Intracortical Inhibition and Facilitation in Different Representations of the Human Motor Cortex

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Intracortical inhibition and facilitation in different representations of the human motor cortex. J. Neurophysiol. 80: 2870–2881, 1998. Intracortical inhibition (ICI) and intracortical facilitation (ICF) of the human motor cortex can be studied with paired transcranial magnetic stimulation (TMS). Plastic changes and some neurological disorders in humans are associated with changes in ICI and ICF. Although well characterized in the hand representation, it is not known if ICI and ICF vary across different body part representations. Therefore we studied ICI and ICF in different motor representations of the human motor cortex. The target muscles were rectus abdominus (RA), biceps brachii (BB), abductor pollicis brevis (APB), quadriceps femoris (QF), and abductor hallucis (AH). For each muscle, we measured the rest and active motor thresholds (MTs), the motor-evoked potential (MEP) stimulus-response curve (MEP recruitment), ICI, and ICF. The effects of different interstimulus intervals (ISIs) were studied with a conditioning stimulus (CS) intensity of 80% active MT. The effects of different CS intensities were studied at ISI of 2 ms for ICI and ISI of 15 ms for ICF. MT was lowest for APB, followed by BB, AH, and QF, and was highest for RA. Except for BB, MEP recruitment was generally steeper for muscles with lower MT. ICI and ICF were present in all the motor representations tested. The stimulus intensity necessary to elicit ICI was consistently lower than that required to elicit ICF, suggesting that they are mediated by separate mechanisms. Despite wide differences in MT and MEP recruitment, the absolute CS intensities (expressed as percentage of the stimulator’s output) required to elicit ICI and ICF appear unrelated to MT and MEP recruitment in the different muscles tested. These findings suggest that the intracortical mechanisms for inhibition and facilitation in different motor representations are not related to the strength of corticospinal projections.

INTRODUCTION

Transcranial magnetic stimulation (TMS) of the human motor cortex can activate corticospinal neurons with monosynaptic connections to upper (Day et al. 1989; Palmer and Ashby 1992) and lower (Brouwer and Ashby 1992) limb spinal motoneurons, producing short latency motor-evoked potentials (MEPs) in contralateral muscles. The motor threshold (MT), defined as the stimulus intensity necessary to produce a minimal MEP, and the rise in MEP amplitudes with increasing stimulus intensities (MEP recruitment) are related to the strength of corticospinal projections. Muscles with strong corticospinal projections, such as intrinsic hand muscles, have lower MT and steeper MEP recruitment than muscles with weaker corticospinal projections, such as biceps or lower limb muscles (Brouwer and Ashby 1990). Intracortical circuits in the hand representations of the motor cortex can also be activated by TMS, at intensities well below that required for activation of corticospinal neurons (Di Lazzaro et al. 1998; Kujirai et al. 1993; Ziemann et al. 1996c). The resulting intracortical inhibition (ICI) and intracortical facilitation (ICF) can be studied by the paired-TMS paradigm, with a subthreshold conditioning stimulus (CS) followed by suprathreshold test stimulus (TS) (Kujirai et al. 1993; Ziemann et al. 1996c). With short interstimulus intervals (ISIs) of 1–4 ms, the test responses are inhibited (ICI), and with longer ISIs of 8–15 ms the test responses are facilitated (ICF) (Kujirai et al. 1993). Abnormalities in ICI and ICF were reported in several neurological disorders, including cortical myoclonus (Brown et al. 1996), Parkinson’s disease (Ridding et al. 1995a), and dystonia (Ridding et al. 1995b). Assessment of intracortical excitability is particularly useful to investigate the mechanisms of cortical plasticity (Cohen et al. 1998), such as the effects of practiced movement or active relaxation (Liepert et al. 1998) or reorganization induced by amputation (Chen et al. 1998) or transient deafferentation (Ziemann et al. 1998a).

Most of the previous studies on ICI and ICF concentrated on the hand area of the human motor cortex. There is little information on ICI and ICF in other motor representations. The aim of this study is to examine intracortical excitatory and inhibitory mechanisms in different areas of the human motor cortex. Our results suggest that ICI and ICF can be demonstrated in different motor representations, and the absolute CS intensities required to elicit ICI and ICF appear unrelated to the strength of corticospinal projections.

METHODS

Subjects

Fourteen healthy volunteers (10 male and 4 female, mean age 37.4 yr, range 20–66 yr) participated in the first set of experiments with a circular coil. Eleven subjects (9 male and 2 female, mean age 37.4 yr, range 20–66 yr) participated in the second set of experiments with a figure-of-eight coil.
The effects of different CS intensities were studied with both steady, minimal background contraction to maintain surface EMG. The effects of different TS intensities for the AH muscle were monitored. For determination of active MT, the subject made a dual variation in the extent of inhibition and facilitation, only CS for each muscle. TMS stimuli were delivered 6 s apart, with five stimuli for each stimulus intensity beginning with the lowest intensity (100% rest MT). The average MEP amplitude at each stimulus intensity was determined from the peak-to-peak MEP amplitude for each trial.

