit muscles: extensor digitorum longus (EDL) and flexor digitorum longus (FDL); and two intrinsic foot muscles: extensor digitorum brevis (EDB) and flexor hallucis brevis (FHB). In monkey C, the EMG leads to PERL were compromised shortly after the implant. As a result, PERL was not included in the data set for monkey C.

All procedures were in accordance with the standards outlined by the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and the U.S. Department of Health and Human Services and approved by the University Animal Care and Use Committee. All surgeries were performed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility using full aseptic procedures. Postoperative analgesics (buprenorphine, 0.01 mg/kg) were administered for five days. Wound edges were inspected daily and treated with topical antibiotic and Betadine (10% povidone-iodine) when necessary.

Data collection

EMG activity, cortical activity and task-related signals were simultaneously monitored and recorded. Glass and mylar-insulated platinum-iridium electrodes (0.5-1.5 MΩ impedances, Frederick Haer) were used to record cortical unit activity and for microstimulation. The electrode was positioned in the recording chamber using a custom-built x-y positioner and advanced using a manual hydraulic
microdrive (Frederick Haer).  Electrode penetrations were systematically made at 1 mm intervals in the
precentral cortex of the left hemisphere.  Data were collected from putative layer V sites in the cortex.
All corticospinal neurons reside in layer V (He et al., 1993, 1995), which is located about 1.5 mm below
the surface of the cortex.  We used first activity as an indicator of the cortical surface and applied
stimulation 1.5 mm below first activity.  In the convexity of the precentral gyrus this was the only site
stimulated because the electrode entered white matter with further advancement beyond the 1.5 mm site.
For penetrations down the bank of the precentral gyrus and the medial wall of the hemisphere, stimulation
continued at intervals of 0.5 mm below the first site at 1.5 mm.  Besides depth, additional criteria for
identifying layer V sites included the nature and size of neuronal spikes as well as the strength of effects
in relation to nearby electrode tracks.  For example, two sites adjacent one another and at the same depth
in the bank of the precentral gyrus were evaluated in terms of spike characteristics and strength of effects
to identify which one was layer V.  Identification of layer V was aided by the fact that layer V pyramidal
cells in hindlimb cortex produce particularly large extracellular spikes.

In rhesus monkeys, the posterior border of SMA is located approximately 7 mm posterior to the
posterior limit of the arcuate sulcus (Mitz and Wise, 1987; Luppino et al., 1991).  The most anterior
points from which we obtained poststimulus effects (PStEs) were all posterior to this boundary (solid
orange lines in Figure 2) so we are confident that all our stimulation sites were in M1 not SMA.
Supporting this conclusion is the fact that the representation of the hindlimb in SMA is very limited using
short train ICMS to evoke movements and what does exist tends to be in the medial wall of the
hemisphere (Mitz and Wise, 1987; Macpherson et al., 1982).  In contrast, most of the hindlimb
representation from M1 we have found is on the dorsal surface rather than the medial wall of the
hemisphere.

At each cortical site, StTAs of EMG activity were collected at 15, 30 and 60 µA (15 Hz) for 19
muscles of the hindlimb as the monkey performed the push-pull task.  Individual stimuli were
symmetrical biphasic pulses, 0.2 ms negative pulse followed by a 0.2 ms positive pulse, applied
throughout all phases of the task.  EMGs were generally filtered at 30 Hz to 1 kHz, digitized at 4 kHz and
full-wave rectified.  StTAs consisted of at least 500 trigger events and were compiled over an 80 ms
epoch, 20 ms pre-trigger and 60 ms post-trigger.  To prevent averaging periods where EMG activity was
minimal or non-existent, segments of EMG activity associated with each stimulus were accepted for
averaging only if the average of all EMG data points over the entire 80 ms epoch was ≥ 5% of full-scale
input (McKiernan et al., 1998).  If PStEs were not detected at 60 µA, high frequency, long duration
intracortical microstimulation (HFLD-ICMS, 15-60 µA, 200 Hz, 500 ms) was applied to identify M1
regions representing muscles not implanted with EMG electrodes (e.g. trunk, tail and forelimb). Sensory cortex was identified by the presence of distinctive spike activity in response to cutaneous stimulation.

EMG-triggered averages were computed to evaluate cross-talk between muscles (Cheney and Fetz, 1980). Averages of EMG activity were compiled for all 19 muscles using each muscle as the source of triggers. This yielded a 'cross-talk peak' for each muscle as a trigger paired with all other muscles. If the ratio of the cortically triggered effect for a pair of muscles was less than twice their cross-talk peak, it was interpreted as an indication that much of the cortical effect may have been due to crosstalk and the effect was eliminated (Buys et al., 1986). No PStf or PStS had to be eliminated based on this criterion.

