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Cortical and spinal motor excitability during the transcranial magnetic stimulation silent period in humans

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We investigated the electromyographic silent period in abductor pollicis brevis (APB) and flexor carpi radialis muscles following transcranial magnetic stimulation of human motor cortex. In APB, we measured cortical stimulation silent period (CSSP) duration as a function of stimulus intensity, motor-evoked potential (MEP) amplitude and muscle twitch force. We used peri-stimulus–time histograms to study the effect of cortical stimulation on single-motor unit firing patterns. We compared F-waves, H-reflexes and magnetic MEPs elicited during the CSSP to control responses elicited at rest and during voluntary contraction. CSSP duration depended on the intensity of cortical stimulation. However, we found no relationship between CSSP duration and MEP amplitude or muscle twitch force, thus the CSSP is not dependent solely on Renshaw cell inhibition or on changes in Ia and Ib afferent activity following the cortically induced muscle twitch. At low intensities of stimulation, the interval to resumption of motor unit firing following the peak in the peri-stimulus–time histogram corresponding to MEP latency sometimes exceeded that which could be accounted for by the motor unit's firing rate prior to the stimulus, suggesting that synchronization of motor unit firing by cortical stimulation cannot account for the CSSP. We found brief inhibition of F-waves during the CSSP in some subjects, reflecting activation of inhibitory corticospinal projections or segmental effects. In contrast, we observed longer inhibition of H-reflexes during the CSSP in all subjects, perhaps resulting from presynaptic inhibition of Ia afferents. Magnetic MEPs also were inhibited during the CSSP, suggesting inhibition of cortical elements by transcranial magnetic stimulation.

INTRODUCTION

Transcranial electrical or magnetic stimulation of motor cortex during voluntary contraction produces a direct electromyographic (EMG) response, the motor-evoked potential (MEP), followed by a period of EMG silence[3,7,9,18,27,33,35,56,57]. Numerous mechanisms have been proposed to mediate the inhibitory effects of stimulation of human motor cortex, including: (1) alterations in segmental spinal motor neuron excitability and muscle afferent function related to the muscle twitch accompanying the MEP[7,9,35]; (2) Renshaw cell inhibition[7,9,36]; (3) activation of intracortical[3,4,9,12,56,57,61] or corticospinal[9,13,33,56,57] inhibitory projections; (4) interruption of cortical command signals mediating voluntary contraction[19]. Previous reports have suggested that studies of the EMG silent period following transcranial magnetic stimulation may provide important insights into motor system pathophysiology[8,27,33,55,56,58]. We therefore undertook this study to characterize the excitability of the human motor system during the EMG silent period following transcranial magnetic stimulation, in an attempt to discern evidence for the contribution of spinal segmental, corticospinal or intracortical inhibitory mechanisms to this phenomenon.

A preliminary account of this work has been published elsewhere[15].

MATERIALS AND METHODS

Nine healthy volunteers (eight men) between 29 and 55 years of age were the subjects of these investigations. All subjects gave informed consent.

We used the Magstim 200 (Magstim, UK) magnetic stimulator with a 9-cm coil centered and fixed over the vertex of the scalp. Current direction in the circular coil was counterclockwise for preferential activation of the left hemisphere[42]. The magnetic pulse shape was largely monophasic with a rise time of less than 100 μs and a duration of less than 1 ms. We determined resting motor threshold in each experimental session for each individual subject, in 5% increments of magnetic stimulator output, as the minimum stimulus intensity which produced at least three motor-evoked po-

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tentials (MEPs) in six consecutive stimulations, using a gain of 100 $\mu V \cdot cm^{-1}$.

Subjects were seated comfortably in a chair. For studies of the abductor pollicis brevis (APB) muscle, each subject's right arm was immobilized using a removable fiberglass resin cast, extending from the distal interphalangeal joints of the hand to the mid-upper arm. An opening permitted free abduction of the thumb. To quantitate abduction, we connected a plastic ring placed around the thumb to a strain gauge (Kulite Semiconductor Products, Leonia, NJ, USA). For studies of the flexor carpi radialis (FCR) muscle, the supinated forearm was fixed to the table using velcro straps positioned proximal to the wrist and distal to the elbow, with the strain gauge resting against the subject's palm. We placed Ag/AgCl surface electrodes over the APB or FCR muscles in a belly–tendon arrangement and used a TE4 Electromyograph (TECA, Pleasantville, NY) to filter EMG signals with a band pass of 16 Hz to 16 kHz. Filtered EMG and analog signals from the strain gauge via a bridge amplifier were digitized with a DAS-20 A/D converter (Keithley Metrabyte, Taunton, MA) coupled to an IBM-compatible computer sampling at 10,000 per s for 400 ms. Data were inspected on-line and stored on the hard drive of the computer for analysis. In some instances the EMG signal was rectified and averaged using a Mystro electromyograph (TECA).

Inhibition of electromyographic activity with transcranial magnetic stimulation

We studied the relationship between the duration of the EMG cortical stimulation silent period (CSSP) and the intensity of cortical stimulation, as well as the relationship between the duration of the CSSP and the amplitude of the accompanying muscle twitch force in APB. Subjects used visual feedback to maintain voluntary contraction at 10% of maximum force while receiving cortical stimulation every 10 s. We started stimulation at 10% of stimulator output below resting motor threshold and increased intensity in 5% increments of stimulator output after every ten stimulations to a maximum of 25% above resting motor threshold. Since the MEP may obscure the development of the CSSP and we have previously described CSSPs in patients with motor neuron disease and absent MEPs[56], we measured CSSP duration from the time of cortical stimulation to the visible resumption of EMG activity following the CSSP, using a gain of 100 $\mu V \cdot cm^{-1}$. This method of determining CSSP duration is at variance with previous reports, most of which determined CSSP duration from the beginning or the end of the MEP. At 25% above resting motor threshold, we also measured peak-to-peak MEP amplitude and used simple regression analysis to examine CSSP duration as a function of the amplitude of the MEP and cortically evoked muscle twitch force for 20 consecutive stimulations. We then asked subjects to attempt to shorten the CSSP by resuming contraction as rapidly as possible following the cortical stimulus and used the Mann–Whitney *U*-test to compare ten CSSPs with and without the subjects attempting to shorten the CSSP; $P < 0.05$ significant. For the FCR muscle, we determined the duration of the CSSP at 25% above resting motor threshold during voluntary contraction at 10% of maximal force for comparison with APB.