**MEP recruitment**

MEP recruitment was studied with the circular coil. The stimulus intensities studied were 100, 110, 120, and 130% of the rest MT for each muscle. TMS stimuli were delivered 6 s apart, with five stimuli for each stimulus intensity beginning with the lowest intensity (100% rest MT). The average MEP amplitude at each stimulus intensity was determined from the peak-to-peak MEP amplitude for each trial.

**Paired TMS: effects of different interstimulus intervals**

The circular coil was used to study the effects of different interstimulus intervals. The paradigm used was similar to that described by Kujirai et al. (1993), with a subthreshold CS followed by a suprathreshold TS. The CS was set at 80% of the active MT to avoid any possible spinal effects from the CS. The TS was adjusted to produce MEPs of ~300 μV peak-to-peak amplitude. The test MEP amplitude was lower than that in previous studies (Kujirai et al. 1993) because in many subjects, it is difficult to obtain MEPs of higher amplitude in BB, RA, and lower limb muscles. The study was performed in 2 blocks of 40 trials, each with single test stimuli and paired stimuli at different ISIs delivered 6 s apart in a pseudorandom order controlled by a laboratory computer. The first block consisted of single TS and paired stimuli at ISIs of 2, 7, and 15 ms. The second block consisted of single TS and paired stimuli at ISIs of 5, 10, and 30 ms. Ten trials were recorded for each ISI, and the conditioned MEP amplitudes were expressed as percentages of the mean MEP amplitude with TS given alone in the same block.

**Paired TMS: effects of different CS intensities**

The effects of different CS intensities were studied with both the circular and figure-of-eight coils. The CS intensities studied were 70% active MT, 80% active MT, 90% active MT, 80% rest MT, and 90% rest MT. The TS intensity was initially adjusted to produce MEPs of ~300 μV peak-to-peak amplitude and then held constant during the experiment. Single TS and paired stimuli at ISIs of 2 and 15 ms were delivered 6 s apart in a pseudorandom order. The lowest CS intensity (70% of active MT) was studied first with CS intensity increasing after each block of 12 trials (4 trials for single TS and for each ISI). The conditioned MEP amplitudes were expressed as a percentage of the mean MEP amplitude with TS given alone in the same block. The block was rejected if any of the control MEPs (TS alone) fell below 100 μV. With CS at 90% of rest MT, the CS very occasionally elicited a MEP. These trials were also rejected.

Because the CS were adjusted as a percentage of the MT and MT differs considerably among different muscles, we also analyzed the data with different CS intensities expressed as a percentage of the stimulator output. The CS intensities were grouped in bins of 10% of the stimulator output. Because of the considerable individual variation in the extent of inhibition and facilitation, only CS intensity bins with three or more subjects were analyzed.

**Paired TMS: effects of different TS intensities**

The effects of different TS intensities for the AH muscle were tested in four male subjects (aged 23–36) with the circular coil.
The CS was set at 80% of the rest MT, and TSs of 105, 110, and 120% of the rest MT were tested. For each TS intensity, single TS and paired stimuli at ISIs of 2 and 15 ms were delivered 6 s apart in a pseudorandom order, with 12 trials for single TS and each ISI.

Comparison of the effects of magnetic CS on magnetic and electric TS for BB muscle

Although it was demonstrated in hand muscles that TMS CS has no effect on transcranial electrical stimulation (TES) TS, supporting the concept that inhibition induced by the CS is intracortical (Kujirai et al. 1993), such effect was not studied in proximal muscles. Therefore we studied the effects of TMS CS on TES test responses in the BB muscle. Surface EMG was recorded from the right BB muscle. A figure-of-eight magnetic coil was placed at the optimal position for activating the right BB muscle. TES was performed with a Digitimer D180 stimulator (Digitimer, Welwyn Garden City, UK). The anode was placed at the optimal position for activating the right BB muscle (at the midpoint of the 8-shaped magnetic coil) and the cathode positioned at the vertex. During the study subjects maintained a mild background contraction of the BB muscle to produce EMG of $\sim 100 \mu V$. This is to ensure that the TES-induced responses would have a substantial contribution from direct (D) activation of corticospinal fibers (Day et al. 1989). The CS was always TMS set at 90% of the active MT. The TS was either TMS or TES to evoke MEPs of $\sim 300 \mu V$. In two subjects, single TES TS and single TMS TS, paired stimuli at 2 ms ISI with TMS (CS) – TES (TS) and TMS (CS) – TMS (TS) were delivered 8 s apart, with 10 trials for each condition (total of 40 trials). In the other two subjects, paired stimuli at 15 ms ISI with TMS (CS) – TES (TS) and TMS (CS) – TMS (TS) were added, giving a total of 60 trials per subject.

Statistical analysis

MOTOR THRESHOLDS. The effect of muscle and subject on rest MT, active MT, and active/rest MT ratio were analyzed with analysis of variance (ANOVA). The paired $t$-test was used for posthoc comparison among different muscles.