**Data analysis**

Poststimulus facilitation (PStF) and suppression (PStS) effects were computer measured as described by Mewes and Cheney (1991). Each average was compiled over an 80 ms epoch, 20 ms pre-trigger and 60 ms post-trigger. A poststimulus effect (PStE) was defined as a peak or trough of EMG activity that rose or fell from baseline EMG activity and maintained a level of activity exceeding 2.25 standard deviations of baseline for a period equal to or greater than 0.75 ms. Baseline EMG activity was measured as the 12 ms period preceding the onset of the effect, initially determined by visual inspection. In cases where the baseline was rising or declining, a shorter baseline close to the onset of the PStE was used. Baseline statistics were then used to determine the onset of the effect as the point where the envelope of the record exceeded two standard deviations of baseline. The magnitude of PStE was expressed as the peak percentage increase (+ppi) or peak percentage decrease (-ppi) in EMG activity above (PStF) or below (PStS) baseline. Figure 1 illustrates the application of these criteria to stimulus-triggered averages obtained from one cortical site at 15, 30 and 60 μA. Asterisks mark records with PStF that were included in the data set. The numbers on the right side give the magnitude of PStF in ppi units, including some that fell below the criterion of activity exceeding 2.25 standard deviations of baseline. Stimulation at 15 μA produced clear PStF in VL and RF as well as marginal effects in two other muscles (SET and BFL). Thirty microamps yielded clear PStF in six muscles, all proximal. At 60 μA, these effects became stronger and clear PStF appeared in three additional proximal muscles (VM, GMAX and ADB) as well as two distal muscles (FHB and TA).

**Unfolding the cortex**

A two-dimensional representation of layer V of the cortex in the medial wall of the hemisphere and the anterior bank of the central sulcus required flattening and unfolding the curvature of the cortex. This process has been described in detail by Park et al. (2001). Briefly, the cortex was unfolded and two-
dimensional maps were generated based on known architectural landmarks, MRI images, observations during the cortical chamber implant surgery, electrode track x-y coordinates, electrode penetration depth and properties of recorded neurons (Figure 2).

RESULTS

Dataset

Table 1 summarizes the data collected from the left M1 in two male rhesus macaques. Three-hundred twelve electrode tracks were made (monkey F, 170; monkey C, 142). Stimulus-triggered averages (15, 30 and 60 μA at 15 Hz) of rectified EMG activity were collected from 19 hindlimb muscles of the hip, knee, ankle, digit and intrinsic foot. Only data from putative layer V sites were analyzed. HFLD-ICMS (15-60 μA, 200 Hz, 500 ms) was performed at 133 sites to identify output effects (movements or EMG responses) when no poststimulus effects were obtained. The presence or absence of sensory responses typical of primary somatosensory cortex (SI) was tested at 65 sites primarily to aid in identifying the M1-S1 border. Figure 1 shows typical results for one cortical site where effects were obtained at 15, 30 and 60 μA as discussed in the Methods.

Table 2 summarizes the mean onset latencies and magnitudes of PStEs for muscles at different joints. Not all sites with PStEs at 30 and 60 μA had PStEs at 15 μA. Only the data from sites with PStEs at all three stimulus intensities were used to analyze the relationship between stimulus intensity and the latency and magnitude of effects (Table 2A). Data from all sites were used to analyze the distribution of effects (Table 2B).

Figure 2 shows the location of electrode penetrations and the complete hindlimb representation maps for each monkey at 15, 30 and 60 μA. Blue represents sites with effects in StTAs of hindlimb muscles. If an effect was not present in StTAs at 60 μA, we applied high frequency ICMS to define the representation as either no effect, trunk (light green), tail (dark green) or forelimb (gray). For simplicity, these representations are only shown in the 60 μA maps. Additionally, cutaneous sensory responses judged to be from S1 are indicated in yellow. As expected, the size of the hindlimb representation expanded with stimulus intensity in both monkeys. There was also a clear difference in the size of the representations between the two monkeys at 15 and 30 μA, with monkey F showing a more limited representation at the lower intensities but expanding at 60 μA to more closely match the size of the representation in monkey C. This difference can be attributed to the fact that the effects in monkey C
were considerably stronger than those in monkey F. At 60 μA the total size of the representation occupied an area of about 8 X 10 mm. The representation was largely on the medial convexity of the precentral gyrus with only a small extension over the midline down the medial wall of the hemisphere. The hindlimb representation was bounded anteriorly, laterally and medially by a trunk representation. Tail representation was located on the medial wall extending onto the convexity of the medial hemisphere. The hindlimb representation extended 12-13 mm anterior to the extrapolated intersection of central sulcus and the midline.