Single-motor unit studies

In four subjects, we examined the effects of transcranial magnetic stimulation on single-motor unit firing patterns. We recorded single motor units in deltoid, FCR, APB or abductor digiti minimi muscles during voluntary activation at a rate of approximately 10 per s using fine concentric needle electrodes. We used the 9-cm mean diameter circular coil centered over the vertex in two subjects; in the other two subjects we used a figure-8 coil with a 6-cm mean lobe diameter (Magstim) centered over the contralateral hand area. We adjusted stimulus intensity between 5–15% of stimulator output below resting motor threshold to avoid producing compound responses (MEPs). Single-motor unit EMG signals were filtered, displayed and digitalized during at least 100 randomly timed cortical stimulations, and stored on digital audio tape for offline analysis.

After careful inspection of all motor unit potentials, we constructed 1-ms bin width peristimulus time histograms of each single-motor unit during the 300-ms intervals before and after cortical stimulation. We used visual inspection of the peristimulus time histogram to arbitrarily define a 5-ms peak of motor unit firing following cortical stimulation, the latency of which approximated the latency of MEPs obtained in the same muscle with stronger intensities of stimulation. We then extracted data from the poststimulus histogram, leaving only those samplings of motor unit firing in which the motor unit fired during the defined poststimulus peak. This modified poststimulus histogram thus displayed the interval to the resumption of motor unit firing after the unit discharged in response to the cortical stimulus. In order to compare this interval to the unit's behavior prior to the cortical stimulus, we created single-motor unit interval histograms relative to the first firing in each series of three consecutive motor unit discharges during the sampled prestimulus time intervals. We then contrasted the interval to resumption of motor unit firing following the poststimulation peak, with that which could be accounted for by the prestimulus firing interval histogram.

H-reflex studies

In four subjects, we studied the effects of preceding transcranial magnetic stimulation at 25% above resting motor threshold on the amplitude of an H-reflex in FCR. We elicited H-reflexes in this muscle using electrical stimulation (square wave pulse, stimulus duration 1.0 ms) of the median nerve in the cubital fossa. In one subject, we also studied an H-reflex in APB using electrical stimulation of the median nerve at the wrist. In most subjects, an APB H-reflex is demonstrable during voluntary contraction, but is uncommonly observed at rest[6]. We adjusted stimulus intensity to obtain an H-reflex of maximal amplitude with the subject at rest, without a preceding M response. We used interstimulus intervals of 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 ms in random sequence to match the development of and recovery from the CSSP. Subjects received an electrical stimulus to the median nerve to elicit a control H-reflex, followed 10 s later by cortical stimulation paired with the same electrical H-reflex stimulus. Subjects alternated between resting and voluntary contraction at 10% of maximum force after each stimulus pair. This process of pairing cortical stimulation with H-reflexes was continued for $n = 10$ for each interval during rest and voluntary contraction. In one subject, we only collected responses at rest. We expressed the peak-to-peak amplitude of each H-reflex elicited after cortical stimulation as a percentage of the amplitude of the control H-reflex obtained 10 s earlier. We also expressed the peak-to-peak amplitude of each H-reflex elicited after cortical stimulation during voluntary contraction as a percentage of the amplitude of the preceding resting control H-reflexes.

In a separate experiment in the subject with an H-reflex in APB, we compared peak-to-peak amplitudes of H-reflexes ($n = 20$) elicited 25 ms after cortical stimulation with the subject at rest and during voluntary contraction.

We used the Mann–Whitney *U*-test to compare H-reflex amplitudes; $P < 0.05$ significant.

F-wave studies

In six subjects, we studied the effects of preceding cortical stimulation at 25% above resting motor threshold on the amplitude of F-waves in APB. We paired cortical stimulation and supramaximal electrical stimulation (duration 0.1 ms) of the median nerve at the wrist using the same 12 interstimulus intervals used in the H-reflex studies (25–300 ms). In two subjects, only three interstimulus intervals were used. As each interval was selected in random sequence, we collected four F-waves paired with preceding cortical stimulation every 5 s during voluntary contraction at 10% of maximum force. We also elicited control F-waves elicited at rest without preceding cortical stimulation. We collected 20 F-waves at each interstimulus interval and 20 F-waves for resting controls. We compared peak-to-peak F-wave amplitudes for each interstimulus interval to resting control F-wave amplitudes using the Mann–Whitney *U*-test; $P < 0.05$ significant.

