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CORTICOCORTICAL INHIBITION IN HUMAN MOTOR CORTEX

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SUMMARY

1. In ten normal volunteers, a transcranial magnetic or electric stimulus that was subthreshold for evoking an EMG response in relaxed muscles was used to condition responses evoked by a later, suprathreshold magnetic or electric test shock. In most experiments the test stimulus was given to the lateral part of the motor strip in order to evoke EMG responses in the first dorsal interosseous muscle (FDI).

2. A magnetic conditioning stimulus over the hand area of cortex could suppress responses produced in the relaxed FDI by a suprathreshold magnetic test stimulus at interstimulus intervals of 1–6 ms. At interstimulus intervals of 10 and 15 ms, the test response was facilitated.

3. Using a focal magnetic stimulus we explored the effects of moving the conditioning stimulus to different scalp locations while maintaining the magnetic test coil at one site. If the conditioning coil was moved anterior or posterior to the motor strip there was less suppression of test responses in the FDI. In contrast, stimulation at the vertex could suppress FDI responses by an amount comparable to that seen with stimulation over the hand area. With the positions of the two coils reversed, conditioning stimuli over the hand area suppressed responses evoked in leg muscles by vertex test shocks.

4. The intensity of both conditioning and test shocks influenced the amount of suppression. Small test responses were more readily suppressed than large responses. The best suppression was seen with small conditioning stimuli (0·7–0·9 times motor threshold in relaxed muscle); increasing the intensity to motor threshold or above resulted in less suppression or even facilitation.

5. Two experiments suggested that the suppression was produced by an action

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on cortical, rather than spinal excitability. First, a magnetic conditioning stimulus over the hand area failed to produce any suppression of responses evoked in active hand muscles by a small (approximately 200 V, 50 μs time constant) anodal electric test shock. Second, a vertex conditioning shock had no effect on forearm flexor H reflexes even though responses in the same muscles produced by magnetic cortical test shocks were readily suppressed at appropriate interstimulus intervals.

6. Small anodal electric conditioning stimuli were much less effective in suppressing magnetic test responses than either magnetic or cathodal electric conditioning shocks. This suggests that neither refractoriness of corticospinal axons nor activity in pyramidal recurrent collaterals was important in producing suppression.

7. The results are consistent with the idea that a weak magnetic conditioning stimulus over the motor cortex can engage intracortical inhibitory circuits. The possible relationship to previously described inhibitory effects from motor cortex stimulation in man and GABAergic inhibitory mechanisms in animals is discussed.

INTRODUCTION

In two previous papers we have used transcranial stimulation with either two magnetic stimulators or with an electric and a magnetic stimulator to demonstrate in normal conscious man probable inhibitory projections to the motor cortex from both the cerebellum (Ugawa, Day, Rothwell, Thompson, Merton & Marsden, 1991) and the contralateral sensorimotor strip (Ferbert, Priori, Rothwell, Day, Colebatch & Marsden, 1992). In the present experiments, we have used a conditioning–test protocol to provide evidence for the existence of an inhibitory action between areas of the motor strip itself. Such inhibition has been described in detail in animal experiments (Krnjević, Randić & Straughan, 1966*a*,*b*,*c*), who showed that a single stimulus to the exposed cortex of cats, rabbits or monkeys could reduce the excitability of cortical neurones for up to 300 ms. They concluded that the major part of the effect was produced by activity in an intracortical inhibitory system. The present results show that a similar inhibitory effect can be seen in conscious man. Some of this work has been published previously in abstract form (Day *et al.* 1987; Rothwell, Ferbert, Caramia, Kujirai, Day & Thompson, 1991; Kujirai, Rothwell, Fong, Thompson & Day, 1993).

METHODS

The experiments were performed with the approval of the local ethical committee on ten normal healthy volunteers (9 men and 1 woman) aged from 27 to 43 years old.

All subjects were seated in a comfortable reclining chair and surface EMG recordings were made from the first dorsal interosseous (FDI) muscle with the active electrode placed over the motor point and the reference electrode on the metacarpophalangeal joint. In some subjects, we also recorded EMG responses from surface electrodes over the tibialis anterior muscle, with the active electrode near the motor point and the reference electrode placed at the head of the fibula, or from the flexor muscles in the forearm, with electrodes placed 3 cm apart over the belly of the flexor carpi radialis muscle. Responses were amplified and filtered by Digitimer D150 amplifiers (Digitimer Ltd, Welwyn Garden City, Herts) with a time constant of 10 ms, and a low-pass filter set at 3 kHz.

Transcranial stimulation was produced using Magstim 200 magnetic stimulators (The Magstim Company, Whitland, Dyfed) or a Digitimer D180 high-voltage electric stimulator. Magnetic stimulation was given either through figure-of-eight-shaped coils, with each loop of the coil

having an outer diameter of 9 cm and a peak magnetic field of 2·4 T or a large round coil 14 cm in external diameter (peak magnetic field 2 T). In most cases we have expressed stimulus intensity relative to relaxed threshold. This is the intensity needed to evoke a minimal EMG response ($> 100\ \mu V$) in 50 % of trials in relaxed subjects. In most individuals this was about 40 % of the maximum output of the stimulator. Threshold was checked throughout the experiments and usually varied by less than 5 % of the stimulator output. In some experiments, both magnetic stimulators were connected to the same stimulating coil through a Bistim module (The Magstim Co., Whitland, Dyfed). Electrical stimulation was given through two 9 mm diameter Ag–AgCl EEG electrodes fixed to the scalp with collodion. In some subjects, H reflexes were elicited in relaxed flexor muscles in the forearm by low-intensity electric stimulation of the median nerve in the cubital fossa.