MEP RECRUITMENT. The effects of stimulus intensity, subject and muscle on MEP amplitude, and %CMAP recruited were tested with analysis of covariance (ANCOVA).

INTRACORTICAL EXCITABILITY. The effects of muscle, subject, and ISI on the conditioned MEP amplitudes were examined by
repeated measures ANOVA, with ISI as the within-group factor (repeated measure) and muscle and subject as the between-group factors. Significant inhibition or facilitation of the conditioned MEP at each ISI compared with the TS alone was tested by the paired t-test.

EFFECTS OF DIFFERENT CS OR TS INTENSITIES ON INHIBITION AND FACILITATION. The effects of muscle, subject and CS intensity on the conditioned MEP amplitudes were examined by repeated measures ANOVA, with CS or TS intensity as the within-group factor (repeated measure) and muscle and subject as the between-group factors. Significant inhibition or facilitation of the conditioned MEP for each CS intensity was tested by the paired t-test.

In all the statistical tests, differences were considered significant if \( P \leq 0.05 \). Bonferroni correction for multiple comparisons was applied when multiple t-tests were performed.

RESULTS

Motor threshold

CIRCULAR COIL. The rest and active MTs are shown in Fig. 1A. The effect of muscle was significant \( (P = 0.0001) \) for both rest and active MT. MTs were highest for RA (\( n = 11 \)), followed by QF (\( n = 12 \)), AH (\( n = 12 \)), and BB (\( n = 14 \)), and APB (\( n = 14 \)) had the lowest threshold. In two subjects, the resting MTs for RA, QF, and AH and in another subject the resting MT for RA were >100% of the stimulator output. In every subject and in all muscles, the active MT was lower than the rest MT. Posthoc comparison with the paired t-test showed that for the rest MT the differences among all five muscles were significant \( (P < 0.005, \) paired t-test) except for BB and AH. For the active MT, the differences among all the muscles tested were also significant except for BB and QF, BB and AH, and QF and AH. The effect of subject was significant (ANOVA, \( P = 0.0001) \) for both rest and active MTs, confirming that there is considerable variation among individuals for TMS MTs.

FIGURE-OF-EIGHT COIL. The rest and active MTs are shown in Fig. 1B. ANOVA showed significant effects for subject \( (P = 0.001) \) and muscle \( (P = 0.001) \). The thresholds were highest for QF and AH \( (n = 10) \), followed by BB \( (n = 11) \) and APB \( (n = 11) \). The rest MT was >100% for AH in one subject and for QF in another subject. Posthoc paired t-test showed significant differences \( (P < 0.001) \) among the four muscles tested except for QF and AH for both rest and active MTs. The active/rest MT ratios were BB 76.5 ± 2.8%, APB 80.6 ± 1.8%, QF 77.6 ± 3.1%, and AH 84.7 ± 2.3%. The effects of muscle and subjects on active/rest MT ratios were not significant.

MEP recruitment

The slope of the MEP recruitment curve was steepest for APB, followed by AH, QF, RA, and least for BB (Fig. 2A). This order is unchanged when responses were expressed as %CMAP (Fig. 2B). ANCOVA showed that the effects of subject \( (P = 0.0001) \), muscle \( (P = 0.0003) \), and stimulus intensity \( (P = 0.0001) \) were significant with the responses expressed as either absolute MEP amplitude or %CMAP. The interaction between muscle and stimulus intensity was also significant \( (P = 0.0001) \), indicating that changes in stimulus intensity have different effects for the muscles studied. The subject and muscle interaction was also significant \( (P = 0.0001) \), indicating that there are considerable individual differences in MEP recruitment in different muscles.

Paired TMS: effects of different ISIs

The conditioned MEP amplitudes at different ISIs for the muscles tested are shown in Fig. 3. The conditioned MEPs were significantly inhibited \( (P < 0.0001) \) at ISI of 2 ms for all the muscles tested. Inhibition remained significant at ISI of 5 ms for RA and BB \( (P = 0.002) \). Although there was...
TABLE 1. **CS intensities expressed as percentage of stimulator output for the circular coil**