Excitability of corticospinal pathways

In four subjects in APB and FCR muscles, we studied the effects of preceding transcranial magnetic stimulation at 25% above resting

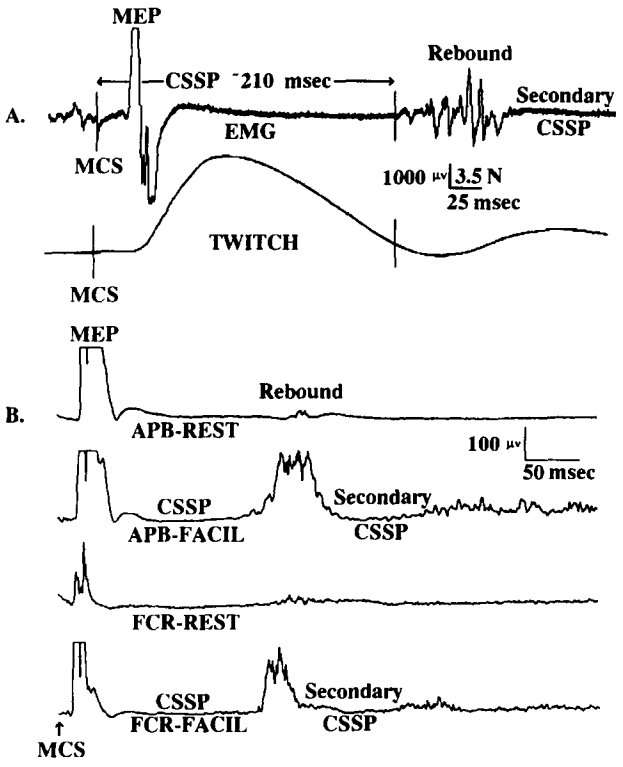

Fig. 1 A: digitalized surface electromyography (EMG, upper trace) and force recording (twitch, lower trace) following transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold (MCS) during voluntary contraction of abductor pollicis brevis at 10% of maximum force, showing the cortical stimulation silent period (CSSP) and muscle twitch force accompanying the motor-evoked potential. The motor-evoked potential (MEP) is clipped at the gain used in the illustration. Note that the falling phase of the cortically evoked muscle twitch occurs well in advance of the rebound phase of excitation terminating the CSSP. B: rectified averaged ($n = 10$) surface electromyography recorded at rest (1st and 3rd traces) and during mild voluntary contraction (FACIL; 2nd and 4th traces) of the abductor pollicis brevis (APB; 1st and 2nd traces) and flexor carpi radialis (FCR; 3rd and 4th traces) muscles showing silent periods and 'rebound' late responses following MCS.

motor threshold on the amplitude of responses to transcranial magnetic stimulation at the same intensity of stimulation. We used a BiStim device (Magstim) to couple the output from two Magstim 200 magnetic stimulators through the same 9-cm coil at interstimulus intervals of 25–300 ms. Subjects alternated between resting and voluntary contraction while this process of paired cortical stimulation was repeated for $n = 10$ for each interstimulus interval. We expressed the peak-to-peak amplitude of each MEP elicited by the second cortical stimulus as a percentage of the amplitude of the immediately preceding (control) MEP. The amplitude of each MEP elicited by a second cortical stimulus during voluntary contraction was also expressed as a percentage of the amplitude of the previous first (control) MEP elicited at rest. We used the Mann–Whitney U-test to compare MEP amplitudes; $P < 0.05$ significant.

RESULTS

Inhibition of electromyographic activity with transcranial magnetic stimulation

Transcranial magnetic stimulation caused a period of EMG silence in APB and FCR in all subjects. At low intensities of stimulation, in single sweeps we sometimes observed brief periods of EMG inhibition without an apparent preceding MEP. However, we never observed a convincing CSSP without a preceding peak of facilitation corresponding to a cortically evoked MEP if the EMG was rectified and multiple sweeps were averaged. As shown in Fig. 1, the CSSP was typically terminated by a rebound period of facilitation most evident in rectified and averaged EMG recordings, the timing of which at 25% above resting motor threshold tended to correlate with the onset of long latency (> 200 ms) 'rebound' responses to cortical stimulation frequently observed in resting subjects[7,35]. Termination of the CSSP was often followed by a second or even multiple periods of inhibition of progressively shorter duration.

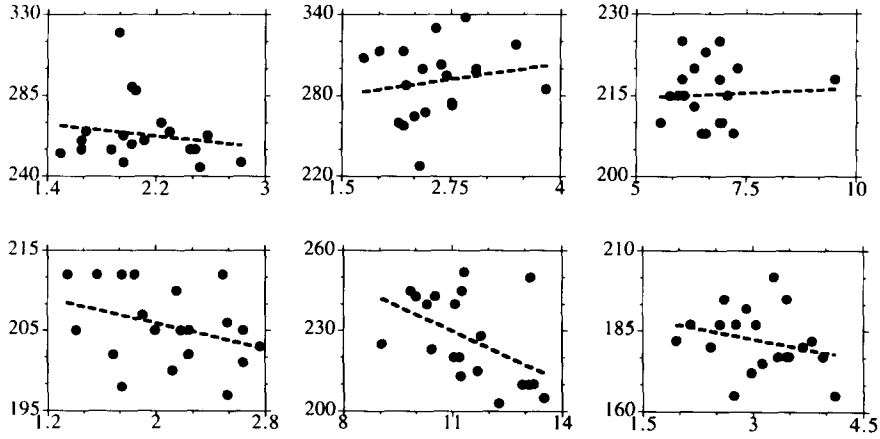

Fig. 2. x-axis = the amplitude of the muscle twitch (newtons) following the motor-evoked potential; y-axis = the duration of the cortical stimulation silent period (ms) in the abductor pollicis brevis muscle. Each graph represents 20 consecutive stimulations at 25% of stimulator output above each subject's resting motor threshold during voluntary contraction at 10% of maximum force. Note absence of significant correlation in all six subjects.

The duration of the APB CSSP during voluntary contraction increased linearly as a function of the intensity of cortical stimulation. At 25% above resting motor threshold, the duration of this CSSP in six subjects was 231 ± 40 (mean ± SD, range 165–338) ms. CSSP duration decreased to 185 ± 36 ms ($P < 0.001$) when subjects attempted to resume contraction as rapidly as possible following the cortical stimulus. However, the strength of voluntary contraction prior to cortical stimulation had no definite effect on the duration of the CSSP. At 25% above motor threshold, the duration of the FCR CSSP in five subjects was 197 ± 10 (185–210) ms, mean 35 (range 10–70) ms shorter than in APB.