In order to investigate the time course of the effects under study, we used a conditioning–test design. The test stimulus was applied over the hand area of the motor cortex, to evoke EMG responses in the contralateral first dorsal interosseous muscle. The conditioning stimulus was applied at the same or different points on the scalp at various times beforehand. A positive conditioning-test interval indicates that the conditioning stimulus came before the test stimulus. In each set of experiments, test and conditioning shocks at different intervals were randomly intermixed and given at intervals of 4–5·5 s. Ten responses per condition were collected and averaged and their peak-to-peak amplitude was measured. Several blocks of trials were performed in order to construct a complete time course. A block consisted of ten trials each of three to six randomized conditions: the response to a test shock given alone, and the response to the same shock when conditioned by a prior stimulus at different conditioning–test intervals. In some experiments we constructed separate time courses using different intensities of conditioning shock, or different positions of the conditioning stimulator. In these cases, a complete time course was made at each intensity or position before increasing the conditioning strength or moving the coil for the next block of trials. Experiments in which different types of conditioning or test stimuli (i.e. magnetic, anodal or cathodal) were compared used a randomized design. At each interstimulus interval studied, we randomized presentation of the different conditioning and test stimuli. In all experiments, the peak-to-peak size of conditioned responses was expressed as a percentage of the size of the unconditioned response (equals 100 %). Signals were collected through a CED 1401 laboratory interface (Cambridge Electronic Design, Cambridge) and fed to a personal computer using data collection and averaging programmes (sampling rate of 5–7 kHz per channel) modified to perform conditional averaging.

In three subjects we conducted experiments on single motor units in the FDI muscle, recorded using concentric needle electrodes (Medelec disposable type DMC25). The details of the technique are given in Day, Dressler, Maertens de Noordhout, Marsden, Nakashima, Rothwell & Thompson (1989*a*). Briefly, subjects voluntarily discharged the unit at about 10 Hz whilst they received magnetic test stimuli every 4–5 s over the contralateral motor cortex. In random trials, this stimulus was preceded by a conditioning stimulus (over the vertex) given 1 or 3 ms earlier. Three different conditions were intermixed: the test stimulus given alone, and the test stimulus conditioned at two different intervals. One hundred trials of each condition were collected and post-stimulus time histograms (PSTH) of unit discharge were constructed.

RESULTS

The principal finding is illustrated in Fig. 1. In this experiment, two magnetic stimuli were given through the same figure-of-eight coil held so that the electric current in the junction region flowed in the anterior to posterior direction over the lateral part of the motor strip. Subjects were relaxed and the conditioning stimulus was adjusted to be of submotor threshold intensity. The test stimulus was supra-motor threshold and produced an EMG response of about 1·5 mV peak-to-peak amplitude. When both stimuli were given, separated by 1–6 ms, the size of the response to the test stimulus was reduced. The time course of this effect is shown for six subjects in Fig. 1*B*. Suppression was followed by excitation at interstimulus intervals of 10 and 15 ms. Despite the fact that suppression was prominent at

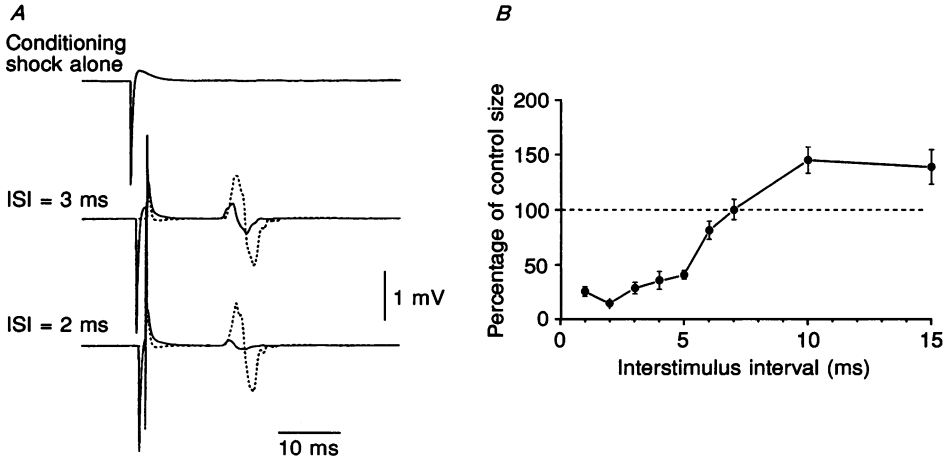

Fig. 1. EMG responses to magnetic cortical stimulation in relaxed first dorsal interosseous are inhibited by a prior, subthreshold, magnetic conditioning stimulus. *A* shows examples of EMG data from a single subject. The first trace shows absence of any responses to the conditioning stimulus given alone. The lower two records have two superimposed traces, the response to the test stimulus given alone, and the response to the test stimulus when given 3 (middle traces) or 2 ms (lower traces) after a conditioning stimulus. The larger of the two traces (dotted line) is the response to the test stimulus alone. It is dramatically suppressed at these two interstimulus intervals (ISI). Note the shorter latency of the conditioned response at an ISI = 3 ms. In this and subsequent figures, each trace is the average of 10 sweeps. *B* shows the mean (± S.E.M.) time course of suppression in 10 subjects. At each interstimulus interval, the size of the conditioned responses is expressed as a percentage of the size of the control response. In both *A* and *B*, the conditioning and test stimuli were given through the same figure-of-eight coil oriented so that electric current in the junction region flowed from anterior to posterior over the lateral part of the motor cortex.

short interstimulus intervals, we frequently observed that the small conditioned response had a shorter latency than the response to the test shock given alone. This can be seen in Fig. 1*A* (and Fig. 4*A*) at an interstimulus interval of 3 ms. The large control response begins about 1 ms later than the smaller conditioned response.

