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Increase in flexor but not extensor corticospinal motor outputs following ischemic nerve block.

Ann-Maree Vallence [1]

Geoffrey R. Hammond [1]

Karen T. Reilly [2, 3]

[1] School of Psychology, The University of Western Australia

[2]INSERM U1028, CNRS UMR5292, Lyon Neuroscience Research Center, ImpAct Team, Lyon, France

[3] University of Lyon 1, Lyon, France

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Correspondence to:

Ann-Maree Vallence

Email: annmaree.vallence@gmail.com

Address: School of Psychology (M304)

University of Western Australia

35 Stirling Highway, Crawley WA 6009

Ph: +61 8 6488 3719

Fax: +61 8 6488 1006

Abstract

The human motor cortex is capable of rapid and long-lasting reorganization. This reorganization is evident globally, in the form of shifts in body part representations, and at the level of individual muscles in the form of changes in corticospinal excitability. Representational shifts provide an overview of how various body parts reorganize relative to each other but do not tell us whether all muscles in a given body part reorganize in the same manner and to the same extent. Transcranial magnetic stimulation (TMS) provides information about individual muscles and can therefore inform us about the uniformity of plastic changes within a body part. Here, we used TMS to investigate changes in corticospinal excitability of forearm flexors *and* extensors after inflation of a tourniquet around the wrist. Motor evoked potential (MEP) amplitudes and input/output (I/O) curves were obtained from wrist flexors and extensors simultaneously before and during block. TMS was delivered to the optimal site for eliciting MEPs in the *flexors* in Experiment 1, the *extensors* in Experiment 2, and both the flexors *and* extensors in Experiment 3. In all experiments flexor MEP amplitude increased during block while extensor MEP amplitude showed no systematic change, and the slope of flexor but not extensor I/O curves increased. Flexor H-reflex amplitude normalised to the maximal M-wave showed negligible changes during block, suggesting that the increase in corticospinal excitability in the flexors cannot be completely explained by increased excitability at the level of the spinal cord. These findings show that forearm flexors and extensors differ in their potential for plastic changes, and highlight the importance of investigating how experimentally-induced plasticity affects anatomically close, but functionally distinct, muscle groups. It also suggests that rehabilitation interventions that aim to alter cortical organization should consider the differential sensitivity of various muscle groups to plasticity processes.

1. Introduction

It is well established that the human primary motor cortex (M1) is capable of both rapid, reversible plastic changes and longer-term, more permanent reorganization. Studies of interventions that either permanently or temporarily affect the periphery, for example amputation, limb replantation, limb transplantation, and ischemic nerve block, show that such interventions induce rapid, substantial, and sometimes long-lasting plasticity of the human motor cortex (e.g. Brasil-Neto et al. 1992; Cohen et al. 1991; Eickhoff et al. 2008; Vargas et al. 2009). This plasticity occurs both at a representational level, e.g. shifts in the location of the hand's representation after amputation and subsequent hand transplant (Giraux et al. 2001), as well as at the level of individual muscles, e.g. increased excitability of the cortical representation of the biceps after amputation above the elbow or ischemic nerve block (INB) applied at the elbow (Brasil-Neto et al. 1993; Röricht et al. 1999; Ziemann et al. 1998a).

While shifts in body part representations provide a global overview of how various body parts reorganize relative to each other they are unable to tell us whether all muscles in a given body part reorganize in the same manner and to the same extent. Since transcranial magnetic stimulation (TMS) can provide specific information about individual muscles it has the capacity to inform us about the uniformity of plastic changes within a given body part. Despite this, TMS studies investigating motor maps after either amputation or ischemic nerve block in the upper limb have only examined individual muscles. Various studies have demonstrated increased excitability in one stump muscle after amputation or one muscle proximal to an INB (Brasil-Neto et al. 1992; Brasil-Neto et al. 1993; Cohen et al. 1991; Irlbacher et al. 2002; McNulty et al. 2002; Ridding and Rothwell 1995, 1997; Röricht et al. 1999; Schwenkreis et al. 2001; Ziemann et al. 1998a), but no information is available about simultaneous changes in different muscle groups (see Table 1 for a list of muscles examined). This is important, as the cortical control of anatomically close but functionally different

muscle groups is not identical, suggesting that distinct muscle groups could be differentially affected by plastic changes.

The control of the flexors and extensors of the forearm differs in several ways: flexion-based movements, like precision grip and whole-hand grasp, are more frequently executed, and require finer force control and independence than extension movements, like releasing a precision grip or opening the hand in order to grasp an object (Oliveira et al. 2008; Schieber 1991; Shim et al. 2007; Yu et al. 2010). There are also differences in the corticospinal control of each muscle group. Brain stimulation studies in humans suggest that there are stronger monosynaptic connections to wrist and finger extensors than flexors (Maertens de Noordhout et al. 1999; Palmer and Ashby 1992), and numerous spike- and stimulus-triggered averaging studies in non-human primates report flexion/extension differences in corticomotoneuronal (CM) connections to forearm muscles. These differences include more and stronger facilitation effects in extensors (Clough et al. 1968; Fetz and Cheney 1980; Kasser and Cheney 1985; McKiernan et al. 1998; Park et al. 2004; Park et al. 2001; Phillips and Porter 1964), more suppression effects in wrist flexors than extensors (Kasser and Cheney 1985; Park et al. 2004), but fewer suppression effects in digit flexors than extensors (Park et al. 2004). Furthermore, the rate-torque slope of extension CM cells is approximately twice that of flexion cells, meaning that for a given increment in firing rate, flexion cells produce a greater increase in torque than extension cells (Cheney and Fetz 1980; Evarts 1968, 1969).

To investigate whether forearm flexors and extensors are differentially affected by a protocol that induces short-term plasticity of the motor cortex we inflated a tourniquet around the wrist and investigated the time course of changes in corticospinal excitability of both forearm flexors *and* extensors. The aim of Experiment 1 was to establish the time course of short-term corticospinal excitability changes in forearm muscles using flexor-appropriate

TMS parameters. In this experiment we recorded motor evoked potentials (MEPs) simultaneously from forearm flexors and extensors while the tourniquet was inflated for 20 minutes. The results showed a very rapid increase in the corticospinal excitability of the flexors but not the extensors, suggesting a differential effect of INB on these two muscle groups. To test whether this effect resulted from stimulating with flexor-appropriate parameters and/or from deflating the cuff too soon we conducted a second experiment in which we used extensor-appropriate TMS parameters and kept the tourniquet inflated for an additional 10 minutes (30 instead of 20 minutes total inflation time). Consistent with the results of Experiment 1, we found that INB increased the excitability of forearm flexors but did not alter the excitability of forearm extensors. As the first two experiments used independent samples (except for one subject who participated in both experiments), and since the flexor and extensor hotspots are very close within the human motor cortex, we conducted a third experiment using a within-subject design to investigate whether stimulating with flexor-appropriate and extensor-appropriate parameters within the same motor cortex would replicate the results of the first two experiments. Consistent with the results of Experiments 1 and 2, regardless of whether stimulation was applied over the flexor or extensor hotspot, excitability increased in the flexors but not the extensors.

2. Materials and methods

2.1 Subjects

Experiment 1 tested 17 right-handed healthy adults (14 females) with age ranging from 19 to 28 years (median age 23 years) and Experiment 2 tested 19 right-handed healthy adults (14 females) with age ranging from 18 to 28 years (median age 21 years) including one subject who had participated in Experiment 1. An independent sample of six right-handed healthy adults (three females) with age ranging from 24 to 37 years (median age 28 years)

were tested in Experiment 3 (in which we examined flexor and extensor excitability within the same subjects as well as H-reflexes and maximal M-waves) and an independent sample of 14 right-handed healthy adults (10 females) with age ranging from 18 to 41 years (median age 24 years) were tested in Experiment 3a (in which we measured H-reflexes). The protocol was in accordance with the Declaration of Helsinki and was approved by The University of Western Australia Human Research Ethics Committee. All subjects gave written informed consent prior to testing.

2.2 Ischemic Nerve Block

Subjects sat comfortably in a slightly reclined position with their neck and head supported and their right forearm resting comfortably on a table in a semi-pronated position with their elbow slightly flexed. The forearm, wrist, and hand were stabilised using a vacuum cast, a sealed plastic bag filled with polystyrene balls was first molded around the forearm and then had the air extracted to secure the arm in position. Nerve block in the upper-limb is normally achieved by inflating the cuff to at least 50 mm Hg above systolic arterial blood pressure (which in young healthy adults is on average 110 to 130 mm Hg). The majority of studies investigating INB-induced excitability changes apply the tourniquet across the elbow and examine changes in upper-limb muscles, but because we were interested in examining the possible differential affect of INB on forearm flexors and extensors we placed a pediatric blood pressure cuff immediately proximal to the wrist. The cuff was inflated to 200 mm Hg for 20 minutes in Experiment 1, 180 mm Hg for 30 minutes in Experiments 2 and 3, and 180 mm Hg for 15 minutes in Experiment 3a.