The amplitude of the muscle twitch force (Fig. 1A) produced by the cortically evoked MEP in APB also increased as a function of the intensity of cortical stimulation. Both MEP amplitude and twitch force showed marked variability at constant stimulus intensity 25% above motor threshold. The relationship between muscle twitch force and CSSP duration is illustrated in Fig. 2. We found no consistent relationship between either MEP amplitude or muscle twitch force and CSSP duration at 25% above motor threshold.

Single-motor unit studies

In all four subjects, transcranial magnetic stimulation caused peaks of motor unit firing, the latencies of which corresponded with MEP latencies, i.e. 17 ms in deltoid and 20–23 ms in FCR, APB and abductor digiti minimi muscles. Prior to cortical stimulation, motor unit firing intervals ranged from 75–100 ms (firing rates of 10–13 per s). In several motor units, the interval to resumption of firing after the motor unit had fired in response to the cortical stimulus was greater than could be accounted for by the unit's firing intervals prior to cortical stimulation (Fig. 3).

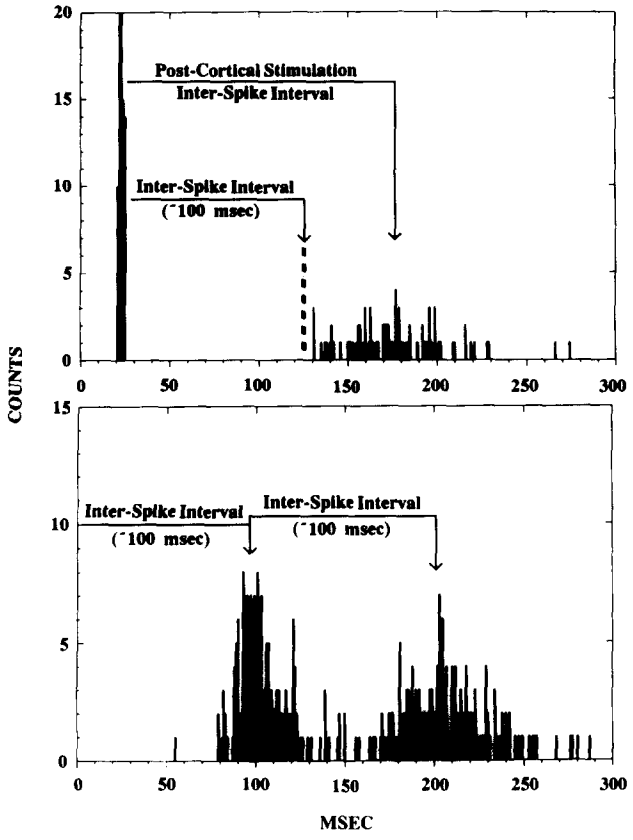

Fig. 3. Upper panel: post-stimulus–time histogram of single-motor unit firing in abductor digiti minimi muscle using focal transcranial magnetic stimulation of the contralateral motor cortex (time 0) during voluntary firing at about 10 per s. x-axis = 300 ms following cortical stimulation, divided into 1-ms width bins; y-axis = motor unit counts per 1-ms bin. The data have been extracted to include only the sampled cortical stimulations after which the motor unit fired at a latency consistent with a direct response to the stimulus. The dashed line indicates when motor unit firing should have resumed following a direct response to cortical stimulation, based on motor unit firing intervals prior to the cortical stimulus (see below). Lower panel: histogram of single-motor unit firing intervals, constructed using each series of three consecutive motor unit discharges *prior* to the cortical stimulus and arbitrarily setting the first discharge in each series to time 0. x-axis = 300 ms, divided into 1-ms bins; y-axis = motor unit counts per 1-ms bin. At a firing rate of approximately 10 per s, these consecutive firing intervals are approximately 100 ms.

H-reflex studies

At rest, transcranial magnetic stimulation at 25% above motor threshold decreased the size of H-reflexes elicited 75–100 ms later (Fig. 4A), with statistically significant inhibition beginning 75–100 ms after cortical stimulation. Recovery of the H-reflex to control levels was typically followed by facilitation between 200–250 ms after cortical stimulation, correlating with the onset of long-latency responses following cortical stimulation (see above and Fig. 1). During the CSSP, H-reflexes were inhibited relative to control H-reflexes elicited during voluntary contraction and at rest (Fig. 4). This inhibition was evident by 50 ms after cortical stimulation in most experiments, 25–50 ms earlier than occurred following cortical stimulation at rest. H-reflex inhibition induced by cortical stimulation appeared greater during voluntary contraction than at rest. Recovery of the H-reflex was followed by facilitation 175–225 ms after the cortical stimulus, often even relative to control H-reflexes facilitated by voluntary contraction. This facilitation was coincident with the peak of rebound facilitation observed to terminate the CSSP in rectified averaged EMG recordings (Fig. 1) and H-reflex recovery typically approximated the duration of the CSSP less H-reflex latency. *However, in one subject, H-reflex recovery preceded the end of the CSSP*[27].

In the subject with an H-reflex in APB, cortical stimulation during voluntary contraction had no effect or decreased the amplitude of H-reflexes elicited 25 ms

later; this same cortical stimulus given at rest caused H-reflex facilitation (Fig. 4A). In a separate experiment, the amplitude of APB H-reflexes elicited 25 ms after cortical stimulation at rest was significantly greater than the amplitude of H-reflexes elicited 25 ms after cortical stimulation during voluntary contraction (cortical stimulation at rest: 2022 ± 219 μV vs. cortical stimulation during voluntary contraction: 1710 ± 307 μV; mean ± S.D., $n = 20$, $P < 0.001$).