Magnetic versus electric test shocks

In order to provide some information on the level of the nervous system at which suppression was occurring we performed a second set of experiments in which we compared the effect of a magnetic conditioning stimulus on test responses evoked by either a magnetic or an electric test shock. This experiment was performed with the subject exerting a small voluntary contraction of the target muscle. This ensured that any response to a small electric test shock (typically around 200 V, 50 μs) would have a substantial contribution from D activation of corticospinal fibres (Day *et al.* 1989*a*). In this experiment, a conditioning magnetic stimulus did not suppress responses evoked by electric stimulation, although the same shock could suppress test responses produced by a

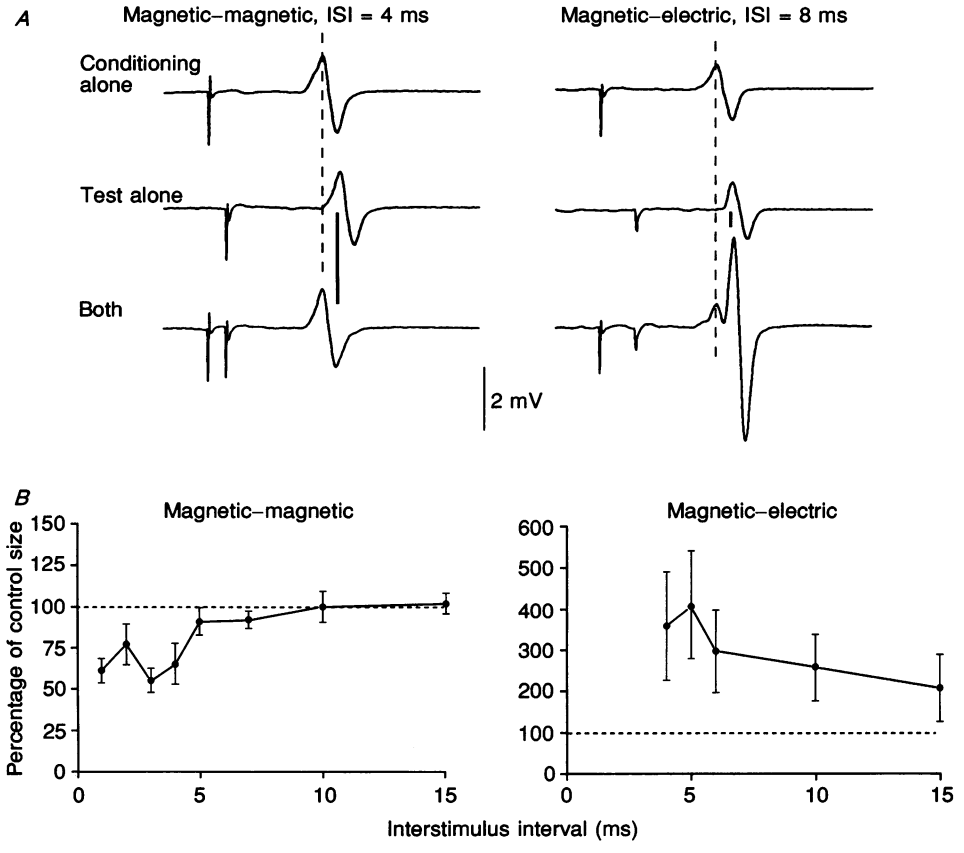

Fig. 2. Comparison of the effect of a magnetic conditioning stimulus on the size of responses evoked in the active first dorsal interosseous muscle by a magnetic or anodal electric test stimulus over the motor cortex. *A* shows a typical example of the effect in EMG responses from one subject. The three rows of traces show (from top to bottom) responses to the conditioning stimulus given alone (note that since the muscle was active, the conditioning stimulus now evokes a clear EMG response, unlike the data from the relaxed subject illustrated in Fig. 1), the response to the test stimulus given alone, and the response to both stimuli given with an interstimulus interval (ISI) of 4 (left-hand panel) or 8 ms (right-hand panel). The left panel shows responses when the test stimulus was magnetic; the right panel shows the responses when the test stimulus was anodal electric. The vertical dashed line aligns the peak of the EMG responses to the conditioning stimulus; the vertical bar aligns the peak of responses to the test stimulus. The conditioning stimulus produced facilitation of anodal test responses, whilst magnetic test responses were virtually abolished. The ISIs of 4 and 8 ms were chosen so that the peak of the magnetic or electric test responses occurred at the same latency after the conditioning shock. In this subject, the latency to the initial peak of the EMG response to magnetic stimulation occurred 4 ms later than that to electric stimulation. *B* shows the average (± S.E.M.) time course of this effect in 5 subjects. The left-hand graph presents data using a magnetic test shock; the right-hand graph presents data with an anodal electric test shock. The magnetic conditioning stimulus was the same in each case. At each interstimulus interval, the size of the conditioned responses is expressed as a percentage of the size of the control response. Note the difference in scales on the *Y*-axis in the two graphs.