**Latency of effects**

At 15 μA, the overall mean PStF onset latency was 15.5 ± 2.5 ms compared with a mean PStS onset latency of 19.4 ± 3.1 ms (Table 2B). These latencies were similar in both monkeys. Changes in onset latency of effects with stimulus intensity are best appreciated from a subset of cortical sites in which effects were present at each of the three stimulus intensities (Table 2A). As stimulus intensity was increased (15-30-60 μA), there was a slight decrease in the mean PStF onset latency (Table 2A) although these changes did not achieve statistical significance. Overall, mean PStF onset latency increased the more distal the joint of a muscle’s action (Table 2B). At 15 μA the PStF onset latencies of the proximal muscles (hip and knee) were significantly different from all other muscles (p<0.05-0.001, 1-way ANOVA) while there were no significant differences between the latencies of the distal muscles (ankle, digit and intrinsic foot). At 30 and 60 μA, all PStF onset latency differences between joints were significant (p <0.05-0.001, 1-way ANOVA) except ankle versus digit at 30 μA. Overall, the mean PStS onset latency increased the more distal the joint of a muscle’s action (Table 2B). At 30 μA, the PStS onset latency of the intrinsic muscles was significantly greater than that of the hip, knee and ankle muscles (p<0.05-0.01, 1-way ANOVA). At 60 μA, all PStS onset latency differences between joints were significant (p<0.01-0.001, 1-way ANOVA) except digit versus knee, digit versus ankle and intrinsic versus ankle. The dataset for mean onset latency of PStS effects was too small for meaningful statistical comparisons of effects across stimulus intensities (Table 2A).

Figure 3 shows the distribution of PStF onset latencies for muscles acting at different joints of the hindlimb at 15, 30 and 60 μA. The distributions at each joint are mostly unimodal, although the hip, knee and ankle latencies do have a suggestion of bimodality at some stimulus intensities that is also evident in the overall results for all muscles. The intrinsic muscles have the narrowest distribution at all stimulus intensities with no tendency toward bimodality. Also noteworthy is the fact that the latency distributions, especially for ankle, knee and hip muscles, extend into the longer latency range. Although slower conduction velocity corticospinal neurons may contribute to these longer latency effects, the results also
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serve to emphasize the potential contribution of non-monosynaptic linkages. Potential non-monosynaptic
linkages to hindlimb motoneurons include spinal interneurons, cortico-bulbar projections, for example,
the cortico-reticulospinal system (Lemon, 2008), as well as propriospinal pathways, although evidence for
a lumbar propriospinal system is much more limited compared to the cervical propriospinal system
(Pierrot-Deseilligny and Burke, 2005).

Magnitude of effects

At 15 μA, the mean PStF magnitude, expressed as peak percent increase (ppi) above baseline,
was 23.8 ± 20.5 compared with -15.8 ± 5.2 for PStS (Table 2B). The mean PStF magnitude increased
significantly as stimulus intensity increased (Table 2A, p<0.001, 1-way ANOVA). Between 15 and 60
μA, ppi increased linearly with stimulus intensity at a rate of 1.16 ppi/μA. Magnitudes based on all
effects (Table 2B) are not appropriate for examining relationships between magnitude and intensity
because higher intensity stimulation recruits new muscles with weak effects that dilute the mean
magnitude. At all stimulus intensities, the magnitude of PStF was strongest in intrinsic muscles (about
double) compared to all other muscle groups and the differences were statistically significant in all cases
(Table 2B, p<0.01-0.001, 1-way ANOVA). The magnitudes of PStF in the digit, ankle, knee and hip
muscles were very similar and the small differences observed were not statistically significant. The
magnitudes of PStS were very similar in all muscles (Table 2B). Only at 60 μA did any differences
become statistically significant with the magnitude of PStS being stronger in the ankle muscles compared
to the hip, digit and intrinsic muscles (p<0.05, 1-way ANOVA). The dataset for magnitude of PStS
effects was too small for meaningful statistical comparisons of effects across stimulus intensities (Table
2A).

Figure 4 shows the distribution of PStF magnitude at 15, 30 and 60 μA for muscles acting at
different joints of the hindlimb. Similar trends are seen at all stimulus intensities. At all joints, the
weakest effects are the most common, as evidenced by the skewed distributions. Muscles at all joints,
except the intrinsic muscles, have a narrow range of magnitudes focused heavily toward weak effects. At
60 μA the vast majority of effects in all muscles, except intrinsic foot muscles, had magnitudes below 50
ppi. In contrast, 46% of effects in intrinsic foot muscles had a magnitude of 50 ppi or greater. Moreover,
the maximum PStF magnitude observed at each stimulus intensity was from one of the intrinsic foot
muscles (149 ppi, FHB, 15 μA; 188 ppi, EDB, 30 μA; 353 ppi, EDB, 60 μA).
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There was a consistent trend among muscles at all joints for shorter PStF onset latencies to be associated with stronger magnitudes. This correlation was statistically significant at the hip, knee and ankle joints at 60 μA (p<0.05-0.01, 1-way ANOVA).