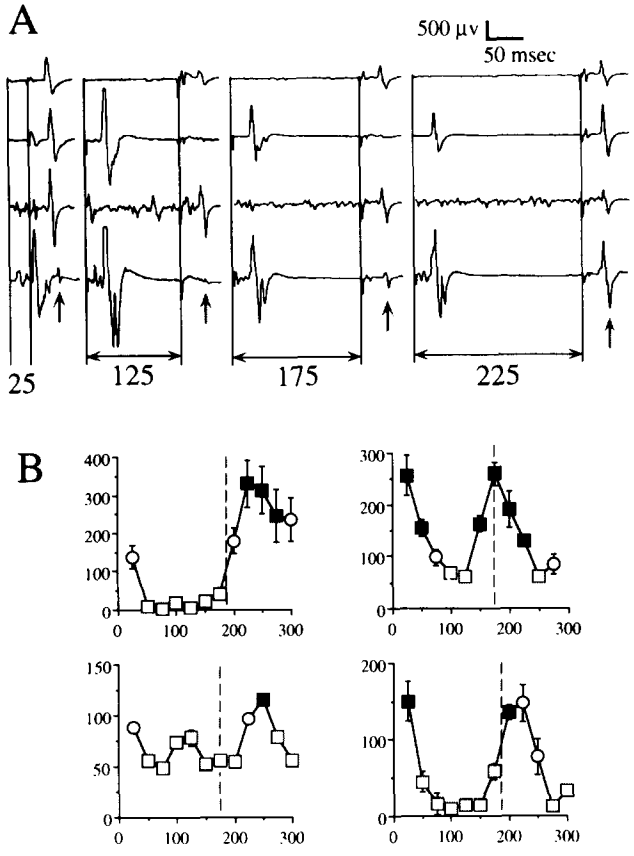

Fig. 4. A: surface electromyography at rest (upper two traces) and during voluntary contraction (lower two traces) of the abductor pollicis brevis muscle at 10% of maximum force, illustrating the effects of preceding transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold (2nd and 4th traces) on the amplitude of the H-reflex elicited 25, 125, 175 and 225 ms later. The effects of cortical stimulation on the amplitude of the H-reflex (vertical arrows) are dependent on both the interstimulus interval and the state of muscle contraction. B: amplitude of the H-reflex following transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold during voluntary contraction at 10% of maximum force in abductor pollicis brevis (upper left graph) and flexor carpi radialis muscles. x-axis = cortical stimulation – H-reflex interstimulus interval (ms); y-axis = H-reflex amplitude relative to control H-reflexes elicited at rest. Each data point represents the mean % of control amplitude ± S.E.M. from ten paired stimulations. Data points represent statistically significant ($P < 0.05$) facilitation (■), inhibition (□) or no difference (○) from control. Dotted line represents mean cortical stimulation silent period duration less H-reflex latency.

F-wave studies

F-waves elicited during the CSSP were inhibited relative to F-waves elicited at rest in three of six subjects (Fig. 5). F-wave amplitudes were decreased at 75 ms after cortical stimulation in one subject, at 75, 100 and 125 ms after cortical stimulation in another and at 50, 75 and 125 ms after cortical stimulation in a third. In all three of these subjects, F-waves recovered relative to resting controls well before the end of the CSSP less F-wave latency.

Excitability of corticospinal pathways

Transcranial magnetic stimulation at 25% above resting motor threshold affected the amplitude of subsequent responses to transcranial magnetic stimulation at the same intensity of stimulation (Fig. 6A). Preceding cortical stimulation at rest increased the amplitude of MEPs elicited 25 ms later. This was followed in each case by decrease in amplitude of the second MEP, beginning 50–75 ms after the first cortical stimulus, with statistically significant inhibition of the second MEP in seven of eight experiments. The second MEP began to recover 125–150 and peaked 225–250 ms after the first cortical stimulus, with statistically significant facilitation of the second MEP in six of eight experiments. Secondary inhibition of the MEP beyond 250 ms was rare.

The size of MEPs elicited during the CSSP decreased as a function of the interstimulus interval (Fig. 6). This decrease reached statistical significance relative to control MEPs elicited at rest between 50–75 ms in three of four experiments in APB and two of four experiments in FCR. The size of the MEPs elicited during the CSSP increased at interstimulus intervals 125–150 ms, preceding the end of the CSSP less MEP latency. MEPs elicited during the CSSP were facilitated relative to control MEPs elicited at rest at interstimulus intervals of 200–275 ms. Inhibition of MEPs did not occur beyond 250 ms.

MEPs elicited during the CSSP also were inhibited relative to control MEPs facilitated by voluntary contraction. However, at an interstimulus interval of 25 ms, second MEPs tended to be inhibited in APB but facilitated in FCR. The size of the second MEP subsequently decreased with increasing interstimulus intervals and statistically significant inhibition occurred 25–100 (mean APB 35, mean FCR 81) ms after the first cortical stimulus. Late facilitation of the second MEP relative to MEPs facilitated by voluntary contraction occurred in only four of eight experiments (one APB, three FCR).

DISCUSSION

We have attempted to characterize the EMG silent period following transcranial cortical stimulation and many of our findings confirm those of previous investigators. Previous studies[8,9,27,33,56,57] have shown that the duration of the silent period increases with the intensity of cortical stimulation, as we observed. However, characterization of this phenomenon as the post-MEP silent period[8,9] may be misleading, since an MEP is not necessarily a prerequisite for the inhibition of voluntary contraction by brain stimulation[3,7,9,16,35,56]. Admittedly, this fact is not readily demonstrable with transcranial magnetic stimulation in normal subjects, presumably because such gross stimulation activates both excitatory and inhibitory intracortical projections, with *relatively* similar thresholds of activation[56].