magnetic shock. An example of this effect is shown in Fig. 2*A*, and mean results and time course in Fig. 2*B*. The left part of Fig. 2*A* shows the usual result of using magnetic test and conditioning stimuli. Unlike the data in Fig. 1*A*, this experiment was conducted in active muscles so that the conditioning stimulus was

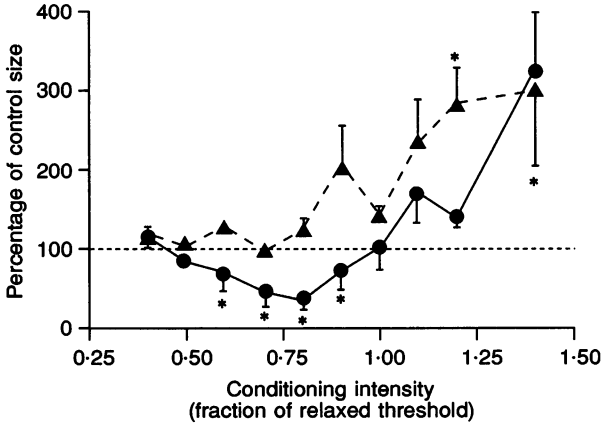

Fig. 3. The effect of changing the intensity of the conditioning shock at interstimulus intervals of 3 (●) and 15 ms (▲). At each interstimulus interval, the size of the conditioned responses is expressed as a percentage of the size of the control response. The graph plots the size of the conditioned responses as a function of the conditioning intensity at the two interstimulus intervals studied. The intensity of the conditioning shock on the *X*-axis is expressed as a percentage of the threshold for evoking EMG responses in contralateral relaxed muscles. With interstimulus intervals of 3 ms, inhibition became evident when the conditioning shock was 0·6 times threshold, and was maximum at 0·8 times threshold. Above this intensity, inhibition began to lessen and change to facilitation at conditioning intensities greater than motor threshold. Using an interstimulus interval of 15 ms, no inhibition was seen at any conditioning intensity. Facilitation began to appear at intensities of 0·9 times threshold and increased as the conditioning intensity was increased. The graph plots the mean data from the relaxed first dorsal interosseous muscle of three subjects. In each subject, 10 control and 10 conditioned trials were collected at each intensity of the conditioning stimulus. Asterisks indicate intensities at which there was a significant effect ($P < 0·05$) of the conditioning shock relative to control values in each of the 3 subjects.

no longer subthreshold for producing a motor response. When the test stimulus was preceded by a conditioning stimulus given 4 ms earlier, the resulting EMG potential was little different from that seen if the conditioning stimulus was given on its own, since the test response had been almost entirely suppressed. On the right, the same conditioning magnetic stimulus preceded an electric test shock. In this case, the time interval between the shocks was 8 ms, in order to take into account the shorter latency of electrically evoked test responses in this subject. The conditioning stimulus produced a pronounced increase in the size of the test response. The mean data in Fig. 2*B* have been plotted after subtracting the response to the conditioning stimulus alone in order to estimate the true size of the response to the test shock. The graph shows that the difference between the effect of a conditioning stimulus on responses produced by magnetic and electric

test stimuli was maintained over a range of interstimulus intervals. Magnetic test responses were suppressed at intervals shorter than 5 ms, whereas responses to electric test stimuli were facilitated at intervals up to 15 ms. Contrary to the situation in relaxed muscles, a magnetic conditioning stimulus did not produce any facilitation of test responses in contracting muscles at interstimulus intervals of 10 and 15 ms. In Fig. 2*B* it should be noted that short magnetic–electric intervals could not be investigated because of the difference in latency between EMG responses to magnetic and electric stimulation of the brain. EMG responses to electric stimulation can occur several milliseconds earlier than those to magnetic stimulation, so that if the magnetic stimulus was given too close to the electric test stimulus, then the electric test response began in the muscle before the volley from the conditioning response had arrived.

Effect of stimulus intensity

In the relaxed state the amount of suppression between two magnetic stimuli depended on the intensity of both the test and the conditioning shock. As expected, the larger the test stimulus the less suppression (not illustrated). Unexpectedly, however, larger conditioning stimuli also resulted in less suppression of the test shock. Figure 3 illustrates the effect of the intensity of the conditioning shock on the size of test responses elicited at interstimulus intervals of either 3 or 15 ms. At an interstimulus interval of 3 ms, test responses were suppressed when the intensity of the conditioning shock was as low as 0·6 times the threshold for activation in relaxed muscles. In the three subjects studied in detail, this was approximately equal to the threshold for evoking a recognizable response in active muscle. Maximum suppression of the test response occurred with a conditioning intensity of 0·8 times threshold. Increasing the conditioning strength even further resulted in less suppression, and at suprathreshold intensities, the suppression was replaced by facilitation. The effect of conditioning intensity was also examined at the longer interstimulus interval of 15 ms. At this interval, there was no suppression at any intensity. Test responses were facilitated when the strength of the conditioning shock was 0·9 times threshold or above.

Magnetic versus electric conditioning shocks

In three subjects we examined the effect of using electric conditioning stimuli. In one set of experiments magnetic conditioning shocks were intermixed with anodal conditioning shocks (cathode at the vertex, anode 7 cm lateral). In another, anodal and cathodal (cathode lateral, anode at the vertex) conditioning shocks were intermixed. In order to match the two types of conditioning stimuli, both were set to be about 0·8 times motor threshold in the relaxed state. At this intensity, the shocks produced responses of about 0·5–1 mV in FDI if the subject tonically activated the muscle. Anodal conditioning stimuli produced much less suppression at these low intensities than either magnetic or cathodal shocks (see Fig. 4). However, if the intensity of the anodal stimulus was increased so that it was nearer to relaxed threshold, or just above, then suppression could be seen (not illustrated).