<table>
<thead>
<tr>
<th>CS Intensity</th>
<th>RA</th>
<th>BB</th>
<th>APB</th>
<th>QF</th>
<th>AH</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% active MT</td>
<td>37.9 ± 4.2</td>
<td>30.8 ± 5.5</td>
<td>28.7 ± 8.3</td>
<td>33.0 ± 5.2</td>
<td>32.6 ± 6.1</td>
</tr>
<tr>
<td>80% active MT</td>
<td>42.5 ± 4.2</td>
<td>35.2 ± 6.2</td>
<td>32.4 ± 9.2</td>
<td>37.4 ± 6.0</td>
<td>36.9 ± 7.0</td>
</tr>
<tr>
<td>90% active MT</td>
<td>47.1 ± 4.5</td>
<td>39.5 ± 6.8</td>
<td>36.1 ± 10.0</td>
<td>41.8 ± 6.7</td>
<td>41.2 ± 8.0</td>
</tr>
<tr>
<td>80% rest MT</td>
<td>53.6 ± 2.9</td>
<td>41.9 ± 9.1</td>
<td>40.8 ± 9.9</td>
<td>52.8 ± 7.3</td>
<td>46.6 ± 6.0</td>
</tr>
<tr>
<td>90% rest MT</td>
<td>60.1 ± 3.4</td>
<td>47.0 ± 10.1</td>
<td>44.6 ± 11.0</td>
<td>58.1 ± 8.3</td>
<td>52.1 ± 6.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. CS, conditioning stimulus; RA, rectus abdominus; BB, biceps brachii; APB, abductor pollicis brevis; QF, quadriceps femoris; AH, abductor hallucis; MT, motor thresholds.

a trend for facilitation in RA, BB, QF, and AH at ISIs of 7–30 ms, it did not reach statistical significance. ANOVA showed that the effects of ISI (P = 0.0001) and subject (P = 0.03) were significant but the effect of muscle was not. However, the interaction between ISI and muscle was significant (P = 0.008), indicating that effects of ISI varied for different muscles.

**Effects of different CS intensities on ICI and ICF: circular coil**

**CS INTENSITIES.** The CS intensities used, expressed as a percentage of the stimulator output, are shown in Table 1. Because the CS intensities were adjusted according to the MT, the lowest CS intensity for RA (70% active MT) was similar to 80% active MT for QF, between 80 and 90% of active MT for AH, and between 90% of active MT and 80% of rest MT for BB and APB. CS intensity of 90% active MT was usually lower than 80% rest MT. However, in some subjects (1 for RA, 4 for BB, 2 for APB, and 1 for AH), 90% active MT was equal to or higher than 80% rest MT. In some subjects, the MTs were too high to obtain adequate test response, and several studies were rejected because the MEP amplitude for the test pulse alone could not be maintained throughout the study. Data from 8 subjects for RA, 13 for BB, 12 for APB, 12 for QF, and 11 for AH were included in the analysis.

**TEST STIMULUS INTENSITY AND AMPLITUDE.** The control MEP amplitudes (test pulse alone) were 0.34 ± 0.07 mV for RA (n = 8), 0.26 ± 0.04 mV for BB (n = 13), 0.49 ± 0.14 mV for APB (n = 13), 0.20 ± 0.06 mV for QF (n = 11), and 0.54 ± 0.09 mV for AH (n = 11). ANOVA did not show a significant effect of muscle on the control test MEP amplitude. The stimulus intensities for the test pulse, expressed as percentages of the resting MT for each muscle, were 115.9 ± 1.3% for RA, 124.4 ± 2.4% for BB, 109.9 ± 2.3% for APB, 113.4 ± 2.0% for QF, and 111.3 ± 1.8% for AH. ANOVA showed a significant effect of muscle on the stimulus intensities expressed as percentages of the resting MT (P = 0.02). Paired t-test with Bonferroni correction showed that significantly higher stimulus intensities were used for BB than for APB (P = 0.0002) and AH (P = 0.004).

**ICI (ISI 2 MS).** Examples from single trials in one subject are shown in Fig. 4; the group data are shown in Fig. 5A. ANOVA showed that the effects of subject (P = 0.003) and CS intensity (P = 0.0006) on the conditioned MEP amplitudes were significant, but the effect of muscle was not significant. However, the interaction of CS intensity and muscle was significant (P = 0.001), indicating that the effects of changes in CS intensity are different for muscles tested.

For RA, the conditioned MEP was significantly inhibited and remained unchanged from CS intensities of 70% active MT to 80% rest MT, but at 90% rest MT inhibition was abolished. Similarly, for AH inhibition was significant and stayed constant for CS of 70% active MT to 90% active MT, but there was no significant inhibition when the CS was increased to 80 and 90% of rest MT. In contrast, for APB and BB, there was no significant inhibition at 70% active MT, but the inhibition increased and became significant with higher CS intensities of 80% active MT to 90% rest MT. For QF, inhibition was significant for all CS intensities, but inhibition increased when CS was increased from 70% active MT to 80% active MT and then remained stable up to 90% rest MT.