Distribution of PStEs

Figures 5A and B show the distributions of PStf and PStS effects in hip, knee, ankle, digit and intrinsic foot muscles at 15, 30 and 60 μA. Of 209 PStF effects at 15 μA, 55% were in distal muscles including 21% in ankle, 5% in digit and 29% in intrinsic foot muscles. Forty-five percent of PStF effects were in proximal muscles including 14% in hip and 31% in knee muscles (Table 2B). At 30 and 60 μA, the number of PStF effects in distal and proximal muscles were nearly equal (51/49% respectively at 30 μA and 49/51% at 60 μA). In contrast, inhibitory effects showed a clear preference favoring distal muscles. Of 50 PStS effects at 15 μA, 68% were in distal muscles including 44% in ankle, 2% in digit and 22% in intrinsic foot muscles. Thirty-two percent of PStS effects were in proximal muscles including 12% in hip and 20% in knee muscles. Similar trends were observed at 30 and 60 μA. The numbers of recorded distal and proximal muscles were nearly equal (9 versus 10 respectively) and cannot account for these differences. Also, electrode tracks were placed systematically throughout the entire hindlimb cortical representation so there was no bias to preferentially sample one part of the representation over another part.

Different numbers of muscles were recorded at each joint (4 hip, 6 knee, 5 ankle, 2 digit and 2 intrinsic foot muscles). Figures 5C and D show the distributions of PStF and PStS effects at each joint after normalizing for the number of muscles sampled. After normalizing, it becomes clear that PStF effects are most common in intrinsic muscles at all stimulus intensities. The number of PStF effects was very similar across all other muscles groups. Suppression effects were more variable than facilitation effects, however, it is clear there tended to be fewer suppression effects in the knee muscles than other muscle groups at 30 and 60 μA. These differences were consistent in the data collected from each of the two monkeys.

Differences in the distribution of effects to flexor and extensor muscles were also evident both in the summed effects for each muscle group after normalizing for number of muscles tested (Figure 6) and in the individual muscle data (Figure 7). At the knee joint, facilitation of the extensors (dark bars) dominated while at the hip joint, the flexors (light bars) were more commonly facilitated. This was true at all stimulus intensities. Taking into account all stimulus intensities, PStF was similarly distributed between flexor and extensor muscles at distal joints. PStF was much more common in all muscle groups
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and all intensities than PStS (Figure 7). However, it should be noted that we only counted PStS effects if the effect was a pure suppression and not preceded by PStF. In many cases a clear dip followed the PStF effect. These biphasic effects were not included for suppression because of uncertainty about the underlying mechanism (real inhibition versus post-excitatory suppression) and uncertainties in measuring latency and magnitude. The prominence of facilitation effects in the intrinsic muscles is also evident (Figures 6 and 7) rivaled only by effects in knee extensor muscles at higher intensities. The smaller number of PStS effects limited interpretation of suppression effects in flexors and extensors. Nevertheless, while suppression was, overall, about equally common in flexors and extensors at 15μA (and other intensities), the distribution was variable when categorized by joint. It is noteworthy that suppression was more common in soleus at 15μA than any other muscle (Figure 7, Hudson et al., 2013). At higher intensities, only two additional muscles (FHB and TFL) had inhibitory effects in the same range as soleus.

DISCUSSION

This study analyzes the cortical output from primary motor cortex to 19 muscles of the hindlimb in terms of the latency, magnitude and distribution of poststimulus facilitation and suppression effects. A similar study of the forelimb from our laboratory was based on stimulus-triggered averaging done in a very similar way except only one intensity (15μA) was used (Park et al., 2004). It was evident early in our data collection that 15μA stimuli were substantially less effective in hindlimb cortex than in forelimb cortex. We had far fewer effects and the effects overall were weaker. Consequently, we performed StTAing using three stimulus intensities (15, 30 and 60μA). Our results are significant in presenting a comprehensive dataset on the properties of cortical output to hindlimb muscles at each joint from the hip to intrinsic foot muscles. The results show that a clear, short-latency PStF can be elicited using stimulus-triggered averaging of EMG activity and this effect can be used to define the borders of the hindlimb representation in primary motor cortex. An important aspect of this study is that the data can be directly compared to data from forelimb M1 obtained using very similar methods.

Comparison of hindlimb and forelimb M1 properties

Table 3 compares the key properties of hindlimb and forelimb M1 output based on StTA data at 15μA. Systematic stimulus-triggered averaging data on forelimb M1 cortex (Park et al., 2004) is not available at the other stimulus intensities. While clear PSTEs were obtained from hindlimb cortex, their magnitude overall was substantially weaker than effects from forelimb cortex for most muscle groups.
However, it is interesting that there was considerable variability by joint in the extent to which forelimb and hindlimb PStF differed. The greatest difference was in digit muscles located in the forearm or lower leg where forelimb PStF was more than five times greater than hindlimb PStF. The difference in PStF magnitude at all other joints was considerably smaller. PStF in muscles acting at the wrist was about three and a half times greater than that of muscles acting at the ankle. The difference for muscles acting at the knee/elbow was smaller yet (2-fold) and muscles at the hip and shoulder were essentially equal in magnitude. The intrinsic foot/hand muscles are an interesting case where the difference in magnitude of PStF for hand muscles is 2.2 times greater than foot muscles – less than the difference for non-intrinsic digit muscles.

There were additional differences in forelimb and hindlimb PStF. The magnitude of forelimb PStF increased consistently at each joint in going from the most proximal muscles (shoulder muscles) to the most distal muscles (intrinsic hand muscles). PStF in hindlimb muscles did not show this consistent increase. Rather, PStF magnitude was similar at all joints except the intrinsic foot muscles, which had a magnitude about double that of all the other muscles.