We chose to inflate the cuff for 30 minutes in Experiments 2 and 3 (as opposed to 20 minutes in Experiment 1) to determine if changes in excitability of the extensor representations took longer to develop than changes in the flexor representations. We also

reduced the inflation pressure to make the procedure more comfortable for the subjects, as a pilot study showed no difference in the excitability changes induced by inflation pressures of 170 and 200 mm Hg. The duration of INB in Experiment 3a was limited to 15 minutes because flexor excitability increases occur rapidly (less than 10 minutes after cuff inflation) when flexor-optimized stimulation parameters are used. INB has been used in this manner for related studies in numerous other laboratories for more than 15 years without any complications (Brasil-Neto et al. 1993; Han et al. 2008; McNulty et al. 2002; Reilly et al. 2008; Ziemann et al. 1998a; Ziemann et al. 2002), and the cuff inflation times used here are well within the medical guidelines for the use of INB (AORN-Recommendations 2007).

2.3 TMS

Electromyographic (EMG) activity was recorded from the relaxed forearm flexors and forearm extensors with Ag-AgCl electrodes placed in a belly-tendon configuration. The EMG signal was amplified (1000x), band pass filtered (10-1000 Hz), and digitized at 4000 Hz for 100 ms beginning 50 ms before the TMS pulse was delivered. A Magstim 200 stimulator generated single-pulse stimuli, delivered through a figure-of-eight coil (90 mm diameter) placed tangentially to the scalp with the handle pointing backward and at a 45° angle away from the midline. Suprathreshold pulses were delivered over the left M1 at a number of sites in order to identify the optimal site for stimulation of the flexors in Experiment 1, the extensors in Experiment 2, and both the flexors *and* extensors in Experiment 3. The optimal site was defined as the site at which five successive suprathreshold pulses produced the largest mean MEP amplitude in the target muscle and all TMS pulses were applied at this site throughout the experiment. A cap marked with the International 10-20 system was used to identify the optimal site and to ensure the precision of coil placement within a testing session. Note that Experiments 1 and 2 were conducted on independent samples (except one subject

who participated in both experiments) and TMS was delivered to different sites in each experiment (flexor or extensor hotspot) whereas in Experiment 3 TMS was delivered to the flexor and extensor hotspot of the same subjects in a fully within-subject design.

The TMS intensity for single pulses was set as the intensity that elicited a MEP of approximately 1mV (SI_{1mV}) in the target muscle at that muscle's hotspot; Experiment 1 - flexors at flexor hotspot; Experiment 2 – extensors at extensor hotspot; Experiment 3; flexors at flexor hotspot and extensors at extensor hotspot. The input/output (I/O) curves in Experiments 1 and 2 were obtained from six blocks of seven single TMS pulses of different stimulus intensities (50, 70, 90, 100, 110, 130, and 150% of SI_{1mV}). In each block a single pulse at each of the seven intensities was presented in random order with randomly selected between-pulse intervals of five, six, or seven seconds. For all three experiments the intensities used for single pulses and I/O curves were determined before cuff inflation (based on SI_{1mV}) and did not change throughout the experiment.

2.4 Cutaneous Sensitivity

Calibrated von Frey filaments were used to measure cutaneous sensitivity of the hand during INB. Stimuli were presented at the tip and the base of the index finger (five stimuli at each site) in randomised order, beginning with the finest filament. Stimuli were presented every 3 seconds and subjects were required to identify the stimulus location (i.e. 'tip' or 'base'); no feedback was given. If more than half of the stimuli were correctly detected the same size filament was used for the next measurement block. If half or fewer of the stimuli were correctly detected the next largest filament was used until more than half of the stimuli were correctly detected; the same filament size was then used for the next measurement block. Cutaneous sensitivity was measured at least 30 seconds after TMS in each measurement period.

2.5 Maximum Voluntary Contraction

In order to track changes in efferent transmission to the hand as a result of the INB EMG activity was measured from FDI during a maximum voluntary contraction pinch of the thumb and index finger. EMG activity was recorded from FDI for 1500 ms beginning immediately after a visual signal to produce a pinch. The visual response signal was presented on a screen in front of the subject to signal the start of each voluntary pinch and removed 1500 ms later to signal the end of each pinch. The pinch was repeated three times and the root mean square of the EMG activity during the 1500 ms trial was calculated and averaged across the three trials. Maximum voluntary contraction was measured after cutaneous sensitivity in each measurement period.

2.6 Procedure

2.6.1   Experiment 1

A pediatric blood pressure cuff was placed around the right forearm immediately proximal to the wrist and inflated to 200 mm Hg for 20 minutes. Measurements of MEP amplitude, cutaneous sensitivity, and maximum voluntary contraction were obtained at four time points: before cuff inflation (baseline), and at five (T5), ten (T10), and twenty minutes (T20) after cuff inflation. The mean MEP amplitude in the flexors and extensors was obtained at each time point from blocks of twelve single TMS pulses delivered to the optimal site for stimulation of the *flexors* at a constant intensity that produced a MEP of 1mV in the flexors at baseline. Pulses were delivered at randomly selected between-pulse intervals of five, six, or seven seconds. I/O curves were also obtained at baseline and T20. Note that it is valid to simultaneously record from multiple muscles, particularly when they are within the same body segment, as TMS thresholds roughly follow a proximo-distal gradient with lower thresholds in more distal muscles. Indeed, several reports exist in which MEPs were reliably

recorded from up to 12 upper-limb muscles simultaneously (Krings et al. 1998; Melgari et al. 2008; Wassermann et al. 1992).

A control condition in which the cuff was inflated to 60 mm Hg was tested in 12 subjects (including nine who participated in the experimental condition). The control pressure was well below that required to induce nerve block but applied cutaneous stimulation at the wrist similar to that applied during the 200 mm Hg condition. The control condition was always tested after the 200 mm Hg condition.

2.6.2   Experiment 2

The procedure for Experiment 2 was the same as Experiment 1 except the cuff was inflated to 180 mm Hg for 30 minutes, and the extensor (not the flexor) hotspot was stimulated. Measurements of MEP amplitude, cutaneous sensitivity, and maximum voluntary contraction were obtained at four time points: before cuff inflation (baseline), and at ten (T10), twenty (T20), and thirty minutes (T30) after cuff inflation. The mean MEP amplitude in the flexors and extensors was obtained at each time point from blocks of ten single TMS pulses delivered to the optimal site for *extensor* stimulation at a constant intensity that produced a MEP of 1mV in the extensors at baseline. Pulses were delivered at randomly selected between-pulse intervals of five, six, or seven seconds. I/O curves were obtained at baseline and T30.

2.6.3   Experiment 3

In this experiment MEP amplitudes were measured simultaneously in the flexors and extensors following stimulation with flexor-optimized parameters at the flexor hotspot and extensor-optimized parameters at the extensor hotspot in a fully within-subject design. The tourniquet was inflated to 180 mm Hg for 30 minutes as the results of Experiment 2 showed a

significant increase in flexor MEP amplitude 30 minutes after inflation of the tourniquet when stimulation parameters were optimized for the extensors. Measurements of MEP amplitude were obtained at four time points: before inflation (baseline), and at ten (T10), twenty (T20), and thirty minutes (T30) after inflation. At each time point, TMS was delivered to the optimal site for flexor stimulation at a constant intensity that produced a MEP of 1 mV in the flexors at baseline *and* to the optimal site for extensor stimulation at a constant intensity that produced a MEP of 1 mV in the extensors at baseline. The order of stimulation (flexor-optimized parameters and extensor-optimized parameters) was counterbalanced across subjects. The mean MEP amplitude in the flexors and extensors was obtained at each time point from blocks of ten single TMS pulses delivered using the flexor-optimized parameters and ten single TMS pulses delivered using the extensor-optimized parameters. Pulses were delivered at randomly selected between-pulse intervals of five, six, or seven seconds.

It is generally assumed that INB-induced changes in MEP amplitude have a cortical origin, but the evidence for this comes from a single study of INB in the lower limb (Brasil-Neto et al., 1993). To investigate whether there was a spinal contribution to the INB-induced excitability changes we observed in upper-limb muscles we also measured flexor H-reflex and M-wave amplitudes. We did not record from the extensors because we observed no increase in corticospinal excitability in the extensors in Experiments 1 and 2, and because H-reflexes are extremely difficult to elicit in forearm extensors while the muscle is at rest (Cowan et al. 1986; Zehr 2002).

The H-reflex and maximal M-wave were obtained by delivering constant-current square wave pulses (duration 0.5 ms) to the median nerve through bipolar electrodes; the cathode was placed approximately 3 cm proximal to the antecubital fossa and the anode was placed approximately 2 cm proximal to the cathode (Lamy et al. 2010). Electrical stimuli

were delivered at increasing intensities (in 10 mA steps starting below the threshold for eliciting both M-waves and H-reflexes) until the maximal M-wave was observed; the point at which peak-to-peak M-wave amplitude reached a plateau (M_{MAX}). Four stimuli were delivered at each stimulus intensity with between-pulse intervals of five seconds. The intensity that elicited the largest H-reflex amplitude for each subject at baseline was used to measure the H-reflex at both baseline and T30. The time taken to obtain the maximal M-wave and the uncomfortable nature of the electrical stimulation meant that M_{MAX} could not be examined at 10 minute intervals (as with MEP amplitude), so M_{MAX} and H-reflex amplitudes were obtained only at baseline and T30. Furthermore, because the intensities required to reach M_{MAX} induced painful sensations in some subjects (which would have interfered with our measures of cortical excitability since pain inhibits both spinal and cortical excitability (Inghilleri et al. 1995; Dube and Mercier 2011; Valeriani et al. 1999; Urban et al. 2004)), we were only able to obtain M_{MAX} data from three subjects (MEP amplitudes were obtained from all six subjects).