The results with different CS intensities, expressed as a percentage of the stimulator’s output, are shown in Fig. 5B. There was prominent inhibition with CS at 30–39% and

TABLE 2. **CS intensities expressed as percentage of stimulator output for the figure-of-eight coil**

<table>
<thead>
<tr>
<th>CS intensity</th>
<th>BB</th>
<th>APB</th>
<th>QF</th>
<th>AH</th>
</tr>
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<tbody>
<tr>
<td>70% active MT</td>
<td>33.6 ± 6.2</td>
<td>30.0 ± 7.0</td>
<td>44.9 ± 9.0</td>
<td>50.2 ± 8.7</td>
</tr>
<tr>
<td>80% active MT</td>
<td>38.6 ± 6.8</td>
<td>33.4 ± 8.5</td>
<td>51.3 ± 10.5</td>
<td>57.3 ± 10.4</td>
</tr>
<tr>
<td>90% active MT</td>
<td>43.6 ± 7.7</td>
<td>37.2 ± 9.5</td>
<td>57.6 ± 11.7</td>
<td>64.5 ± 11.6</td>
</tr>
<tr>
<td>80% rest MT</td>
<td>51.9 ± 10.8</td>
<td>40.4 ± 8.0</td>
<td>66.7 ± 9.6</td>
<td>65.3 ± 8.2</td>
</tr>
<tr>
<td>90% rest MT</td>
<td>58.2 ± 12.5</td>
<td>45.0 ± 9.1</td>
<td>73.3 ± 9.9</td>
<td>71.4 ± 7.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. See Table 1 for definitions.
The results with CS intensities expressed as a percentage of the stimulator’s output are shown in Fig. 6B. There was no facilitation in any of the muscles with CS of 20–29% or 30–39% of the stimulator’s output. Significant facilitation began with CS of 40–49% of the stimulator’s output for all the muscles tested.

CS INTENSITIES TO ELICT ICI AND ICF. From the group data, the CS threshold intensities for eliciting ICI were lower than those for ICF in all the muscles studied (Figs. 5A and 6A). Additionally, in 27 individual studies that showed significant ICI and ICF, the CS threshold was lower for ICI than for ICF (P = 0.0013, paired t-test).

Effects of different CS intensities on ICI and ICF: figure-of-eight coil

CS INTENSITIES. The CS intensities used, expressed as a percentage of the stimulator output, are shown in Table 2. Because 40–49% of the stimulator’s output for all muscles. For QF, AH, and APB, the relationship between CS intensity and inhibition of the conditioned MEP followed a U-shaped curve, with inhibition most marked in the midrange of stimulus intensities tested and less inhibition at higher or lower intensities. Inhibition decreased with higher CS intensities for RA, but CS intensities <30% were not tested. In contrast, inhibition increased with higher CS intensities for BB, but CS intensities >60% were not tested.

ICI (ISI 15 ms). Examples of single trials in one subject are shown in Fig. 4; the group data are shown in Fig. 6A. ANOVA showed that the effects of muscle (P = 0.0002) and CS intensity (P = 0.0001) were significant, but the effect of subject was not. The interaction of CS intensity and muscle was significant (P = 0.0001), indicating that the effects of changes in CS intensity are different for the muscles tested.

For all muscles, there was no significant facilitation of the conditioned MEP with CS of 70% active MT; facilitation tended to increase with increasing CS intensity. For RA, significant facilitation began at 80% active MT, which rapidly increased with higher CS intensities. Significant facilitation began at 90% active MT for QF, 80% rest MT for AH and BB, and 90% rest MT for APB. In every muscle, the minimum CS intensity necessary to elicit facilitation was higher than that for inhibition (Figs. 5A and 6A).

FIG. 4. Examples of single trials from one subject showing the effects of different CS intensities on ICI and ICF tested with a circular coil. For RA, QF, and AH, inhibition and facilitation of the conditioned MEPs can be elicited at lower CS intensities (expressed as percent of MT) than BB and APB.

FIG. 5. ICI at ISI of 2 ms for different CS intensities with the circular coil. A: CS intensity expressed as percent of MTs (A = active MT, R = rest MT). B: CS intensity expressed as percent of the stimulator’s output. Error bar = 1 SE. * Significant difference from test pulse alone.
ICI (ISI 2 MS). The conditioned MEP amplitudes at different stimulus intensities for the muscles tested are shown in Fig. 7A. ANOVA showed that the effect of subject was significant ($P = 0.03$), but the effect of CS intensity or muscle was not significant. There was a trend for significant interaction between CS intensity and muscle ($P = 0.07$). There was significant inhibition for each muscle at all stimulus intensities except for QF at 90% rest MT. For APB, inhibition increased from 70% active MT to 90% active MT and remained stable up to 90% rest MT. In contrast, inhibition was most prominent at low stimulus intensities of 70–90% active MT for QF and at stimulus intensities of 70–80% active MT for AH and diminished at higher stimulus intensities. For BB, inhibition was most marked at 90% active MT and diminished at higher or lower stimulus intensities.

The results of CS intensities expressed as percentages of the stimulator output are shown in Fig. 7B. Similar to the findings for the circular coil in three muscles, the relationship between the conditioned MEP amplitude and CS intensity followed a U-shaped curve, but in this case for all muscles studied, with prominent inhibition at 40–49% of the stimulator's output.
Effects of different TS intensities on ICI and ICF for AH muscle

TEST MEP AMPLITUDES. The mean control test MEP amplitudes were 0.32 ± 0.14 mV for TS of 105% rest MT, 0.55 ± 0.15 mV for TS of 110% rest MT, and 0.88 ± 0.16 mV for TS of 120% rest MT.