How can the differences in magnitude of PStF effects in hindlimb muscles compared to forelimb muscles be interpreted? Using 0.5 mA surface anodal stimulation, Jankowska et al. (1975) reported that cortically evoked EPSPs in distal hindlimb motoneurons ranged from 0.2 - 2 mV in amplitude with mean values for different motoneurons groups of 0.15 mV to 0.6 mV. Motoneurons belonging to different muscle groups were identified by stimulating peripheral nerves. Although directly comparable data does not exist for the forelimb, Clough et al. (1968) reported EPSP amplitudes ranging from 0.2 - 9.5 mV for distal muscles of the forelimb. However, it was noted that only five motoneurons had EPSPs exceeding 5 mV. Mean values for different motoneuron groups ranged from 1.6 – 3.4 mV with motoneurons of EDC and intrinsic hand muscles showing the largest EPSPs. However, this data was based on maximal EPSPs obtained by increasing stimulus intensity until amplitude plateaued making direct comparison to the hindlimb data difficult. Intensities up to 11.8 mA were used although it was noted that EPSPs generally became maximal at much lower intensities. Given the difference in methods of the forelimb and hindlimb EPSP studies, comparisons are tenuous at best. Nevertheless, comparing the mean EPSP amplitudes suggests that hindlimb EPSPs are weaker than forelimb EPSPs. Our data provide a direct comparison of the strength of PStF across a wide range of muscles in the forelimb and hindlimb. The results show that the synaptic linkage to forelimb motoneurons from M1 cortex, compared to hindlimb, is stronger for muscles at all joints except the hip/shoulder joints. Stronger PStF could result from a greater density of corticospinal neurons in the cortex, greater connectivity among cortical neurons and/or a more robust synaptic linkage in the spinal cord. Based on the work of Strick and colleagues, the density of
corticospinal neurons in forelimb and hindlimb cortex is similar (Dum and Strick, 1991; He et al., 1993, 1995; see also Cheney et al., 2004, Table 1). Another potential contributing factor might be differences in cortical connectivity within forelimb and hindlimb cortex. If cortical connectivity was greater in forelimb cortex, physiological spread of activation from stimulation might result in excitation of a greater number of neurons for the same stimulus intensity. Although it is known that ICMS activates some cortical cells at a distance from the site of stimulation through physiological spread (Tehovnik et al., 2006; Histed et al., 2009), the extent to which this occurs should be substantially reduced with the single pulse ICMS method (stimulus-triggered averaging) used in this study compared to high frequency ICMS. Of the factors that could influence the strength of the synaptic linkage, it seems mostly likely that the greater amplitude of forelimb PSTF is due in large part to a greater monosynaptic input to forelimb compared to hindlimb motoneurons.

Distribution of PSTF to hindlimb compared to forelimb muscles

The distribution of output effects from M1 cortex to different muscle groups of the hindlimb and forelimb provides another interesting contrast (Table 3). After normalizing for the number of muscles recorded at each joint, the percent of forelimb PSTF effects in wrist, digit and intrinsic hand muscles was essentially equal. The number of effects dropped off slightly for elbow muscles and quite drastically for shoulder muscles. In contrast, the percent of hindlimb PSTF effects was similar across hip, knee and ankle muscles but was three and a half times greater for intrinsic foot muscles. The low number for non-intrinsic digit muscles at 15 μA is puzzling (Table 3). At 30 and 60 μA, the relative number of PSTF effects in non-intrinsic digit muscles was nearly the same as the ankle, knee and hip (Figure 5C).

What stands out about the data in Table 3 is that, unlike the forelimb, hindlimb intrinsic foot muscles had a disproportionately large percentage (47%) of the total number of effects obtained (Table 3). The number of effects obtained for each muscle group will be dependent on the size of the representation of the muscles in M1 cortex – the greater the territory devoted to a particular muscle group, the larger the number of effects that will be observed. Of course, this assumes the strength of the linkage is above a minimum threshold for detection.

Interpretation of differences in effects at forelimb and hindlimb

Assuming that monosynaptic linkages will produce the strongest PSTF effects compared to polysynaptic linkages, just as they produce the strongest effects in spike-triggered averages of EMG activity, the differences in magnitude of PSTF for hindlimb muscles compared to forelimb muscles might be explained as differences in the strength of the monosynaptic component of the synaptic linkage to
motoneurons. The data would then suggest a clear gradation in the contribution of the monosynaptic linkage to forelimb motoneurons in going from the most proximal to the most distal muscles, with the most distal muscles receiving the strongest monosynaptic input. Based on the magnitude of PSTF across muscles, this gradation of magnitude does not exist for the hindlimb. The results suggest that the strength of the synaptic linkage is similar for motoneurons at all joints except those of the intrinsic foot muscles, which have a much stronger linkage, possibly related to a much more prominent monosynaptic component.