Given the relatively small sample from which H-reflexes and M_{MAX} data could be obtained, and the importance of investigating the potential contribution of changes in spinal excitability to the increased corticospinal excitability evident in the flexors during INB, we conducted an additional experiment in which we measured flexor H-reflex amplitude (but not M_{MAX}; Experiment 3a). For Experiment 3a the cuff was inflated to 180 mm Hg for a total of 15 minutes and flexor MEP amplitudes and H-reflexes were measured before and 15 minutes after cuff inflation (T15). For each time point, mean MEP amplitude in the forearm flexors was obtained from blocks of 12 single TMS pulses delivered to the optimal site for flexor stimulation at an intensity adjusted to produce a MEP of about 1mV in the flexors at baseline. Pulses were delivered at randomly selected between-pulse intervals of five, six, or seven seconds. Mean H-reflex amplitude was obtained from 12 constant-current square wave pulses

(duration 1 ms) using the same procedure as outlined in Experiment 3 (above) with the exception that the electrical stimulus intensity was adjusted for each subject to evoke an H-reflex amplitude that was 50% of the maximum H-reflex amplitude measured at baseline and this same intensity was used at T15.

2.7 Data Analysis

The peak-to-peak MEP amplitude in millivolts (mV) was obtained from the 40 ms of EMG activity beginning 10 ms after the pulse was delivered. The mean was used as the measure of central tendency for all analyses. Measurements of cutaneous sensitivity were analysed using Kruskal-Wallis one-way ANOVAs. A repeated-measures ANOVA was used to analyse the maximum voluntary contraction data for FDI. Each subject's mean MEP amplitudes obtained from the blocks of single TMS pulses following inflation of the tourniquet were expressed as a ratio of that subject's mean MEP amplitude obtained at baseline. The ratios were log transformed prior to analysis to normalize the distributions, and back transformed means are reported. One-sample t-tests comparing the log-transformed ratios to zero (baseline) were performed with Bonferroni-adjusted alpha levels for multiple comparisons. Repeated-measures ANOVA was used to analyse the I/O curve data, with data from flexors and extensors always analysed separately. Greenhouse-Geisser corrections were used for analyses in which the assumption of sphericity was violated. I/O curves were fitted with linear regression through the five middle stimulus intensities (0.7, 0.9, 1.0, 1.1, and 1.3 x SI_{1mV}) to obtain the slope of each individual's curve (Rosenkranz et al. 2007b). Paired-samples t-tests were performed on the slopes at baseline and T20 for both muscles in Experiment 1, and at baseline and T30 for both muscles in Experiment 2.

In Experiments 3 and 3a the peak-to-peak M-wave amplitude (in mV) was obtained from the EMG activity beginning 2 ms after the electrical stimulus was delivered (visual

inspection of each EMG trace was conducted to ensure no stimulus artefact or H-reflex was included in the peak-to-peak scoring) and the peak-to-peak H-reflex amplitude (in mV) was obtained from the EMG activity between 8 and 20 ms after the electrical stimulus was delivered (visual inspection of each EMG trace was conducted to ensure that H-reflex amplitude measures were not contaminated by the M-wave (see Figure 7 in the Results for raw EMG traces of M-waves and H-reflexes). In Experiment 3, each subject's mean H-reflex amplitude was expressed as a ratio of their M_{MAX} (H/M_{MAX} ratio), and the difference between these two ratios at baseline and T30 was calculated for each subject. In Experiment 3a, we examined the percentage change in both H-reflex and M-wave amplitudes between baseline and T15. Each subject's mean H-reflex and M-wave amplitude (the small M-wave elicited when the H-reflex was tested) obtained at T15 were expressed as a ratio of their mean H-reflex and M-wave amplitude obtained at baseline. One-sample t-tests were used to compare the log-transformed ratios to zero (baseline) and correlational analyses were performed on the M-wave and H-reflex amplitude ratios as well as the MEP and H-reflex amplitude ratios. Within-subject error bars are presented in all figures (Loftus and Masson 1994). All analyses were performed using SPSS version 18.0.

In all experiments individual trials were excluded if the root mean square of EMG activity in the 40 ms prior to the TMS pulse exceeded 50μV and subjects were excluded from analyses if more than half of the trials in a measurement block were excluded due to excessive pre-TMS EMG. In Experiment 1, one subject was excluded from both extensor analyses (MEPs and I/O curves) in the 200 mm Hg and 60 mm Hg conditions; for the remaining subjects between 0 and 7 trials were excluded for any one subject from any one muscle, and three subjects were excluded from the extensor MEP and I/O curve analyses because of recording problems. In Experiment 2, one subject was excluded from the extensor

MEP analysis and five from the flexor MEP analysis; for the remaining subjects between 0 and 9 trials were excluded. In Experiment 3, between 0 and 7 trials were excluded.

For all included trials the root mean square (RMS) of EMG activity in the 50 ms immediately preceding each TMS pulse was calculated for both the flexors and the extensors and a repeated-measures analysis of variance (ANOVA) was used to test whether EMG levels changed across time (separate analyses for each muscle in each experiment). There was no systematic change in RMS EMG activity in either the flexors or the extensors in any of the experiments [Experiment 1: Flexors ($F(3,48)=0.08$, $P>.05$), Extensors ($F(3,36)=2.72$, $P>.05$); Experiment 2: Flexors ($F(3,39)=1.26$, $P>.05$), Extensors ($F(3,51)=0.32$, $P>.05$); Experiment 3: Flexor Stimulation Parameters: Flexors ($F(3,15)=0.75$, $P>.05$), Extensors ($F(3,15)=0.26$, $P>.05$); Extensor Stimulation Parameters: Flexors ($F(3,15)=2.11$, $P>.05$), Extensors ($F(3,15)=0.38$, $P>.05$); Experiment 3a: Flexors ($t(13)=0.33$, $P>.05$)].

In Experiment 2, 11 (out of 19) subjects were included in the extensor and flexor I/O curve analyses; eight subjects were excluded because their 1mV intensity was too high to permit us to obtain a complete I/O curve (the stimulus intensity for 150% of SI_{1mV} was greater than the maximum stimulator output).

3. Results

3.1 Experiment 1: TMS pulses delivered to the optimal site for flexor stimulation

Cutaneous sensitivity of the index finger decreased from baseline to T5 and then remained constant to T20, with a large and statistically significant effect of Time ($\chi2$ (3) = 17.15, $P<.05$). Despite the rapid decrease in cutaneous sensitivity following cuff inflation there was only a slight decrease in maximum voluntary contraction of FDI from baseline to T20 (a mean decrease of 9% from baseline), and the ANOVA revealed no significant effect of Time (F(3, 48) = 0.49, $P>.05$).

The mean SI_{1mV} intensity for the flexors (at the flexor hotspot) was 55% of maximum stimulator output. Figure 1 (left panel) shows the mean normalized MEP amplitude in both flexors and extensors following inflation of the tourniquet as well as raw EMG traces from one representative subject (right panel). A very rapid increase in flexor MEP amplitude was evident at T5 (mean increase of 33% from baseline) and further increases were evident at T10 and T20 (47% and 54% above baseline respectively) with normalized MEP amplitudes at all time points significantly greater than baseline (all $t(16)>2.83$, $P<.017$). In contrast to the flexors, extensor MEP amplitudes showed a non-systematic change following inflation of the tourniquet (mean increase of 2%, and mean decreases of 6% and 8% from baseline at T5, T10, and T20 respectively); none was significantly different from baseline at any time point (all $t(12)<0.4$, $P>.017$).

Figure 2 shows I/O curves from both of the muscles at baseline and T20. For both muscles MEP amplitude increased systematically with increasing stimulus intensity. At the five highest intensities the flexor I/O curve showed larger MEP amplitudes at T20 than at baseline, and a two-way repeated-measures ANOVA revealed a significant interaction of Time and Stimulus Intensity ($F(6, 96) = 13.2$, $P <.05$). Further, the slope of the best-fitting straight line through the middle five stimulus intensities in the flexor I/O curve was significantly steeper at T20 than at baseline ($t(16) = 5.2$, $P<.05$). In contrast, but consistent with the absence of an increase in extensor MEP amplitudes (Figure 1), no systematic change was observed in the extensor I/O curve between baseline and T20 (interaction of Time and Stimulus Intensity $F(6, 72) = 0.8$, $P>.05$) and there was no significant difference in slope at baseline and T20 ($t(12) = 0.4$, $P>.05$).

In the control condition (inflation pressure 60 mm Hg) cutaneous sensitivity of the index finger showed no change from baseline to T20. Maximum voluntary contraction of FDI showed a slight decrease from baseline to T20 but this was not systematic (mean decreases of

14%, 5%, and 12% from baseline at T5, T10, and T20 respectively) and there was no significant effect of Time ($F(3, 33) = 0.01$, $P>.05$). There was no systematic change in normalized MEP amplitude in either the flexors or extensors following inflation of the tourniquet in this condition [Flexors (all $t(11)<1.0$, $P>.017$); Extensors (all $t(10)<1.9$, $P>.017$)]. The I/O curves in Figure 3 show slightly larger MEP amplitudes in the flexors and extensors at the two highest stimulus intensities at T20 than baseline but the interaction of Time and Stimulus Intensity was not statistically significant for either the flexors ($F(6,66) = 1.6$, $P>.05$) or the extensors ($F(6, 60) = 1.5$, $P>.05$). The slope of the best-fitting straight line through the middle five stimulus intensities was not significantly different at T20 than at baseline in either the flexor or the extensor I/O curve (Flexors: $t(11)=2.1$, $P>.05$); Extensors: $t(10)=1.2$, $P>.05$).