The CSSP may be due to peripheral mechanoreceptive effects of superimposing a muscle twitch on a voluntary contraction[7,35]. Such effects include a pause in excitatory Ia input from muscle spindles[31] and an increase in inhibitory Ib input to motoneurons[29]. However, we found no relationship between CSSP duration and muscle twitch amplitude, indicating that the CSSP is not due solely to changes in segmental excitability attributable to the muscle twitch. If the CSSP were due to changes in muscle afferent function, then the rebound excitation which ends the CSSP should accompany the falling phase of the muscle twitch with muscle stretch and increased spindle discharge[29], rather than following the muscle twitch (Fig. 1). Rothwell et al.[50] even observed increased muscle spindle discharge after cortical stimulation, suggesting activation of fusimotor neurons by the stimulus. Further, Cantello et al.[9] found that the silent period following cortical stimulation exceeds that with stimulation of peripheral nerve, even when the muscle twitch with nerve stimulation exceeds that with cortical stimulation.

Activation of Renshaw cells via spinal motoneurons[47] may contribute to the silent period following cortical[7,9,36] or peripheral[39,51] stimulation. However, we found no consistent correlation between CSSP duration and MEP amplitude. This is similar to the experience of other investigators using different experimental paradigms[3,9,57]. Since MEP amplitude should reflect the number of simultaneously activated spinal mo-

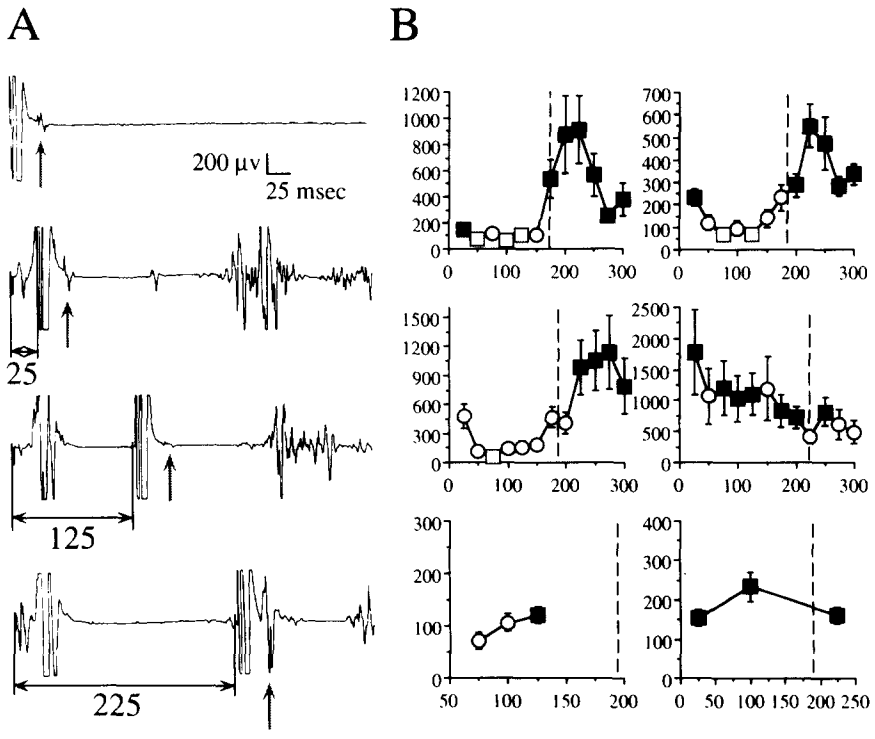

Fig. 5. A: surface electromyography at rest (upper trace) and during voluntary contraction (lower traces) of the abductor pollicis brevis muscle at 10% of maximum force, illustrating the effects of preceding transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold on the amplitude of the F-wave elicited 25, 125 and 225 ms later. In this subject, the effects of cortical stimulation on the amplitude of the F-wave depend on the interstimulus interval. B: amplitude of the F-wave following transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold during voluntary contraction at 10% of maximum force in the abductor pollicis brevis muscle. x-axis = cortical stimulation–F-wave interstimulus interval (ms); y-axis = F-wave amplitude relative to control F-waves elicited at rest. Each data point represents the mean % of control ± S.E.M. from at least 20 paired stimulations. Data points represent statistically significant ($P < 0.05$) facilitation (■), inhibition (□) or no difference (○) from control. Dotted line represents mean cortical stimulation silent period less F-wave latency.