The electric conditioning shocks that were used in these experiments were well below threshold for activating leg or trunk muscles. Because of this we presume the

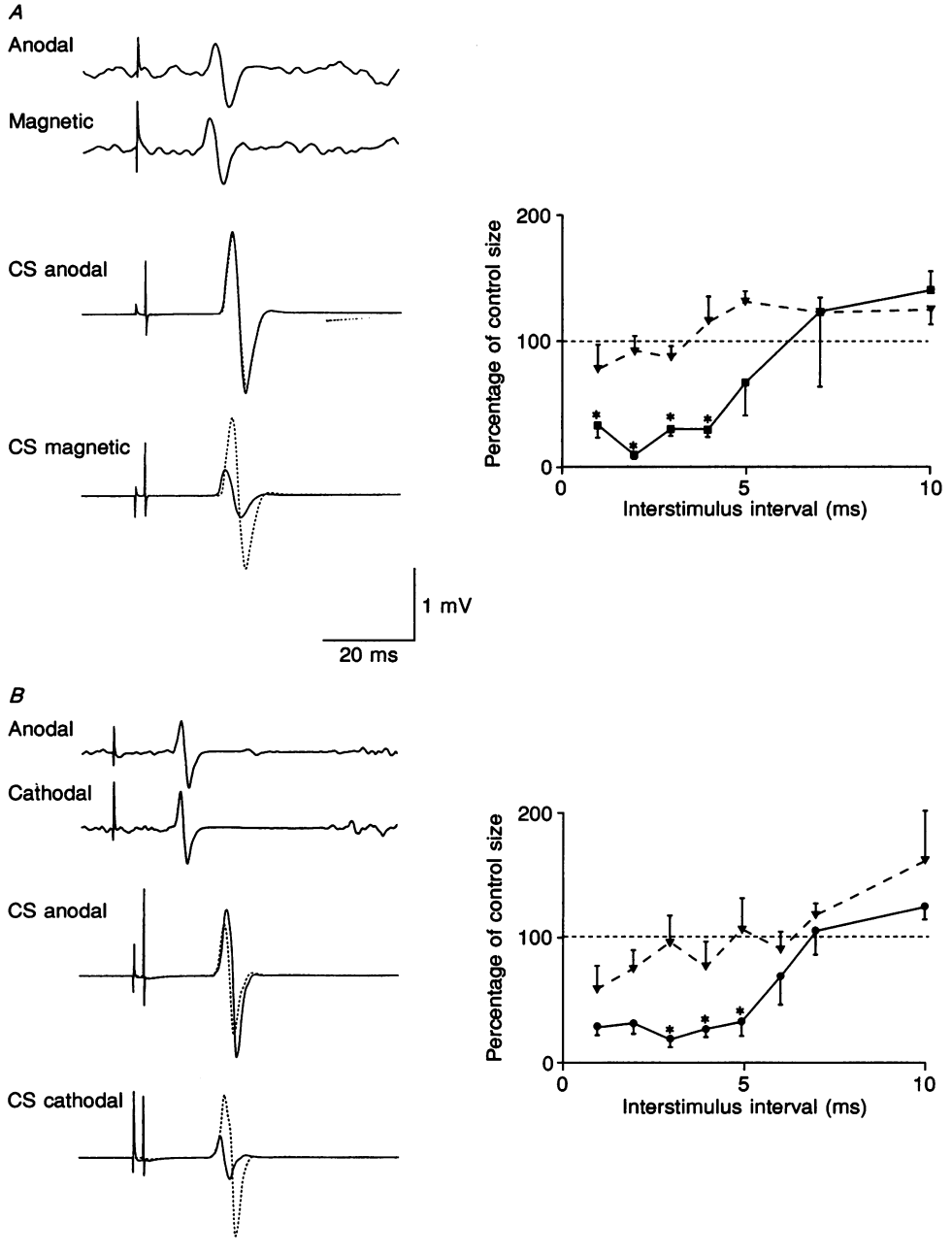

Fig. 4. Comparison of the effectiveness of anodal *versus* magnetic conditioning shocks (*A*) and anodal *versus* cathodal conditioning shocks (*B*), on the size of test responses evoked in the relaxed first dorsal interosseous by magnetic stimulation of the motor cortex. The EMG traces on the left show typical examples of the results from one subject. The four sets of records in *A* and *B* show (from top to bottom) responses evoked in active muscle by the two types of conditioning stimulus given alone. The intensity of the shocks has been adjusted to give responses of equal size. The bottom two records from relaxed muscles show, superimposed, responses to the test stimulus given alone (the larger of the two potentials

vertex electrode was inactive, and that the effects observed were due to stimulation through the lateral lead.

Movement of the conditioning stimulus

In three subjects we used two separate coils to explore the effect of moving the conditioning coil away from the motor strip. In these experiments, the conditioning stimulus was delivered via a figure-of-eight coil held so that the current in the junction zone of the coil flowed in the medial to lateral direction over the hand area of cortex. The test coil was a large round (14 cm external diameter) coil centred at the vertex. For a given intensity of conditioning shock, there was less suppresion ($P < 0{\cdot}05$) when the conditioning coil was moved 3 cm anterior or posterior to the motor strip. In a second set of experiments, the conditioning stimulus intensity was increased when the coil was moved anterior or posterior to the motor strip, so that it evoked responses (in active contralateral muscles) of the same size as had been seen when the coil was placed over the motor strip. Under these conditions, if the coil was moved anteriorly or posteriorly, there was no significant difference in the amount of suppression ($P > 0{\cdot}05$) compared to that seen with the standard coil position.