3.2 Experiment 2: TMS pulses delivered to the optimal site for extensor stimulation

Cutaneous sensitivity of the index finger decreased from baseline to T30, with a large and statistically significant effect of Time ($\chi2(3) = 50.24$, $P<.05$). Maximum voluntary contraction of FDI decreased over time (mean decrease of 48% from baseline) with a significant effect of Time ($F(3, 54) = 6.1$, $P<.05$).

The mean SI_{1mV} intensity for the extensors (at the extensor hotspot) was 61% of maximum stimulator output. Figure 4 (left panel) shows the mean normalized MEP amplitude from both the flexors and extensors following inflation of the tourniquet as well as raw EMG traces from one representative subject (right panel). The normalized flexor MEP amplitude increased over time, with mean increases of 14%, 24%, and 55% from baseline at T10, T20, and T30 respectively. The increase in normalized MEP amplitude did not reach statistical significance at T10 ($t(13)=1.0$, $P>.017$) or T20 ($t(13)=1.5$, $P>.017$), but was significantly different from baseline at T30 ($t(13)=2.9$, $P<.017$). In contrast to the flexors,

extensor MEP amplitudes decreased following INB with a mean decrease of 19% from baseline, but this decrease was not significantly different from baseline at any time point (all $t(17)<2.1$, $P>.017$).

Figure 5 shows I/O curves from both of the muscles at baseline and T30. The flexor I/O curve shows that MEP amplitudes at the five highest stimulus intensities differed as a function of Time; MEP amplitudes were greater at T30 than baseline in the flexors and a two-way repeated measures ANOVA showed a significant interaction of Time and Stimulus Intensity ($F(6, 60) = 3.6$, $P<.05$). Further, the slope of the flexor I/O curve was steeper at T30 than baseline, with this difference just failing to reach significance ($t(10)= 2.1$, $P=.059$). As in Experiment 1, there was no systematic change in extensor I/O curves from baseline to T30, with no interaction of Time and Stimulus Intensity ($F(6, 60) = 0.7$, $P>.05$), and no difference in the slopes at baseline and at T30 ($t(10)= 1.0$, $P>.05$).

3.3 Experiment 3

MEP amplitude (flexor- and extensor-optimized parameters)

As the first two experiments used independent samples, and since the flexor and extensors hotspots are very close within the human motor cortex, here TMS was delivered to the flexor and extensor hotspot of the same subjects in a fully within-subject design. Five out of the six subjects showed a 1 cm difference between the two optimal stimulation sites; for three subjects the extensor site was 1 cm anterior to the flexor site, for one subject the extensor site was 1 cm posterior to the flexor site, and for one subject the extensor site was 1 cm lateral to the flexor site. The mean SI_{1mV} intensity was 66% of maximum stimulator output for the flexors (at the flexor hotspot) and 60% of maximum stimulator output for the extensors (at the extensor hotspot).

Figure 6 shows the mean normalized MEP amplitude for both the flexors and the extensors with flexor-optimized parameters (left) and extensors-optimized parameters (right) as well as raw EMG traces from one representative subject (bottom). Consistent with the results of Experiments 1 and 2, the normalized flexor MEP amplitude increased over time under both stimulation conditions. For the flexor-optimized parameters (Figure 6, top left panel), mean flexor MEP amplitude increased 56%, 75%, and 86% from baseline to T10, T20, and T30 respectively. The increase in normalized MEP amplitude was significantly different from baseline at all time points (all $t(5)>4.8$, $P<.05$). For the extensor-optimized parameters (Figure 6, top right panel), mean flexor MEP amplitude increased 35%, 64%, and 95% from baseline to T10, T20, and T30 respectively. The increase in normalized MEP amplitude did not reach statistical significance at T10 ($t(5)=1.9$, $P>.05$), consistent with the results of Experiment 2 (in an independent sample), but was significantly different from baseline at T20 and T30 (both $t(5)>3.8$, $P<.05$).

In contrast to the flexors, but consistent with the results of Experiments 1 and 2, extensor MEP amplitudes showed no systematic change following inflation of the tourniquet with either flexor- or extensor-optimized parameters (Figure 6); the change in extensor MEP amplitude (increase or decrease) was not significantly different from baseline at any time point (all $t(5)<1.7$, $P>.05$).

H/M$_{MAX}$ ratio

Table 2 shows flexor H-reflex and M$_{MAX}$ amplitudes and H/M$_{MAX}$ ratios at baseline and T30 for the three subjects in whom we could measure H-reflexes and M$_{MAX}$ and Figure 7 shows raw EMG traces from one subject showing the small M-wave and the H-reflex (top) as well as M$_{MAX}$ (bottom). The mean M$_{MAX}$ showed a small increase from baseline to T30; the mean M$_{MAX}$ at baseline was 5.62 mV and at T30 it was 6.02 mV (7% increase). H/M$_{MAX}$

ratios in each of these three subjects were similar at baseline and T30 and there was no relationship between change in H/M_{MAX} ratio and change in MEP amplitude in the flexors from baseline to T30 (change in H/M_{MAX} ratio : change in MEP amplitude – S_1; -10%:120%, S_2; 10%:43%, S_3; 8%:55%).

H-reflex

Flexor H-reflexes and MEP amplitudes were obtained at baseline and T15 from a sample of 14 subjects. The mean SI_{1mV} intensity for the flexors (at the flexor hotspot) was 60% of maximum stimulator output. Consistent with results from the previous three experiments, normalized MEP amplitude in the flexors increased from baseline to T15 by 93% ($t(13)= 8.3$, $P<.05$). In Experiment 3 the H/M_{MAX} ratio did not change from baseline to T30; here the flexor H-reflex amplitude increased significantly from baseline to T15 (mean increase of 25% from baseline; $t(13)= 2.7$, $P<.05$) but there was no significant relationship between the percentage change in H-reflex amplitude and the percentage change in MEP amplitude (from baseline to T15; $r=0.33$; 95% confidence interval: -0.24, 0.73; see Figure 8). Furthermore, three subjects showed increased MEP amplitudes and either no change or a reduction in H-reflex amplitude. In addition to the H-reflex we also measured the small M-wave (not the M_{MAX}) elicited when the H-reflex was measured (see Methods); the mean small M-wave amplitude increased by 20% from baseline to T15 and while this increase was not statistically significant ($t(12)=1.4$, $P>.05$) there was a significant positive relationship between the change in M-wave and H-reflex amplitude from baseline to T15 ($r=0.86$; see Figure 9).

4. Discussion

This is the first study to examine changes in corticospinal excitability of two anatomically close but functionally different muscle groups during a protocol that induces short term plastic changes in the motor system. In three separate experiments we found that INB at the wrist increased the excitability of forearm flexors but induced little or no change in the excitability of forearm extensors. We also found that the slope of the flexor but not the extensor I/O curve increased during INB. The increased excitability and steeper slope of the forearm flexors in the absence of a change in the forearm extensors did not depend upon the stimulation site, as stimulation at the optimal site for the flexors (Experiments 1 and 3) produced the same results as stimulation at the optimal site for the extensors (Experiments 2 and 3). The differential effect of INB on the excitability of forearm flexors and extensors irrespective of stimulation site was observed both in independent samples (Experiments 1 and 2) and within the same sample (Experiment 3). The rate at which the excitability increase occurred did, however, depend upon stimulation intensity. With a stimulation intensity that produced baseline flexor MEPs of approximately 1 mV (Experiments 1 and 3), the effect was present and significant at all post-inflation time points. With a stimulation intensity that produced baseline flexor MEPs of approximately 0.6 mV (i.e. an extensor-adjusted SI_{1mV}; Experiments 2 and 3), the effect was present at all post-inflation time points but the increase was not statistically significant at the first time point (T10) in Experiments 2 and 3 or at the second time point (T20) in Experiment 2.

It is unlikely that the INB-induced changes we observe arise from modifications in the efficacy of synaptic connections brought about by long term potentiation or depression, or from synaptogenesis or dendritic arborisation. They could, however, occur as a result of the activation of previously silent synapses, or by alterations in membrane excitability. Even though such changes probably do not involve plasticity at the level of the synapse, nor

morphological changes within neural networks, we and others (e.g. Classen and Ziemann 2003) believe that excitability changes reflect plasticity within the motor system. Numerous other studies also interpret short-term excitability changes as evidence for reorganization or plasticity (Butefisch et al. 2000; Classen et al. 1998; Rosenkranz et al. 2007a; Thickbroom et al. 2006; Ziemann et al. 1998a; Ziemann and Siebner 2008). As such, throughout the discussion we have interpreted our results in terms of motor system plasticity.

Spinal or Cortical Plasticity?

In order to better understand the source of the rapid increase in MEP amplitude observed in the flexors during INB, flexor MEP and H-reflex amplitudes were examined during INB. When H-reflex amplitude was normalized to M_{MAX} we observed no change in the H/M_{MAX} ratio from baseline to 30 minutes following inflation of the tourniquet. We also observed no significant relationship between the magnitude of the increase in H-reflex amplitude and the magnitude of the increase in flexor corticospinal excitability (Experiment 3a), and subjects with significant increases in MEP amplitude sometimes showed no change or a decrease in H-reflex amplitude (Experiment 3) or H/M_{MAX} ratio (Experiments 3). Together, these data suggest that changes in the excitability of spinal circuits cannot completely explain the increase in flexor MEP amplitude during INB.