ICI (ISI 2 MS). The conditioned MEP amplitudes (as percent of control) were 24.9 ± 17.8% for TS of 105% rest MT, 20.9 ± 19.2% for TS of 110%, and 13.7 ± 13.4% for TS of 120% of rest MT. Data from each subject are shown in Fig. 9A. ANOVA showed significant effects of TS intensity ($P = 0.03$) and subject ($P = 0.0002$). Posthoc paired $t$-tests showed significantly more inhibition for TS of 120% MT compared with TS of 105% MT ($P = 0.03$).

ICF (ISI 15 MS). The conditioned MEP amplitudes (as percent of control) were 174 ± 58% for TS of 105% rest MT, 150 ± 36% for TS of 110%, and 166 ± 13%. Data from each subject are shown in Fig. 9B. ANOVA showed the effect of subject was significant ($P = 0.03$) but the effect of TS intensity was not.

ICF (ISI 15 MS). The conditioned MEP amplitudes at different CS intensities for the muscles tested are shown in Fig. 8A. ANOVA showed significant effect of CS intensity ($P = 0.001$), but the effects of muscle and subjects were not significant. However, the interaction of CS intensity and muscle was significant ($P = 0.005$), indicating that the effects of changes in CS intensity are different for the muscles tested. Significant facilitation began at 80% rest MT for BB and APB. For AH, facilitation was achieved at 80% active MT. For QF, the MEPs appeared to be facilitated from CS of 80% MT but were not significantly different from the baseline until 90% rest MT because of large individual variations. In every muscle, the minimum CS intensity necessary to elicit facilitation was higher than that for inhibition (Figs. 7A and 8A).

The results of CS intensities expressed as percentages of the stimulator’s output are shown in Fig. 8B. There was no facilitation with CS below 40% of the stimulator’s output for all the muscles tested. Significant facilitation began at 40–49% of the stimulator’s output for BB, APB, and QF and at 50–59% for AH. Facilitation was more marked with higher stimulus intensities.
adjacent to the central sulcus, away from the stimulating coil.

Site of inhibition and facilitation induced by CS

Kujirai et al. (1993) showed that, with CS at 80% of rest MT, the inhibition of the conditioned MEP observed in the paired-TMS paradigm is likely to be a cortical phenomenon for hand and forearm muscles because the CS did not suppress responses to a small anodal electric test stimulus and had no effect on forearm H-reflexes. We now show that, for a proximal muscle (BB), CS sufficient to cause inhibition and facilitation of the magnetic test response did not change the electric test response. In addition, we reported that, in six normal subjects, CS at 80% rest MT (circular coil) for the QF muscle did not change spinal excitability, as tested by H-reflex amplitudes for the QF muscle (Chen et al. 1998). Therefore it is likely that the inhibition and facilitation observed at CS of 80% rest MT or lower, even in muscles with high MT, is due to cortical mechanisms.

Effects of varying CS intensities on ICI

With a constant CS of 80% active MT, inhibition of the test response can be obtained in proximal upper limb, lower limb, and axial muscles at ISIs similar to those for intrinsic hand muscles (Fig. 3). Facilitation was relatively weak because the CS intensity used (80% of active MT) was below that required to elicit significant facilitation in our experimental setup (Fig. 6).

The effects of using different CS intensities, expressed as percentages of MT, appeared to be different for the muscles tested (Figs. 5A and 7A). For the muscles with the lowest MTs (APB and BB for the circular coil, APB for the figure-of-eight coil), inhibition increased with increasing CS and then remained constant up to an intensity of 90% rest MT. The findings for the APB are similar to those reported for other intrinsic hand muscles (Kujirai et al. 1993; Ridding et al. 1995c; Schäfer et al. 1997; Ziemann et al. 1996c). In contrast, for RA and AH, the muscles with relatively high MT, inhibition was prominent with low CS intensities but diminished with higher CS intensities. Some of these differences could be explained if the absolute CS intensities necessary for eliciting ICI in different muscles are to some extent independent of MT, and there is a U-shaped relationship between inhibition and absolute CS intensity for most of the muscles. For muscles with low MT such as APB, the CS intensities used are low, and the effects resemble the left one-half of a U-shaped curve. On the other hand, for muscles with high MT such as RA, the CS intensities used are high, and the effects resemble the right one-half of a U-shaped curve. The findings for QF appear to be an exception in that prominent inhibition was seen at all the CS intensities tested (Figs. 5A and 7A). The reasons for this discrepancy are unclear. With CS intensities expressed as percentages of the stimulator output (Figs. 5B and 7B), the CS necessary to produce significant inhibition was similar among different muscles, despite wide differences in MTs. Stokic et al.

Comparison of the effects of magnetic CS on magnetic and electric TS for BB muscle

At ISI of 2 ms, the CS produced prominent inhibition of the responses to magnetic TS but had little effects on the responses to electric TS in all four subjects (Fig. 10). At ISI of 15 ms, the conditioned TMS MEPs were facilitated in both subjects (199 and 175% of baseline), and there was no facilitation of the conditioned TES MEPs (80 and 80% of baseline).