Figure-of-eight coil stimulation over leg and hand areas

The experiments described above refer to anteroposterior movement of the conditioning coil. The results were different when the conditioning coil was placed at the vertex. Thus a conditioning stimulus delivered from a figure-of-eight coil over the vertex produced clear suppression of EMG responses in the hand evoked by stimulation over the hand area with a separate figure-of-eight coil, without itself evoking any EMG responses in active hand muscles. An example of the effect is shown in Fig. 5*A*, and the combined time course of suppression from six subjects shown in Fig. 6*A*. The time course of suppression was similar to that seen when the conditioning stimulus was given over the hand area. There was clear suppression with interstimulus intervals as short as 1 ms, but none when the conditioning stimulus was given after the test shock (at an interval of −1 ms). Unlike the situation with conditioning stimuli over the hand area, responses suppressed by conditioning shocks over the vertex never had a shorter latency than control responses. In addition, with this position of the conditioning coil, there was no evidence of facilitation of test responses at interstimulus intervals of 10 and 15 ms (cf. Fig. 1). In three subjects, the conditioning shock had no effect on H reflexes in wrist flexor

potentials; dotted line), and the responses conditioned by a prior conditioning shock (CS) of each type given at an interstimulus interval of 3 ms. Magnetic conditioning stimuli produced more inhibition of test responses than anodal conditioning stimuli (*A*); cathodal conditioning stimuli also produced more inhibition than anodal conditioning stimuli (*B*). The graphs on the right show the average time course of the conditioning effects in 3 subjects. At each interstimulus interval, the size of the response conditioned by the two forms of stimulation (▼, anodal; ●, cathodal; ■, magnetic) is expressed as a percentage of the size of the control. At each interval, 10 control and 10 conditioned responses were collected from each individual. Asterisks indicate interstimulus intervals at which anodal conditioning shocks produced significantly less ($P < 0{\cdot}05$) inhibition of test responses relative to control values in each of the 3 subjects than magnetic (*A*) or cathodal (*B*) stimulation.

muscles, even though it produced substantial suppression of cortically evoked responses in the same muscle (Fig. 6*B*). As with conditioning stimuli applied over the hand area, the effect of a vertex conditioning shock was highly dependent upon the

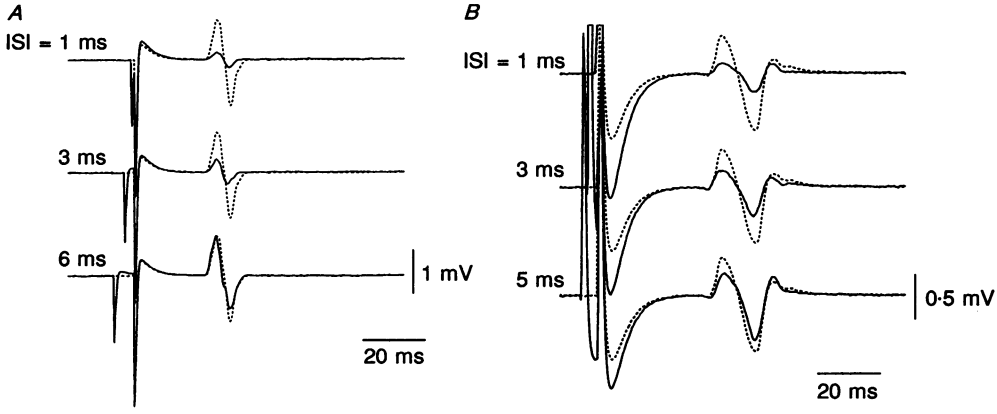

Fig. 5. Effects obtained between hand area and leg area of motor cortex using two separate figure-of-eight coils. *A*, suppression of responses elicited by magnetic test stimulation in the relaxed first dorsal interosseous of one subject by conditioning magnetic stimulus applied at the vertex; *B*, suppression of responses elicited by magnetic test stimulation over the vertex in the relaxed tibialis anterior muscle by conditioning magnetic stimuli applied over the hand area of motor cortex. Data are mean (of 10 trials) EMG responses from one subject. The three rows show superimposed responses to the test stimulus given alone (the larger of the two responses; dotted line) and responses conditioned at three different interstimulus intervals (ISI). The conditioning stimuli were subthreshold for evoking an EMG response in the muscle under test even when active. Note the clear suppression at interstimulus intervals of 1 and 3 ms, and less suppression at 5 (*B*) or 6 ms (*A*).

anteroposterior location of the coil. When the coil was moved forwards or backwards from the vertex, the amount of suppression decreased substantially (not illustrated).

A conditioning magnetic stimulus over the hand area of the motor cortex could also suppress the test responses elicited by test magnetic stimulus over the vertex in active tibialis anterior (Fig. 5*B*; 3 subjects).