Our results suggest that at least part of the INB-induced increase in corticospinal excitability likely occurs at the cortical level. This is consistent with a previous study showing that INB in the lower limb increases cortical but not spinal excitability (Brasil-Neto et al., 1993), and is coherent with the suggestion that the increase in corticospinal excitability is due to disinhibition of the primary motor cortex, probably via the unmasking of existing excitatory connections (Jacobs and Donoghue 1991; Sanes and Donoghue 2000; Ziemann et al. 1998a; Ziemann et al. 1998b). This idea is supported by the results of studies showing that

GABAergic inhibition plays a role in INB-induced plasticity (Werhahn et al. 2002; Levy et al. 2002). Lorazepam, a $GABA_A$ agonist, has been shown to suppress the INB-induced increase in corticospinal excitability (Werhahn et al. 2002), and it has also been shown that GABAergic function (measured with magnetic resonance spectroscopy) is reduced during INB (Levy et al. 2002). Ziemann and colleagues (1998a) used paired-pulse TMS to examine an intracortical inhibitory process mediated by $GABA_A$ receptors (short latency intracortical inhibition; SICI), but found no change in SICI acting on muscles proximal to an INB. Overall, it appears that the INB-induced increase in corticospinal excitability is related to a reduction in GABAergic inhibition, but the exact relationship between this reduction and the increase in corticospinal excitability remains unclear. Moreover, INB-induced excitability increases are probably modulated by several mechanisms, one of which is a reduction in GABAergic inhibition.

Greater plasticity of forearm flexors than extensors

In Experiments 1 and 3, where stimulation parameters were optimized for the flexors, increased flexor MEP amplitudes were recorded at the first post-inflation measurement block (as early as 5 minutes after cuff inflation). Rapid changes were also reported by Brasil-Neto and colleagues (1993) who found that nerve block across the elbow increased the excitability of biceps brachii seven to eight minutes following cuff inflation. Consistent with the idea that cortical plasticity after INB and amputation is primarily driven by the loss of sensory input from the body part distal to the block and not the increase in sensory input generated by cuff inflation (Brasil-Neto et al. 1992), these rapid changes were not observed when the cuff was inflated to only 60 mm Hg.

There is evidence from both human and non-human experiments for differences in the control of forearm flexors and extensors. Non-human primate studies show that basal ganglia

cells exert a greater inhibitory effect on wrist extensors than flexors (Mink and Thach 1991), and that the red nucleus facilitates finger flexors but not extensors (Lawrence and Kuypers 1968; Keifer and Houk 1994). Corticomotoneuronal control of forearm flexors and extensors also differs, with stronger monosynaptic connections to wrist and finger extensors in both humans (Maertens de Noordhout et al. 1999; Palmer and Ashby 1992) and non-human primates (Clough et al. 1968; Fetz and Cheney 1980; Kasser and Cheney 1985; McKiernan et al. 1998; Park et al. 2004; Park et al. 2001; Phillips and Porter 1964). The stronger and more numerous CM connections to forearm extensors might appear to be incompatible with our finding of INB-induced plasticity of forearm flexors but not extensors, but since CM cells constitute only a small proportion of the corticospinal tract, the predominance of extensor-related CM cells does not imply greater descending control of extensors than flexors. Indeed, Cheney and Fetz (1980) suggested that flexor motoneurons might receive a greater contribution from other (non-corticomotoneuronal) descending systems than extensor motoneurons, and that this might explain their apparently paradoxical finding of greater CM inputs to extensor motoneurons but a greater increase in static torque in flexors than extensors for a given increment in firing rate.

If, as suggested above, INB-induced plasticity is at least partially driven by GABAergic disinhibition of M1 then the presence of a larger number of flexor-related corticospinal cells would likely be accompanied by greater cortical inhibitory control over flexors than extensors, which could in turn explain the differential effect we observed in flexors and extensors. The evidence for an imbalance in the number of cells projecting to flexor and extensor motorneuron pools remains indirect, however, and the exact mechanisms underlying the differential modulation of corticospinal excitability in flexors and extensors remain unclear. What is clear is that manipulations other than INB also produce differential plasticity of forearm flexors and extensors. For example, Koganemaru and colleagues (2010)

showed a significant increase in MEP amplitudes from a forearm flexor but not a forearm extensor following 5 Hz repetitive TMS over M1, and McMillan and colleagues (2004) showed a greater MEP amplitude increase in a forearm flexor than a forearm extensor during the foreperiod of a warned reaction time task. Together, these findings suggest that flexors are more sensitive to experimental manipulation and have a greater potential for plasticity than extensors. The cortical contribution to this differential plasticity is likely to be regulated by intracortical inhibitory circuits, but further studies are necessary to confirm this.

Clinical Relevance

The greater sensitivity of flexors than extensors to INB-induced plasticity shares some similarities with the debilitating hypertonicity observed in upper-limb flexors, but not extensors, following stroke (e.g. Mayer 1997). While it is generally accepted that hypertonicity in the flexors following stroke is due primarily to changes at the spinal level, there is also evidence that a change in descending control contributes to this hypertonicity (Koganemaru et al. 2010; Sheean 2002). Specifically, a reduction of inhibitory drive from the cortex following lesion of the corticobulbar fibres results in a net excitation of spinal cord activity (Sheean 2002). Given this, and the results of the current study, we suggest that the forearm flexors have a greater potential for plasticity (both beneficial and maladaptive) than the forearm extensors, and that rehabilitation interventions that aim to alter cortical organization should take this difference into account.

Acknowledgements

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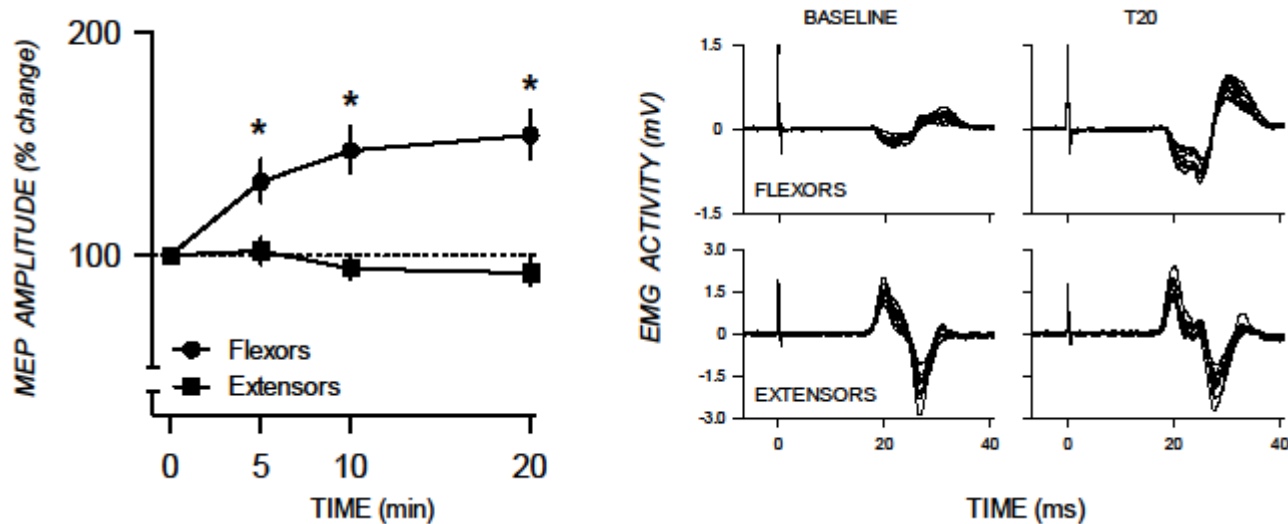
Figure Legends