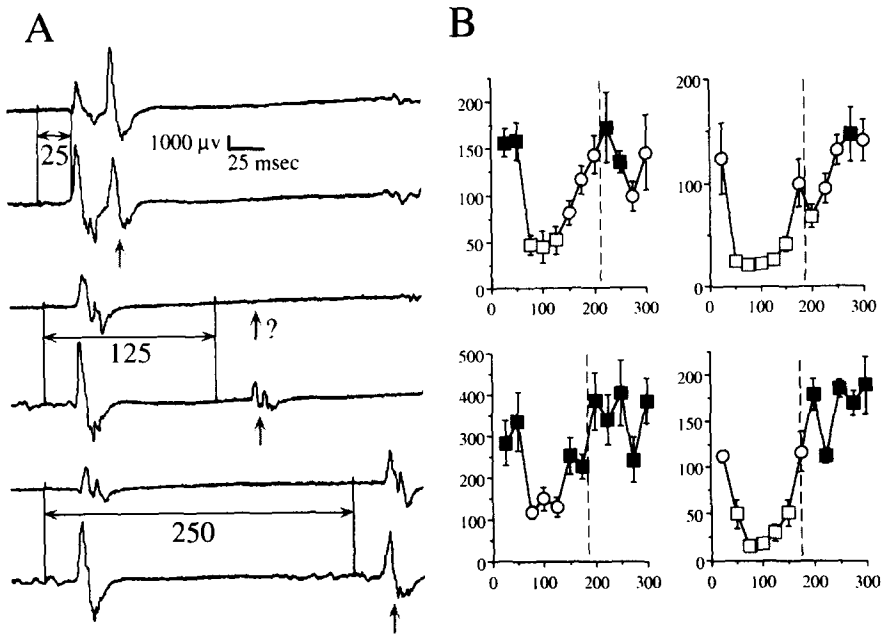

Fig. 6. A: surface electromyography at rest (upper trace in each pair) and during voluntary contraction (lower trace in each pair) of the abductor pollicis brevis muscle at 10% of maximum force, illustrating the effects of paired transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold at interstimulus intervals of 25, 125 and 250 ms. The amplitude of the second motor-evoked potential in each pair (vertical arrows) is dependent on both the interstimulus interval and the state of muscle contraction. B: amplitude of the second motor-evoked potential (MEP) following paired transcranial magnetic stimulation at 25% of stimulator output above resting motor threshold during voluntary contraction at 10% of maximum force in the abductor pollicis brevis muscle. x-axis = interstimulus interval for paired magnetic stimulation (ms); y-axis = amplitude of the second MEP relative to control MEPs elicited at rest. Each data point represents the mean % of control ± S.E.M. from ten paired cortical stimulations. Data represent statistically significant ($P < 0.05$) facilitation (■), inhibition (□) or no difference (○) from control. Dotted line represents mean cortical stimulation silent period less motor-evoked potential latency.

toneurons, this finding suggests that Renshaw cell activation is not a pivotal mechanism in the generation of the CSSP[56]. However, the absence of correlation between MEP amplitude and CSSP duration does not exclude collateral activation of Renshaw cells following the MEP.

If the CSSP is not produced by peripheral afferent activity or Renshaw cell activation, might it be caused simply by synchronization of motor unit discharge? CSSP duration does not correlate with MEP amplitude, suggesting that post-discharge afterhyperpolarization is not a sequelae to facilitation of spinal motoneuron firing. MEP amplitude is increased, while CSSP duration is decreased in parkinsonism[8]. Cortical motoneuron spike-triggered averaging of forearm muscle EMG in monkeys during trained movements typically shows post-spike facilitation without a succeeding period of inhibition, while a facilitation of similar magnitude followed by a period of inhibition or even inhibition without preceding facilitation, may be observed with intracortical microstimulation of the cortical region immediately surrounding the same cortical motoneuron[41]. These observations indicate that synchronization or refractoriness of spinal motoneurons play

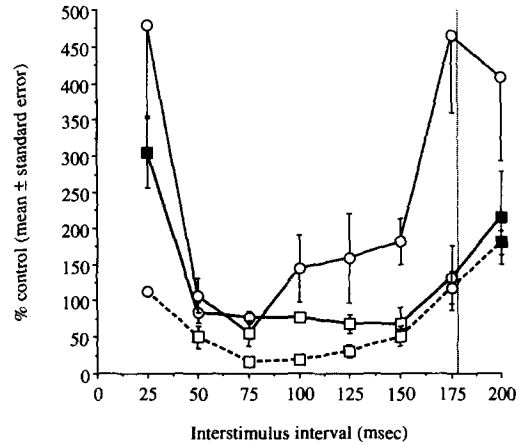

Fig. 7. Effects of transcranial magnetic stimulation given at time 0 on H-reflexes (solid line), F-waves (dotted line), and magnetic motor-evoked potentials (MEPs, dashed line) in a single subject. F-waves and MEPs were elicited in the abductor pollicis brevis muscle while the subject attempted to maintain voluntary contraction at 10% of maximum force. H-reflexes were elicited in the flexor carpi radialis muscle with the subject at rest. x-axis = interstimulus interval (ms); y-axis = amplitude of H-reflexes, F-waves and MEPs elicited after magnetic stimulation, relative to control responses elicited at rest (mean % of control ± S.E.M.). Data represent statistically significant ($P < 0.05$) facilitation (■), inhibition (□) or no difference (○) from control. The dotted vertical line approximates cortical stimulation silent period less H-reflex, F-wave, and MEP latency.

little role in the CSSP. Our analysis of single-motor unit firing following transcranial magnetic stimulation is consistent with this conclusion, since we found that motor units discharging in direct response to a cortical stimulus may show an interval to the resumption of firing after responding to the stimulus in excess of that which could be accounted for by the pre-stimulus firing rate. Other investigators using single-motor unit post-stimulus time histograms[7,46] or surface recordings[3,9,16] have suggested that stimulation of human motor cortex below the threshold for motor unit excitation may inhibit motor unit firing.

We found the H-reflex to be markedly suppressed during the CSSP, as others have observed[9,27]. Inhibition of the H-reflex by cortical stimulation may reflect activation of inhibitory corticospinal projections. Disynaptic inhibition of spinal motoneurons by cells in the primary motor cortex has been documented in monkeys and is probably mediated by the Ia inhibitory interneuron[37,38]. Lemon et al.[41] attributed intracellular excitatory and inhibitory post-synaptic potentials in primate spinal motor neurons following intracortical microstimulation to temporal and spatial summation of descending corticospinal volleys at the interneuronal level. Transcranial electrical stimulation below motor threshold may inhibit an H-reflex in FCR at an interstimulus interval consistent with the existence of similar disynaptic inhibitory corticospinal projections in man[13].