ACKNOWLEDGEMENTS

We thank Ian Edwards for his expert electronics, computer and instrumentation assistance. This work was supported by National Institute of Health Grant NS064054 and National Institute of Health Center Grant HD02528, the Kathleen M. Osborn Endowment (PDC) and the KUMC Biomedical Research Training Program (DMG).

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REFERENCES


Cortical output to hindlimb muscles


Cortical output to hindlimb muscles


Cortical output to hindlimb muscles


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FIGURE CAPTIONS

Figure 1. Stimulus-triggered averages (StTA) of 19 muscles of the hindlimb at 15, 30 and 60 μA from one site in hindlimb primary motor cortex. Stimulus occurs at time zero. Range of trigger events listed at bottom of each stimulus column. Numbers on the right side of records give the magnitude of PSTF expressed as ppi (peak percent increase over baseline). Asterisks mark records with clear PSTF that were included in the database. GMAX, gluteus maximus; ADB, adductor brevis; GRA, gracilis; TFL, tensor fascia latae; RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; BFL, biceps femoris; SEM, semimembranosus; SET, semitendinosus; PERL, peroneus longus; MG, medial gastrocnemius; LG, lateral gastrocnemius; SOL, soleus; TA, tibialis anterior; EDL, extensor digitorum longus; FDL, flexor digitorum longus; EDB, extensor digitorum brevis; FHB, flexor hallucis brevis.

Figure 2. Maps of hindlimb primary motor cortex organization in two monkeys (F and C), represented in two dimensions after unfolding the medial wall and central sulcus. Maps of hindlimb muscles were based on PSTF effects at 15, 30 and 60 μA. If no effects were obtained with stimulus-triggered averaging at 60 μA, HFLD-ICMS was used to evoke movements at joints whose muscles were not implanted with EMG electrodes. HFLD-ICMS was performed at 15, 30 and 60 μA. For clarity, evoked movements from HFLD-ICMS are represented only in the 60 μA maps. Cutaneous responses of cortical neurons were used to identify boundaries with sensory areas. Cortical areas producing hindlimb muscle facilitation are represented in blue. Black dots are sites that produced a poststimulus effect; open circles are sites that did not produce poststimulus effects in the hindlimb muscles tested but were tested with HFLD-ICMS. For mapping, an effect in only one muscle was considered sufficient to identify the site as hindlimb. Light blue line is the midline. Above the light blue line represents the bank of the medial wall of the hemisphere. Solid black curved line is the central sulcus. Dotted black curved line is the fundus of the central sulcus. Solid purple line is the superior precentral sulcus. Solid red line is the arcuate sulcus. Solid orange line represents the posterior border of the supplementary motor area. Intersection of the grid lines represents the center of the recording chamber. ANT: anterior. POST: posterior. MED: medial. LAT: lateral.

Figure 3. Distribution of PSTF onset latencies for muscles at the hip, knee, ankle, digit and intrinsic foot joints at 15, 30 and 60 μA stimuli. The values given in parentheses for each graph represent the means ± SD of the onset latency of the PSTF.

Figure 4. Distribution of PSTF magnitudes for muscles at the hip, knee, ankle, digit and intrinsic foot joints at 15, 30 and 60 μA stimuli. The magnitudes are expressed as peak percent increase (ppi) above
Cortical output to hindlimb muscles

baseline. The values given in parentheses for each group represent the means ± SD of the magnitude of
the PStF.

Figure 5. Distribution of PStF (A) and PStS (B) in hip, knee, ankle, digit and intrinsic foot muscles at 15, 30 and 60 μA stimuli. Distribution of PStF (C) and PStS (D) after normalizing for the number of
recorded muscles at each joint (dividing the total number of effects obtained by the number of muscles
recorded at each joint).

Figure 6. Distribution of PStF (A) and PStS (B) in extensor and flexor muscles of the hip, knee, ankle, digit and intrinsic foot joints at 15, 30 and 60 μA stimuli. Distributions have been normalized for the
number of recorded muscles at each joint.

Figure 7. Distribution of PStF (right) and PStS (left) obtained from 19 muscles of the hindlimb. Dotted
lines separate muscles belonging to different muscle groups (joints). Muscle abbreviations are the same
as in Figure 3. Data show the total number of effects obtained and is not normalized for the number of
muscles of each group recorded.
Cortical output to hindlimb muscles

TABLES

Table 1. Summary of data collected

<table>
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<tr>
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<td>312</td>
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<tr>
<td>HFLD-ICMS sites*</td>
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<td>41</td>
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<td>Stimulus-triggered averaging</td>
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<td>Sites stimulated (all)</td>
<td>130</td>
<td>152</td>
<td>282</td>
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<td>StTA records (all)</td>
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<td>2692</td>
<td>5162</td>
</tr>
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<td>Layer V sites†</td>
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<td>142</td>
<td>259</td>
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<td>StTA records †</td>
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<td>2524</td>
<td>4747</td>
</tr>
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<td>Sites yielding PSTEs†</td>
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<td>180</td>
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<td>344</td>
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<td>6328</td>
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<td>5789</td>
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<td>6328</td>
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<tr>
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</tr>
<tr>
<td>60 μA</td>
<td>4747</td>
<td>2670</td>
<td>5843</td>
</tr>
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</table>