Discussion

Thresholds and MEP recruitment

We found that, in the intrinsic hand muscle (APB), the stimulus intensity necessary to elicit motor responses (MT) is lower and the MEP recruitment is steeper than in other muscles tested, similar to previous findings in humans (Benecke et al. 1988; Brouwer and Ashby 1990; Rothwell et al. 1987). This likely relates to the findings that cortical stimulation resulted in larger excitatory postsynaptic potentials (EPSPs) in spinal motoneurons for hand muscles compared with proximal arm or lower limb muscles (Palmer and Ashby 1992; Phillips and Porter 1964), which we refer to as strength of corticospinal projections. This may be due to a number of mechanisms, such as differences in the number or density of pyramidal tract neurons in motor cortex and the size of EPSPs generated from each pyramidal tract neuron.

BB has a lower threshold but much lower rate of MEP recruitment compared with RA and lower limb muscles. Single-unit studies showed that TMS caused inhibition with no preceding facilitation in some BB motor units (Palmer and Ashby 1992). It is possible that with increasing stimulus intensity the inhibitory effects on BB partially cancel out the facilitatory effects, leading to a slower MEP recruitment rate compared with other muscles. Alternatively, the higher MT for leg muscles compared with BB may be due to their representation on the medial surface of the motor cortex


Effects of varying CS intensities on ICF

We found that the threshold for eliciting facilitation was higher than that for inhibition not only in intrinsic hand muscles as previously reported (Kujirai et al. 1993; Ziemann et al. 1996c) but also in proximal upper limb, proximal and distal lower limb, and truncal muscles. This is consistent with the suggestion that ICI and ICF are mediated by separate mechanisms (Ziemann et al. 1996c). The ICF at 15 ms ISI is different from I-wave facilitation in the motor cortex, which requires much shorter ISIs (1–4 ms) and suprathreshold first stimulus (Tokimura et al. 1996; Ziemann et al. 1998b).

Although all the muscles studied showed increased facilitation with increasing CS intensity, there is considerable variation in the threshold for significant facilitation with CS expressed as a percentage of MT (Figs. 6A and 8A). The threshold for facilitation is higher in muscles with low MT such as APB than in muscles with high MT such as RA, QF, and AH. However, with CS expressed as percentage of the stimulator’s output (Figs. 6B and 8B), the threshold for facilitation was remarkably uniform among the different muscles despite their wide differences in MT and MEP recruitment. This strongly suggests that the mechanisms for facilitation are different from those for determining MT and generating MEP. This finding also supports the idea that facilitation, at least at low CS intensities, is due to intracortical mechanisms (Ziemann et al. 1996c) rather than subthreshold facilitation of spinal motoneurons (Kujirai et al. 1993). If facilitation were due to corticospinal volleys elicited by the CS, it can be expected that muscles with low MT, such as APB, should have a lower threshold for facilitation (with CS expressed as percentage of the stimulator’s output) than muscles with high MT, such as RA. When CS intensity approaches that of the rest MT, it is likely that facilitation of spinal motoneurons also contributes to the facilitation observed.

The dissociation between MTs and thresholds for ICI and ICF can be explained if the intracortical connections for inhibition and facilitation are similar in different representations of the human motor cortex. MTs are likely related to variations in the strenght of corticospinal projections. Muscles with weak corticospinal projections have high MTs because it is necessary to activate a higher proportion or produce stronger activation of corticospinal neurons to generate MEPs of a certain amplitude (50 μN in our definition of rest MT), compared with muscles with strong corticospinal projections. Thus intracortical mechanisms are more activated at a fixed percentage of MT for muscles with weak corticospinal projections such as RA than for muscles with stronger corticospinal projections such as APB.

Effects of varying TS intensities on ICI and ICF

Because of the differences in the recruitment curves (Fig. 2), it was not possible to match both the control MEP amplitudes and TS intensities (as percentage of rest MT) when examining the effects of different CS intensities. We attempted to obtain similar MEP amplitudes, resulting in small differences in TS intensities (as percentage of MT) being used for the muscles tested. We studied the effects of varying TS intensities (and MEP amplitudes) and showed that ICF was not consistently affected by changes in TS intensities between 105 and 120% rest MT. Therefore the slight differences in TS intensities among the different muscles tested are unlikely to influence the results of ICF. Because we found that higher TS intensities and test MEP amplitudes lead to a small increase in ICI (Fig. 9), we did not directly compare the extent of ICI among the different muscles tested. However, we were able to obtain similar maximum ICI in all the muscles tested. The maximum ICIs were between 35 and 50% of the TS given alone for the circular coil (Fig. 5) and between 32 and 45% for the figure-of-eight coil (Fig. 7). Thus slight variations in TS intensities (but similar test MEP amplitudes) are unlikely to account for the observation that CS intensity for maximum inhibition did not correlate with MTs.