FIG. 1. Left: Mean of each subject's normalized mean MEP amplitude for the flexors (round symbols) and extensors (square symbols) at each of the measurement blocks in Experiment 1. There is no variance in the data at baseline as this is not a real data point; it has been included in the figure to illustrate the change in MEP amplitude following inflation of the tourniquet. Asterisks represent a significant increase from baseline (flexors only). Error bars show +/- within subject error. Right: EMG traces (from 12 trials) from a representative subject showing raw MEPs recorded from the flexors (top) and the extensors (bottom) at baseline and T20.
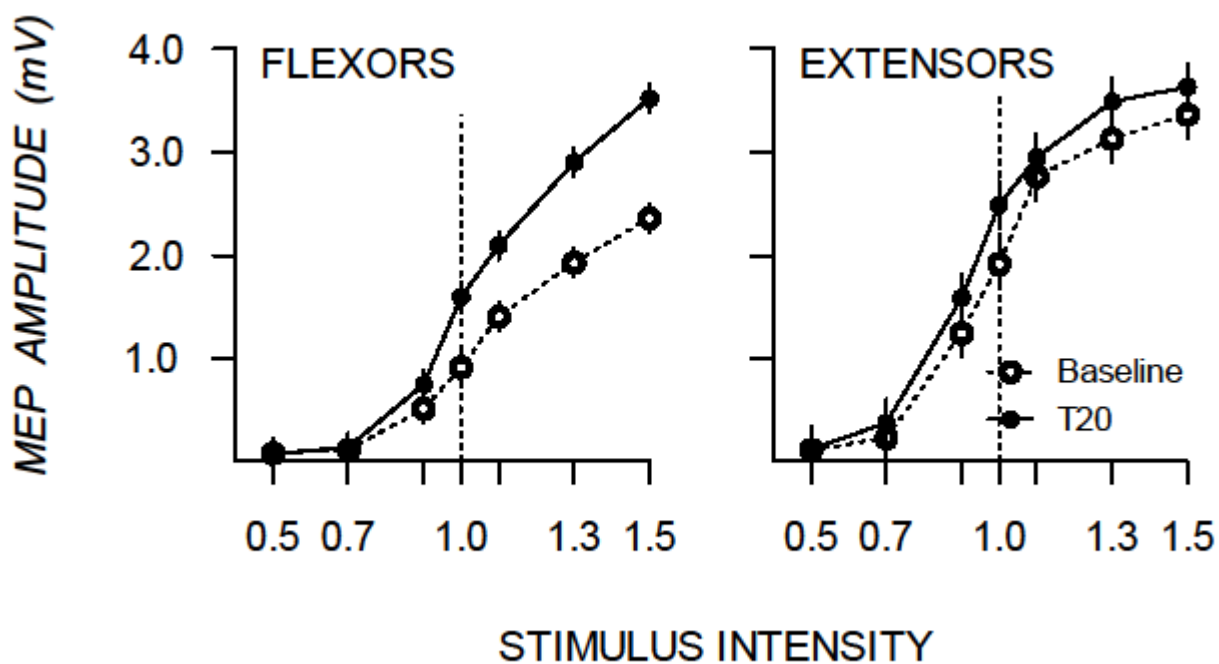
FIG. 2. Mean of each subject's mean MEP input/output curves (in mV) for the flexors (top) and extensors (bottom) in Experiment 1. Open symbols represent baseline measures and closed symbols represent T20 measures. TMS intensity expressed as a ratio of SI_{1mV}. Error bars show +/-within subject error.
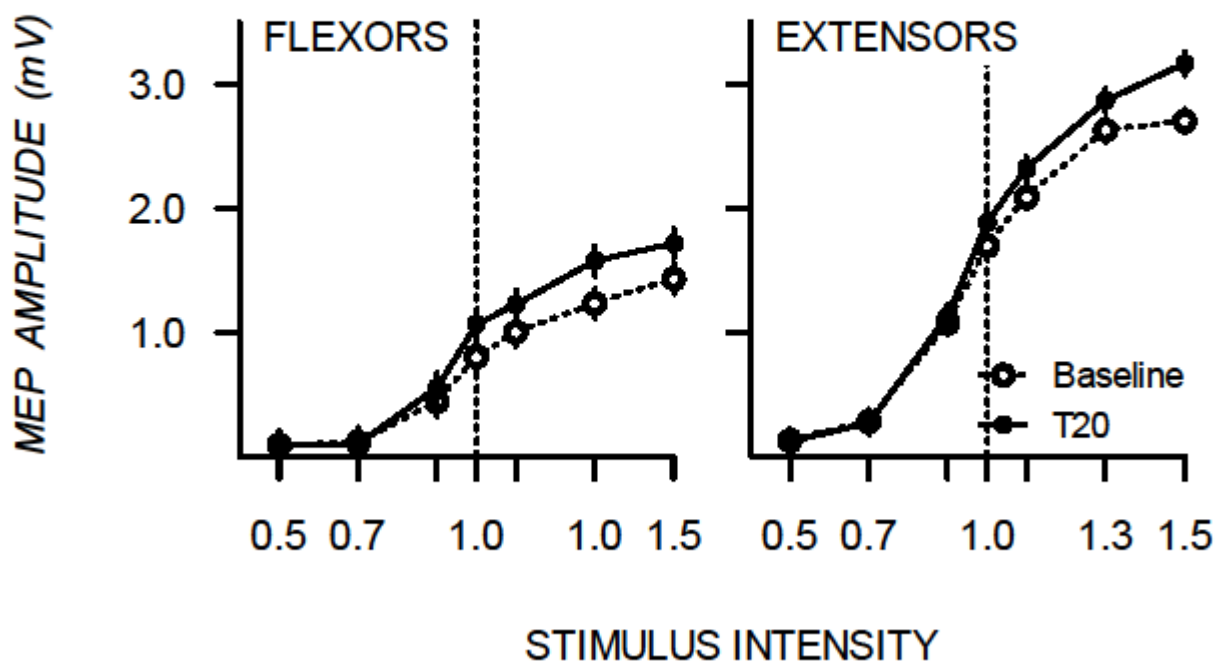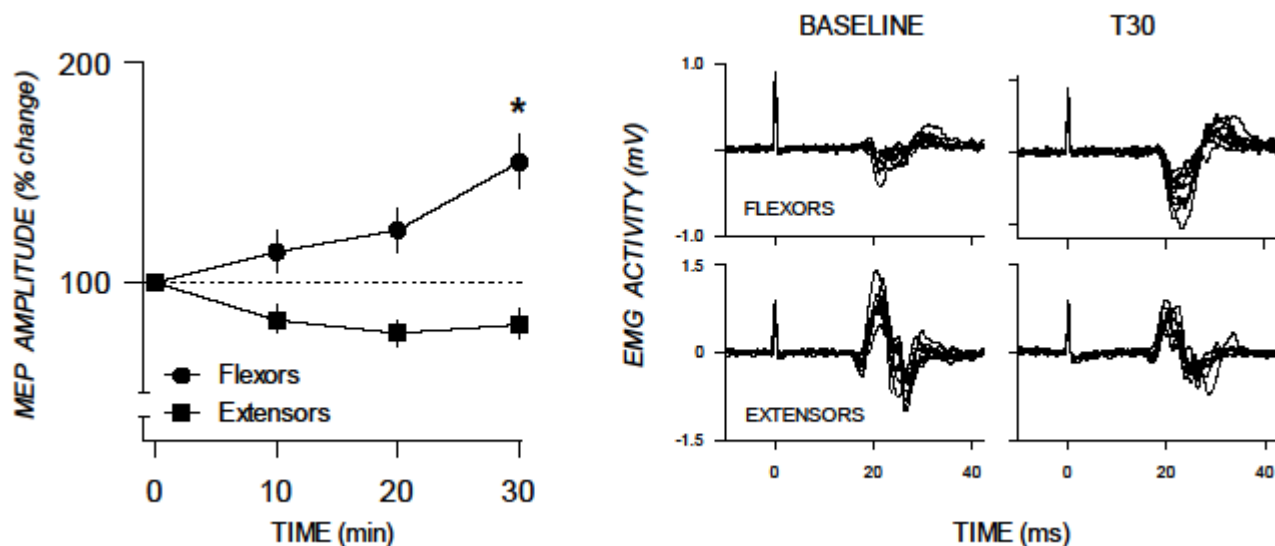
FIG. 3. Mean of each subject's mean MEP input/output curves (in mV) for the flexors (top)

and extensors (bottom) in the control condition with cuff inflation pressure of 60 mm Hg in

Experiment 1. Open symbols represent baseline measures and closed symbols represent T20

measures. TMS intensity expressed as a ratio of SI_{1mV}. Error bars show +/-within subject

error.