Inhibition of the H-reflex by cortical stimulation could conceivably result from presynaptic depolarization of Ia afferents[28], rather than postsynaptic inhibition of spinal motoneurons. The size principle[34] suggests that recruitment of spinal motoneurons in the H-reflex should favor the small, tonic motoneurons activated during gentle voluntary contraction[14] and by cortical stimulation[13]. In contrast, motoneurons of all sizes may produce F-waves at similar frequencies following antidromic invasion[20,40]. In theory, however, the F-wave is more likely to result from activation of larger motoneurons, particularly since smaller tonic motoneurons may be more subject to recurrent inhibition[22,30]. While the distinction between reflex and antidromic activation of motoneurons is obscured during voluntary activation[32,59], it seems reasonable to distinguish H-reflexes and F-waves elicited during EMG silence. We found that F-waves in APB were unaffected by transcranial magnetic stimulation in three of our subjects, one of whom had an H-reflex in FCR inhibited throughout the CSSP. In our remaining three subjects, F-waves were inhibited by cortical stimulation, but the duration of this inhibition was substantially shorter than that of the H-reflex and was nearly negligible in one of these subjects (Fig. 7). If F-waves do result from activation of larger spinal motoneurons than those recruited in the H-reflex[6], our findings might suggest that cortical stimulation causes preferential inhibition of smaller spinal motoneurons. However, this conclusion would contradict the size principle[34]. Further, we and others[33,57] have observed no effect of increasing the strength of voluntary contraction prior to cortical stimulation on the duration of the CSSP, suggesting that activation of the entire spinal motoneuron pool is equally inhibited by cortical stimulation. Thus, if F-waves do provide a measure of motoneuron excitability[23–26,44,45,54], then the relatively selective inhibitory effects of cortical stimulation on the H-reflex may be mediated by presynaptic inhibition of Ia afferents[28,43]. In animal models, presynaptic inhibition of Ia afferents has been observed to follow electrical stimulation of the brain stem[10] and cerebellum[11] and after muscle stretch[21].

We found that voluntary contraction potentiated the inhibitory effects of transcranial magnetic stimulation on the H-reflex. It seems reasonable to attribute this difference to the effects of muscle stretch on Ia afferents[21]. However, we found that voluntary contraction enhanced H-reflex inhibition 25 ms after cortical stimulation; 5 ms before the onset of the MEP muscle twitch. In APB, cortical stimulation at rest facilitated the H-reflex 25 ms later. However, cortical stimulation during voluntary contraction either facilitated to a lesser degree or even failed to facilitate H-reflexes elicited 25 ms later. Thus, a cortical stimulus given during voluntary contraction produced less facilitation of the H-reflex than the same cortical stimulus at rest, suggesting that voluntary contraction enhances the activation of inhibitory corticospinal projections.

This finding may exemplify yet another inhibitory effect of magnetic stimulation of human cerebral cortex. Other examples include: (1) suprathreshold stimulation of motor cortex delays initiation of movement for up to 150 ms[19]; (2) subthreshold cortical stimulation influences motor programming[4] and activates intracortical inhibitory circuits[49]; (3) stimulation of ipsilateral motor cortex has inhibitory effects on motor unit activity[12,61]; (4) stimulation of premotor cortex has complex inhibitory effects on motor processing[1]; (5) stimulation of occipital cortex suppresses visual perception for 60–100 ms[2]. Electrical stimulation of the corticospinal tract[52] or the cortical surface[48] produces IPSPs in feline cortical motoneurons. This hyperpolarization occurs independently of antidromic invasion, and is attenuated by anesthesia, suggesting interneuron-mediated recurrent collateral inhibition of cortical motoneurons[52]. Cortical stimulation may produce IPSPs with-

out preceding spike discharge[48], thus changes in cortical motoneuron membrane potentials do not reflect afterpotentials. Hyperpolarization of cortical motoneurons may last for 80–100 ms after the stimulus[48,52], implying spatial and temporal summation of IPSPs. Clearly, intracortical inhibitory effects of cortical stimulation may play a role in the CSSP[3,9,16,27,56,57].

We assessed the excitability of the cortical motor elements activated by transcranial magnetic stimulation during the CSSP. We found that MEPs were frequently inhibited during the CSSP, relative to MEPs elicited at rest, indicating inhibition rather than disfacilitation of corticospinal elements. We also found that inhibition of responses to paired magnetic stimulation at rest seemed to correspond to inhibition of voluntary EMG by a single magnetic stimulus, as Valls-Solé et al.[60] observed. Conceivably, these findings could result from changes in spinal or cortical excitability. However, the duration of inhibition of MEPs during the CSSP exceeded that of F-waves, in those subjects in whom F-waves were inhibited at all (Fig. 7), suggesting that inhibition of MEPs reflects changes in cortical excitability or corticospinal input.

Inhibition of magnetic MEPs during the CSSP relative to magnetic MEPs elicited at rest probably indicates cortical inhibition, since the results of electrical brain stimulation indicate that spinal excitability is not decreased by transcranial magnetic stimulation[18,19]. Day et al.[17] suggest that magnetic stimulation produces preferentially indirect, trans-synaptic activation of human cortical motoneurons, whilst electrical stimulation produces preferentially direct cortical motoneuron activation. Thus, recovery of magnetic MEPs prior to the end of the CSSP may indicate temporal and spatial summation of voluntary command signals mediating voluntary contraction with the excitatory effects of the second magnetic stimulus, at the level of the cortical motoneuron. However, magnetic stimulation also may be capable of direct activation of human cortical motoneurons at high intensities of stimulation[5,53] and thus bypassing a presumed locus of cortical inhibition during the CSSP. It seems reasonable to suggest that either direct activation of cortical motoneurons or perhaps increased summation of synaptic inputs from voluntary motor command with excitatory cortical afferent inputs activated by the first magnetic stimulus may account for our observations in which MEPs elicited during the CSSP were inhibited relative to MEPs elicited during voluntary contraction, but not relative to MEPs elicited at rest.

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