Single motor unit studies

In the Discussion we suggest that the suppression described here is due to synaptic activity in corticocortical inhibitory circuits. If so, an interstimulus interval of 1 ms seems remarkably short for inhibition to travel from the leg to the hand area of cortex. However, this is probably an underestimate of the true minimum interstimulus interval for the following reason. The test stimulus evokes several descending volleys in the pyramidal tract which may last for 3–6 ms (Day *et al.* 1989*a*). The size of the final EMG response depends upon the combined action of all these volleys on spinal motoneurones. At an interstimulus interval of 1 ms, inhibition might not arrive at the hand area of cortex early enough to affect the initial descending volley, but could be in time to depress later volleys. The EMG

response would be decreased, but the interstimulus interval would not represent the minimum time taken for inhibition to arrive at the hand area. We have used a similar argument to explain the short interstimulus interval for the onset of transcallosal inhibition (Ferbert *et al.* 1992).

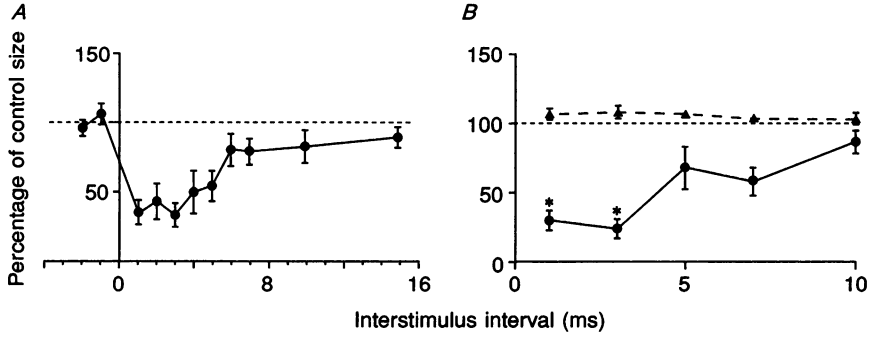

Fig. 6. *A*, mean (± S.E.M.) time course of suppression of test responses evoked by magnetic stimulation in the relaxed first dorsal interosseous muscle of eight subjects by conditioning magnetic stimuli applied at the vertex. At each interstimulus interval, the size of the conditioned response is expressed as a percentage of the size of the control. Note the clear suppression at interstimulus intervals less than 6 ms. There is no excitation at 10 and 15 ms. *B*, comparison of the effect of a vertex magnetic conditioning shock on EMG responses elicited in the relaxed forearm flexor muscles by either a magnetic test stimulus over the hand area of motor cortex (●) or an H reflex stimulus to the median nerve in the cubital fossa (▲). The conditioning stimulus has no effect on the size of H reflexes, despite producing clear suppression of the response to magnetic test stimulation. Data are the means from 3 subjects. In these subjects, the latency of the H reflex was the same as that of the response to magnetic stimulation of motor cortex. In each subject at each interstimulus interval, 10 conditioned and 10 control responses were elicited. Asterisks show times when there was a significant ($P < 0{\cdot}05$) difference between conditioning effects on the H reflex and responses to magnetic stimulation in all 3 of the subjects.

In order to confirm this line of reasoning, we tested the hypothesis in three subjects using single motor units recorded from the first dorsal interosseous muscle. The experiment was performed with the test stimulator over the hand area of cortex, and the conditioning stimulus over the vertex. An example of the results is given in Fig. 7. The test stimulus on its own produced a period of increased firing probability in the post-stimulus time histogram of unit firing, which lasted for around 3 ms. Based on data from previous studies of single motor unit behaviour to magnetic stimulation (see Day *et al.* 1989*a*), this period of increased firing probability probably represents two separate peaks close to each other. This broad period has therefore been divided arbitrarily (vertical lines of Fig. 7) into an early and a late part, which we term the first and second peak. When the conditioning stimulus at the vertex was given 1 ms beforehand, the size of the second peak in the post-stimulus time histogram was decreased in size, but the size of the first peak was unaffected. We suggest that at this interval, inhibition arrived late at the hand area, and could affect only the second descending volley set up by the test shock but not the first. When the conditioning stimulus was given 3 ms