PStE, poststimulus effect; PStF, poststimulus facilitation; PStS, poststimulus suppression (PStS only counted if it was the first effect. Suppression preceded by facilitation was not counted as PStS)

* 500 ms train, 200 Hz, 15-60 μA. For testing sites outside the hindlimb representation.
† Putative layer V sites identified based on criteria given in the text.
Table 2. Latency and magnitude of PStEs

**A. Effects present across all three stimulus intensities (15, 30 and 60 μA)**

<table>
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<th>PStF</th>
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<th>Magnitude, %</th>
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<tr>
<td></td>
<td>15 μA</td>
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<tr>
<td>Muscle</td>
<td>n</td>
<td>Mean n Mean</td>
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<tr>
<td>Hip</td>
<td>24</td>
<td>12.6 ± 1.8</td>
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<tr>
<td>Knee</td>
<td>54</td>
<td>13.8 ± 1.4</td>
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<tr>
<td>Ankle</td>
<td>34</td>
<td>16.8 ± 2.1</td>
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<tr>
<td>Digit</td>
<td>8</td>
<td>16.1 ± 1.0</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>58</td>
<td>17.1 ± 0.9</td>
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<tr>
<td>Total</td>
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<td>15.4 ± 2.3</td>
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</table>

<table>
<thead>
<tr>
<th>PStS</th>
<th>Onset Latency, ms</th>
<th>Magnitude, %</th>
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<td></td>
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<td>30 μA</td>
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<tr>
<td></td>
<td>15 μA</td>
<td>30 μA</td>
</tr>
<tr>
<td>Muscle</td>
<td>n</td>
<td>Mean n Mean</td>
</tr>
<tr>
<td>Hip</td>
<td>1</td>
<td>15.0</td>
</tr>
<tr>
<td>Knee</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Ankle</td>
<td>9</td>
<td>17.6 ± 1.2</td>
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<tr>
<td>Digit</td>
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<tr>
<td>Intrinsic</td>
<td>2</td>
<td>19.0 ± 0.7</td>
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<tr>
<td>Total</td>
<td>12</td>
<td>17.6 ± 1.4</td>
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**B. All effects**

<table>
<thead>
<tr>
<th>PStF</th>
<th>Onset Latency, ms</th>
<th>Magnitude, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td>30 μA</td>
</tr>
<tr>
<td>Muscle</td>
<td>n</td>
<td>Mean n Mean</td>
</tr>
<tr>
<td>Hip</td>
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<td>12.9 ± 1.8</td>
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<tr>
<td>Knee</td>
<td>64</td>
<td>14.0 ± 1.6</td>
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<tr>
<td>Ankle</td>
<td>44</td>
<td>17.2 ± 2.6</td>
</tr>
<tr>
<td>Digit</td>
<td>11</td>
<td>16.2 ± 1.1</td>
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<tr>
<td>Intrinsic</td>
<td>60</td>
<td>17.1 ± 0.9</td>
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<tr>
<td>Total</td>
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<td>15.5 ± 2.5</td>
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<table>
<thead>
<tr>
<th>PStS</th>
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<th>Magnitude, %</th>
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<tbody>
<tr>
<td></td>
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<td>30 μA</td>
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<tr>
<td></td>
<td>15 μA</td>
<td>30 μA</td>
</tr>
<tr>
<td>Muscle</td>
<td>n</td>
<td>Mean n Mean</td>
</tr>
<tr>
<td>Hip</td>
<td>6</td>
<td>18.1 ± 3.8</td>
</tr>
<tr>
<td>Knee</td>
<td>10</td>
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<tr>
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<tr>
<td>Intrinsic</td>
<td>11</td>
<td>20.8 ± 1.5</td>
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<td>Total</td>
<td>50</td>
<td>19.4 ± 3.1</td>
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Values are mean ± SD. %, peak percent change from baseline. PStE, poststimulus effect. PSiF, poststimulus facilitation. PStS, poststimulus suppression.
Cortical output to hindlimb muscles

<table>
<thead>
<tr>
<th>Table 3. Comparison of M1 hindlimb and forelimb StTA (15 μA) data.</th>
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<td><strong>Magnitude of PStF (ppi)</strong></td>
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<tr>
<td>Hip/Shoulder</td>
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<td>Knee/Elbow</td>
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<tr>
<td>Ankle/Wrist</td>
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<tr>
<td>Digit</td>
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<td>Intrinsic Foot/Hand</td>
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<table>
<thead>
<tr>
<th>Onset Latency of PStF (ms)</th>
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<tr>
<td>Hip/Shoulder</td>
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<td>Knee/Elbow</td>
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<tr>
<td>Ankle/Wrist</td>
</tr>
<tr>
<td>Digit</td>
</tr>
<tr>
<td>Intrinsic Foot/Hand</td>
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<table>
<thead>
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<th>Distribution of PStF by joint*, %</th>
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<td>Hip/Shoulder</td>
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<tr>
<td>Ankle/Wrist</td>
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<tr>
<td>Digit</td>
</tr>
<tr>
<td>Intrinsic Foot/Hand</td>
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</tbody>
</table>