Mechanisms of ICI

Our findings suggest that ICI and ICF in different areas of the motor cortex are not related to the strength of corticospinal projections. ICI is likely due to the activity of inhibitory GABAergic interneurons. γ-Aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the cortex (Jones 1993), and drugs that enhance GABA increase ICI as tested by paired TMS (Ziemann et al. 1996a,b). GABA neurons of the cerebral cortex are aspy nonpyramidal neurons and constitute 25–30% of cortical neurons (Jones 1993; White 1989). In the motor cortex (area 4), layer II has the highest concentration of GABAergic neurons (Jones 1993), and there are prominent vertical GABAergic projections (Keller 1993). Cortical pyramidal cells receive extensive GABAergic synapses (Jones 1993). In a series of experiments, Krnjevic et al. (1966a–c) showed that cortical stimulation, especially in the superficial one-half of the cortex, can inhibit spontaneous discharges of Betz cells and their responses to glutamate. Similarly, intracortical microstimulation (ICMS) of the motor cortex can inhibit...
voluntary EMG activity (Cheney et al. 1985; Lemon et al. 1987; Schmidt and McIntosh 1990). The zones where ICMS produced inhibition were interspersed among zones that produced excitation for the same muscle (Schmidt and McIntosh 1990). However, the inhibitory effects of ICMS may be due to spinal in addition to cortical mechanisms.

It seems unlikely that TMS activates small nonpyramidal GABAergic neurons directly. This is because ICI can be evoked at low stimulus intensities, and it extends over a long range across the motor cortex (Kujirai et al. 1993), although GABAergic neurons have only limited horizontal connections (DeFelipe and Jones 1985; Keller 1993). A possible explanation for these findings is that TMS does not activate GABAergic neurons directly, but instead activates corticocortical pyramidal neurons or their axons that project to nonpyramidal GABAergic neurons (Keller 1993; White 1989).

Mechanisms of ICF

ICF may be due to activation of corticocortical pyramidal cells and their axons. These axons have excitatory, glutaminergic synapses (Keller 1993; White 1989). The cells are mainly located in layers II and IIIA, and there are intracortical connections between layers III and V (Asanuma and Rosén 1973; Gatter and Powell 1978). Labeling studies revealed extensive, long (3 mm) horizontally oriented intrinsic axons of pyramidal cells within the monkey motor cortex (Huntley and Jones 1991; Keller 1993). The pattern and extent of intrinsic connections are similar throughout the motor cortex irrespective of topographical representations (Gatter and Powell 1978). In addition, there is little variation in the size of ipsilateral corticocortical cells within different areas of the sensorimotor cortex, whereas the size of corticospinal cell varies considerably from area-to-area and within the same area of the sensorimotor cortex (Jones and Wise 1977). The relatively uniform properties of corticocortical pyramidal cells and their connections across the different areas of the motor cortex are consistent with the remarkably constant threshold for ICF in the different muscle representations.

Implications for studies of ICI and ICF

Assessment of intracortical excitability in the proximal arm, truncal, and lower limb representations of the motor cortex have many potential applications, especially when noninvasively evaluating the mechanisms of plasticity (Chen et al. 1998; Cohen et al. 1998; Lipert et al. 1998; Ziemann et al. 1998a). The finding that the minimum CS intensities necessary to elicit ICI and ICF are not strongly related to MT have implications for studies of intracortical excitability. CS is usually set as a fixed percentage of MT because MT varies widely among subjects and CS is adjusted to a level below that expected to produce spinal effects (Rothwell 1996). The MT is altered in some conditions, such as amputation (Chen et al. 1998) or stroke (Binkofski et al. 1996; Rapisarda et al. 1996; Turton et al. 1996), or with administration of drugs, such as carbamazepine (Ziemann et al. 1996b) or phenytoin (Chen et al. 1997). Testing with CS intensities adjusted for changes in MT can produce results different from testing at CS intensities without such adjustment. Therefore testing with the same CS intensity (Ziemann et al. 1996b), in addition to testing with MT-adjusted CS intensity, can provide additional information. Similar consideration also applies for interhemispheric comparisons and for comparing patients and normal subjects if there are differences in MT between the two populations.

For every muscle studied, there is a range of CS intensities in which the degree of inhibition is near maximum and relatively constant (Figs. 5A and 7A). This range of CS intensities is higher (as percent of MT) for muscles with low MT than for muscles with high MTs. For all muscles except AH with the figure-of-eight coil, CS of 90% active MT achieved near maximum inhibition. For ICI studies that require repeated measurements in the same subject or comparison between different subjects, it may be desirable to use the middle of this range of CS intensity because slight variation in the CS intensity used will have little effect on the degree of inhibition.

In conclusion, we found that ICI and ICF can be demonstrated in proximal arm, trunk, and lower limb muscles, in addition to hand muscles. ICI can be elicited at lower CS intensities than ICF in all the muscles tested. The absolute CS intensities required to elicit ICI or ICF appears unrelated to the strength of corticospinal projection.

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