FIG. 4. Left: Mean of each subject's normalized mean MEP amplitude for the flexors (round symbols) and extensors (square symbols) at each of the measurement blocks in Experiment 2. There is no variance in the data at baseline as this is not a real data point; it has been included in the figure to illustrate the change in MEP amplitude following inflation of the tourniquet. Asterisk represents a significant increase from baseline (flexors only). Error bars show +/- within subject error. Right: EMG traces (from 10 trials) from a representative subject showing raw MEPs recorded from the flexors (top) and the extensors (bottom) at baseline and T30.
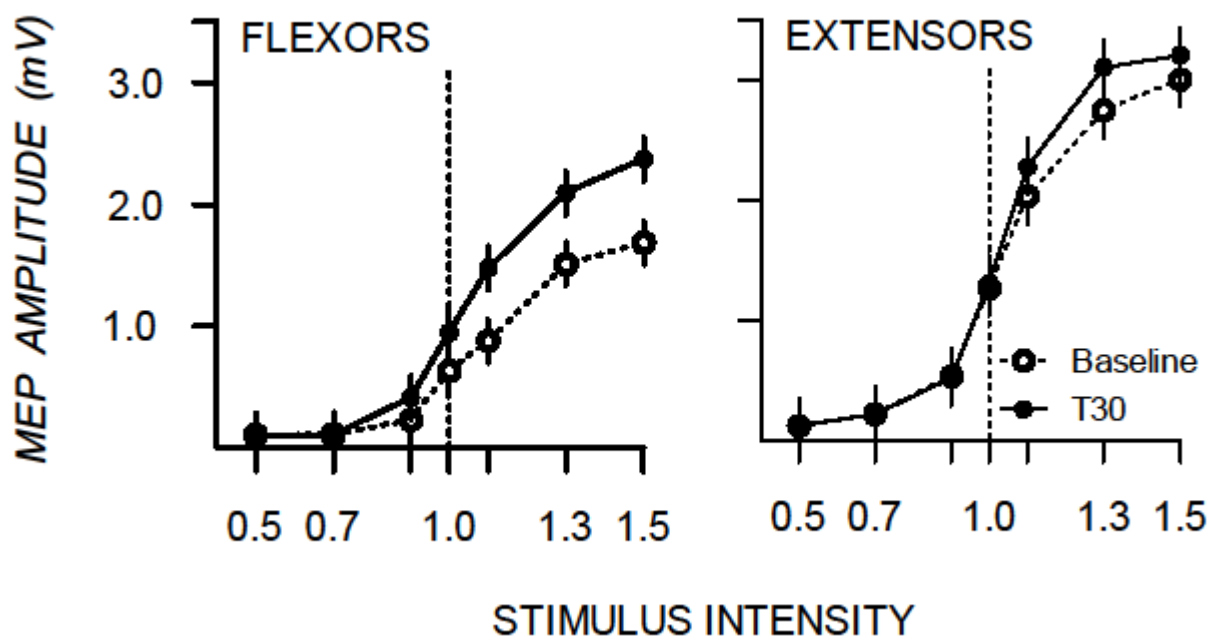
FIG. 5. Mean of each subject's mean MEP input/output curves (in mV) for the flexors (top) and extensors (bottom) in Experiment 2. Open symbols represent baseline measures and closed symbols represent T30 measures. TMS intensity expressed as a ratio of SI_{1mV}. Error bars show +/-within subject error.
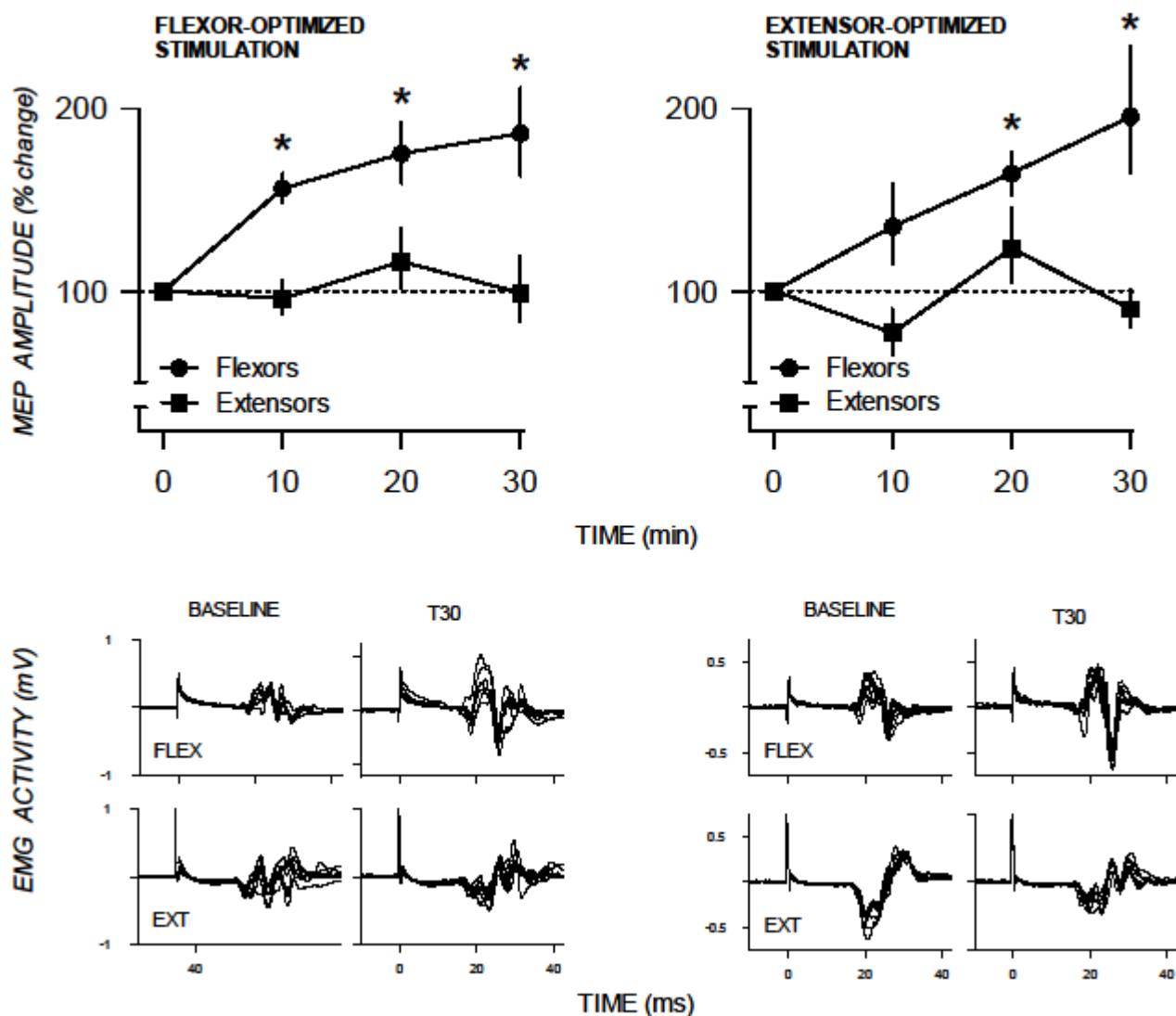
FIG. 6. Top: Mean of each subject's normalized mean MEP amplitude for the flexors (round symbols) and extensors (square symbols) for flexor-optimized parameters (left) and extensor-optimized parameters (right) at each of the measurement blocks in Experiment 3. There is no variance in the data at baseline because this is not a real data point; it has been included in the figure to illustrate the change in MEP amplitude following the inflation of the tourniquet. Asterisks represent a significant increase from baseline (flexors only). Error bars show +/- within subject error. Bottom: EMG traces (from 10 trials) from a representative subject showing raw MEPs recorded from the flexors and the extensors at baseline and T30 with flexor-optimized stimulation parameters (left) and extensor-optimized parameters (right).

Note the different scales on the flexor-optimized (left) and extensor-optimized (right) EMG traces.
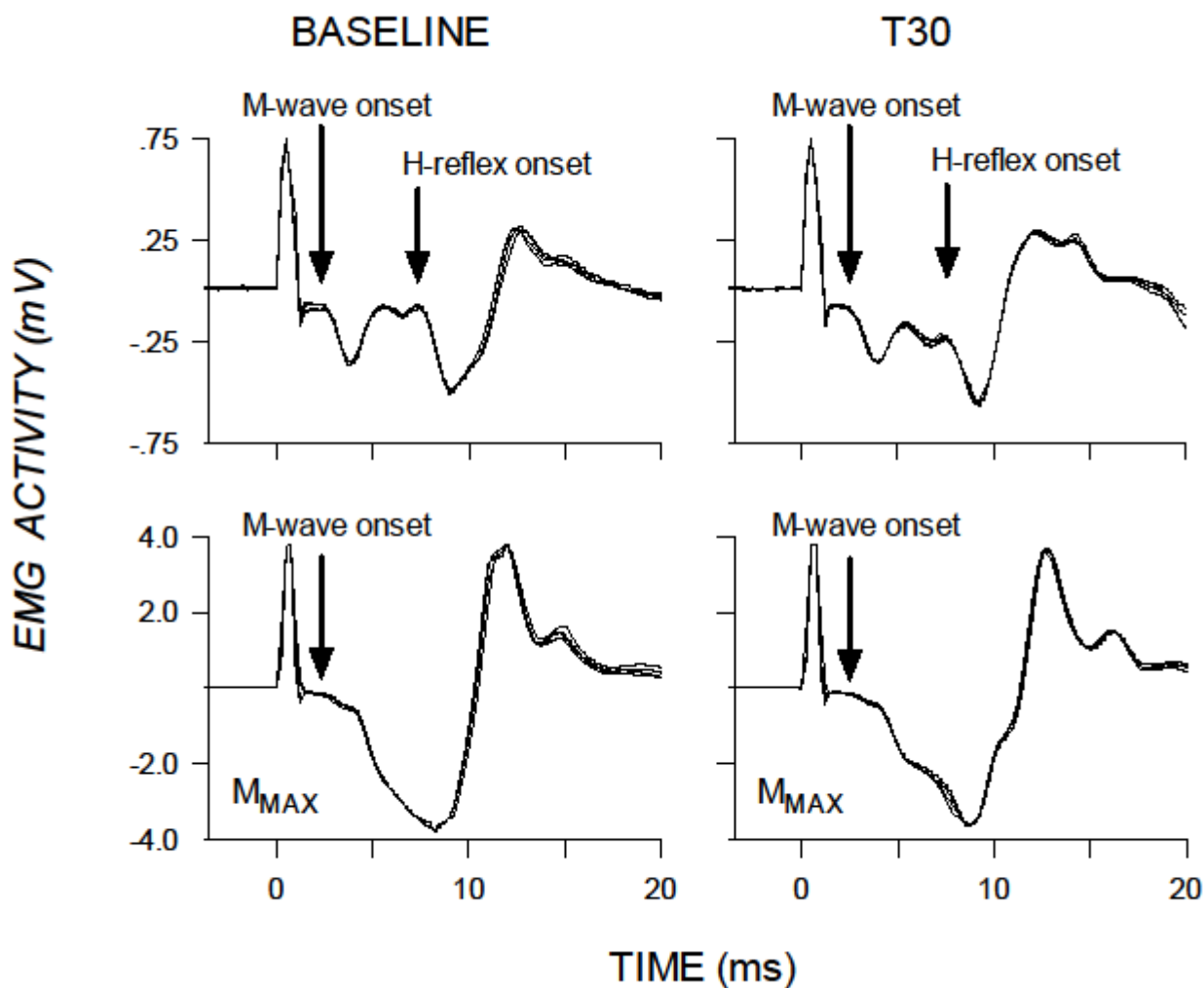