<table>
<thead>
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<th>Distribution of PSIS by joint*, %</th>
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<td>Ankle/Wrist</td>
</tr>
<tr>
<td>Digit</td>
</tr>
<tr>
<td>Intrinsic Foot/Hand</td>
</tr>
</tbody>
</table>

Parentheses give number of observations. Magnitude expressed as mean ppi ± SD.
* data normalized by number of recorded muscles.
† data adapted from Park et al., 2004.
### Table 1

<table>
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<tr>
<th>Muscle</th>
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<th>Resting Current</th>
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<td>15 mA</td>
<td>0-200 mV</td>
<td>5-20 ms</td>
</tr>
<tr>
<td>EDB</td>
<td>15 Hz</td>
<td>20 mA</td>
<td>0-300 mV</td>
<td>5-20 ms</td>
</tr>
</tbody>
</table>

### Figure 1

The figure shows the response of various muscles to electrical stimulation. The muscles are categorized into INTRINSIC, DIGIT, ANKLE, KNEE, and HIP. Each muscle is represented by a waveform graph. The graphs are labeled with conditions such as "22F1, 15Hz, 15uA" and "22F1, 15Hz, 30uA." The number of trials for each condition is indicated in the graphs. For example, the graph labeled "ppi = 10" shows the response with a particular stimulation protocol.

- **INTRINSIC**: FHB, EDB
- **DIGIT**: FDL, EDL
- **ANKLE**: TA, SOL, LG, MG, PERL
- **KNEE**: SET, SEM, BFL, VM, VL, RF
- **HIP**: TFL, GRA, ADB, GMAX

The graphs include annotations such as "*" and numbers indicating significant responses or trial counts.
Figure 2

Poststimulus Facilitation

15 μA

30 μA

60 μA

Monkey F

Monkey C

Stimulus-Triggered Averaging

Hindlimb
PSfE
No PSfE
HFLD-ICMS
Forelimb
Tail
Trunk
Sensory
CS
CS (fundus)
Midline
1 mm

15/280
30/280
60/280

ANT
MED
POST
LAT
Figure 3

15 μA

30 μA

60 μA

Latency (ms)

Number of PStF

(12.7 ± 1.9)

(13.2 ± 2.6)

(13.3 ± 2.0)

(14.0 ± 1.7)

(13.9 ± 1.5)

(14.0 ± 1.8)

(17.6 ± 3.1)

(16.1 ± 2.3)

(16.0 ± 2.6)

(16.0 ± 1.0)

(15.4 ± 1.3)

(15.2 ± 1.5)

(12.7 ± 1.9)

(13.2 ± 2.6)

(13.3 ± 2.0)

(17.2 ± 0.8)

(17.3 ± 1.5)

(17.1 ± 1.2)

(15.8 ± 2.6)

(15.3 ± 2.4)

(15.0 ± 2.3)
Figure 4

**15 µA**

- Hip: $23.0 \pm 7.1$
- Knee: $22.2 \pm 11.5$
- Ankle: $25.7 \pm 14.8$
- Digit: $20.7 \pm 4.2$
- Intrinsic: $40.0 \pm 30.6$
- All Muscles: $29.6 \pm 22.3$

**30 µA**

- Hip: $26.4 \pm 11.9$
- Knee: $25.9 \pm 13.9$
- Ankle: $26.2 \pm 14.0$
- Digit: $20.7 \pm 6.2$
- Intrinsic: $50.6 \pm 35.3$
- All Muscles: $31.6 \pm 23.5$

**60 µA**

- Hip: $31.7 \pm 20.3$
- Knee: $34.9 \pm 21.8$
- Ankle: $32.8 \pm 20.1$
- Digit: $29.3 \pm 11.8$
- Intrinsic: $70.0 \pm 60.7$
- All Muscles: $39.6 \pm 34.5$
Figure 5

A. Facilitation

B. Suppression

C. Number of PStF / Muscle

D. Number of PStS / Muscle

Legend:
- Black: 15 µA
- Gray: 30 µA
- White: 60 µA
Figure 6

A. Facilitation

B. Suppression

C. 30 μA

D. 60 μA

E. 60 μA

F. 60 μA
Figure 7

Number of PStEs at 15 μA

Number of PStEs at 30 μA

Number of PStEs at 60 μA

Intrinsic

Digit

Ankle

Knee

Hip

GMAX

ADB

GRA

TFL

RF

VL

VM

BFL

SEM

PERL

MG

LG

SOL

TA

EDL

FDL

EDB

FHB

Number of PStF

Number of PStS

Number of PStF

Number of PStS

Number of PStS

Extensors

Flexors