FIG. 7. EMG traces (from 4 trials) from a representative subject showing raw M-waves and H-reflexes (top) and M_{MAX} (bottom) recorded from the flexors at baseline (left) and T30 (right) in Experiment 3. The M-wave latency is 3 ms and the H-reflex latency is 8 ms. Both H-reflex and M_{MAX} waveforms are similar at baseline and T30 (note there is no H-reflex when the M-wave is maximal). Note the different Y-axis scales for the H-reflex (top) and M_{MAX} (bottom) figures.
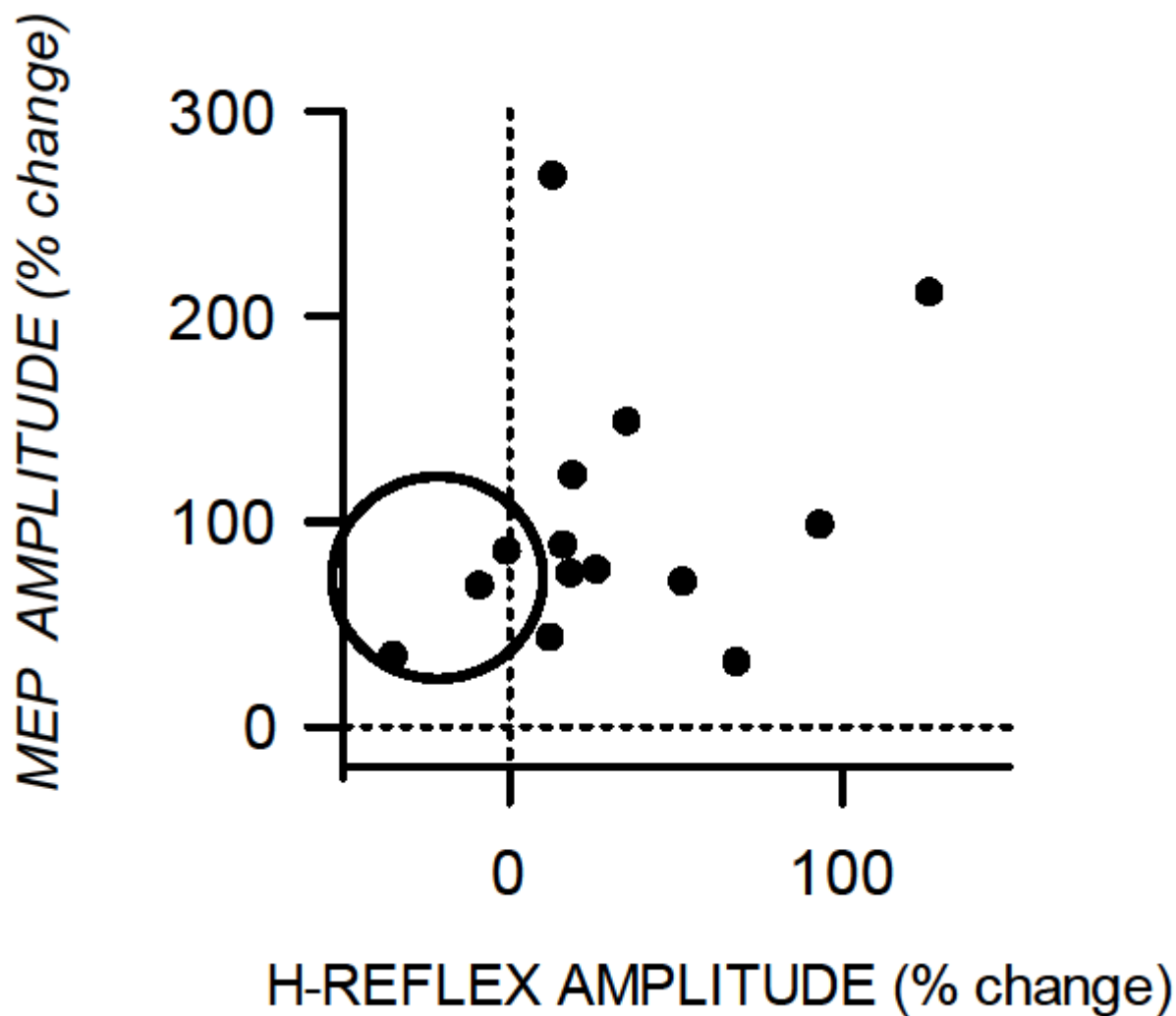
FIG. 8. Scatter diagram of the relationship between the change in MEP and H-reflex amplitude from baseline to T15. Note the three subjects who showed an increase in MEP amplitude from baseline to T15 but no change or a decrease in H-reflex amplitude from baseline to T15 (circled data).
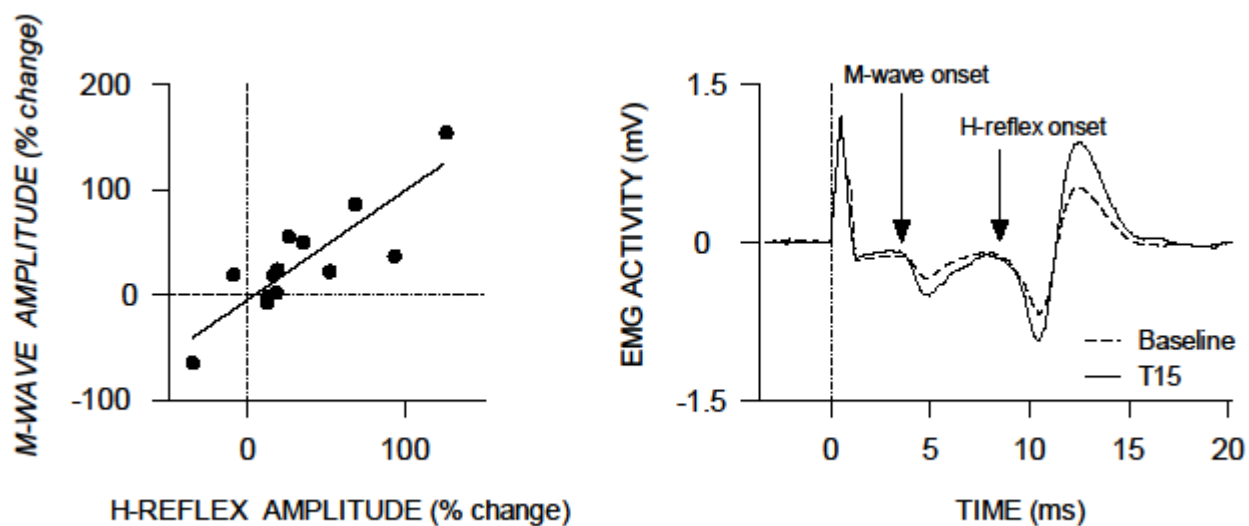
FIG. 9. Left: Scatter diagram of the relationship between the change in H-reflex amplitude and change in M-wave (elicited by the same stimulus used to measure H-reflex amplitude) from baseline to T15 (Expt 3a). Right: EMG traces from a representative subject showing a single trial with raw M-waves and H-reflexes recorded from the flexors at baseline and T15. The latency of the small M-wave is 4 ms and the latency of the H-reflex is 9 ms.

Table 1. *Target muscles, sample sizes, and findings of previous reports of changed corticospinal excitability following amputation or during INB or local anesthesia of the upper limb.*

	Manipulation(s)	Muscle(s); sample size	Finding(s)
Brasil-Neto et al. (1992)	INB and local anesthetic below elbow	biceps and deltoid; N=3	larger MEPs in biceps and deltoid
Brasil-Neto et al. (1993)	INB below elbow	biceps; N=2 deltoid; N=2	larger MEPs in biceps and deltoid
Cohen et al. (1991)	Amputation below elbow above elbow	biceps; N=3 deltoid; N=4	larger MEPs, decreased rMT, and increased area of biceps and deltoid
Irlbacher et al. (2002)	Amputation below elbow	biceps; N=10	larger MEPs and area in biceps
McNulty et al. (2002)	INB below elbow	biceps; N=7	larger MEPs in biceps
Ridding & Rothwell (1995)	Amputation lower forearm INB below elbow	FCR; N=2 biceps; N=2	larger MEPs and increased area of FCR increased MEPs in biceps
Ridding & Rothwell (1997)	Amputation lower forearm mid-arm INB at wrist	forearm flexor; N=1 deltoid; N=1 biceps; N=8	increased MEPs and steeper I/O curves in flexor and deltoid increased MEPs and steeper I/O curve in biceps
Röricht et al. (1999)	Amputation forearm upper arm or shoulder	biceps; N=8 deltoid or trapezoid; N=7	increased MEPs and area in biceps in 7/8 patients increased MEPs and area in only 2/7 patients
Schwenkreis et al. (2001)	Amputation forearm upper arm	biceps; N=7 deltoid; N=4	increased area of biceps and deltoid
Ziemann et al. (1998)	INB at elbow	biceps; N=7	increased MEPs in biceps
Ziemann et al. (2002)	INB at elbow	biceps; N=7	no change in MEPs in biceps

Table 2. Each subject's mean peak-to-peak H-reflex and M_{MAX} amplitudes, and H/M_{MAX} ratios at baseline and T30 in Experiment 3.

Subjects	H-reflex (in mV)		M_{MAX} (in mV)		**H/M_{MAX} ratio**	
	Baseline	T30	Baseline	T30	**Baseline**	**T30**
S_1	1.17	1.12	6.79	7.24	**0.17**	**0.15**
S_2	0.34	0.48	2.76	3.58	**0.12**	**0.13**
S_3	0.81	0.86	7.31	7.23	**0.11**	**0.12**