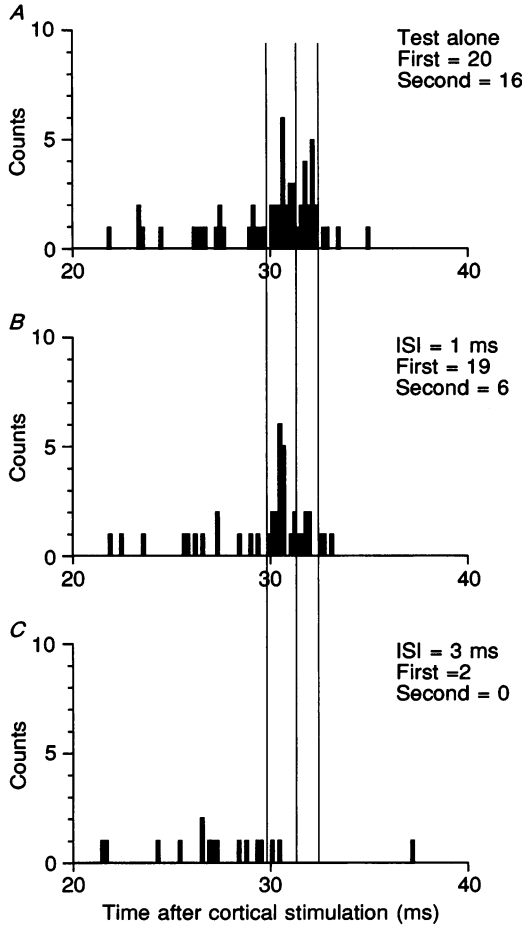

Fig. 7. Post-stimulus time histograms of the firing pattern of a single motor unit in the first dorsal interosseous after a magnetic test stimulus given over the contralateral motor cortex at $t = 0$ ms. The traces start 20 ms after the stimulus was applied. The three traces show the histograms to the test shock given alone (*A*), and when preceded (on random trials) by a conditioning magnetic stimulus to the vertex given 1 (*B*) or 3 ms (*C*) earlier. The vertical lines indicate the durations of the two main peaks of increased firing in the post-stimulus time histograms. The number of counts in each peak is noted to the right of each trace. With an interstimulus interval of 1 ms, only the second peak is reduced in size (second peak, $\chi^2 = 4{\cdot}14$, 1 degree of freedom, $P < 0{\cdot}05$). At 3 ms, both the peaks are affected (first peak, $\chi^2 = 14{\cdot}76$, 1 degree of freedom, $P < 0{\cdot}001$; second peak, $\chi^2 = 15{\cdot}29$, 1 degree of freedom, $P < 0{\cdot}001$). One hundred stimuli were given to construct each histogram.

before the test, then both peaks were reduced in size. Similar results were obtained in the other subjects.

DISCUSSION

The present experiments have shown that a magnetic conditioning stimulus given over the motor cortex at intensities below threshold for obtaining EMG responses in relaxed hand muscles can suppress responses evoked in the same muscles by a

suprathreshold magnetic test stimulus given approximately 1–6 ms later. Although the suppression can be observed whether muscles are relaxed or active, we have concentrated on the results seen in relaxed muscle. Under these conditions, the conditioning stimulus is, by definition, subthreshold and this makes it easier to measure effects on the size of the responses to the test shock.

Level at which suppression of magnetic test responses occurs

Suppression of magnetic test responses most probably occurred through an inhibitory action at the level of the cerebral cortex rather than at the spinal cord. The arguments are similar to those previously presented by Datta, Harrison & Stephens (1989), Day, Riescher, Struppler, Rothwell & Marsden (1991), Ugawa *et al.* (1991), and Ferbert *et al.* (1992). They rely on two related findings. First, conditioning stimuli over either the hand area (Cowan, Day, Marsden & Rothwell, 1986) or vertex (present data) never produce a 5 ms period of inhibition in forearm H reflexes even though the same stimuli can suppress responses evoked in the same muscles by a suprathreshold magnetic test shock. Second, conditioning stimuli failed to suppress test responses evoked by low-intensity anodal test stimuli to the motor cortex. Although the precise mechanism of action of anodal and magnetic stimulation of motor cortex has yet to be agreed, most recent work suggests that there is an important difference in the site at which they activate neurones in the brain. With threshold levels of stimulation, anodal stimulation is likely to activate corticospinal axons within the white matter, whilst an appropriately directed magnetic stimulus activates either the axon at the initial segment region or excitatory inputs to corticospinal neurones (Day *et al.* 1989*a*; Edgley, Eyre, Lemon & Miller, 1990; Burke, Hicks & Stephen, 1990; Amassian, Quirk & Stewart, 1990). If the intensity of either type of stimulus is raised sufficiently then impulses may be generated at more than one site, leading to sequential descending volleys in corticospinal neurones (D and I waves). Nevertheless, under conditions similar to those in the present experiments, results on single motor units suggest that near threshold anodal stimuli can produce isolated D waves without contamination from I waves (Day *et al.* 1989*a*). This has been confirmed by direct recording from the spinal cord in anaesthetized patients (Boyd *et al.* 1986; Berardelli, Inghilleri, Cruccu & Manfredi, 1990; Thompson *et al.* 1991) and in a small number of patients in whom spinal recordings have been made after withdrawal of volatile anaesthetics (Hicks, Burke, Stephen, Woodforth & Crawford, 1992). Indeed, the intensities of electric stimulation which produced pure D wave activity in the latter experiments (150 V, 100 μs in their Fig. 3) were similar to the intensities used in the present experiments ($\sim$200 V, 50 μs). Given this difference in their manner of corticospinal activation, the responses to magnetic stimulation should be more affected by changes in the level of corticospinal excitability than the responses to anodal stimulation. Thus, a differential suppression of magnetic but not anodal test responses is compatible with a cortical inhibitory mechanism. Although cortical inhibition seems the simplest explanation available for the present results, it is not possible to exclude other actions such as, for example, presynaptic effects on the H reflex pathway, postsynaptic effects on possible interneurones (e.g. propriospinal neurones) in the corticospinal pathway, or activation of quite separate short latency descending pathways by anodal electric and magnetic stimulation of the motor cortex.

Hand area conditioning stimuli may produce concurrent spinal cord facilitation and cortical suppression

Two features of the present results suggest that suppression of test responses at interstimulus intervals of 1–6 ms is a complex phenomenon. First, the latency of test response in the period of suppression was often shorter than that of control responses. Second, the amount of suppression did not increase progressively as the intensity of the conditioning stimulus was raised: maximum suppression occurred at 0·8 times relaxed threshold and then got smaller as the intensity was increased.

In the absence of any direct recordings of the descending activity set up by the conditioning and test stimuli, any explanation must be somewhat speculative. However, it is known that subthreshold conditioning stimuli can facilitate spinal H reflexes in the absence of any discharge of the spinal motoneurones (Cowan *et al.* 1986). This is probably caused by subthreshold facilitation of motoneurones by a small corticospinal volley set up by the conditioning shock. If the same occurs in the present experiments, the subliminal increase in excitability of spinal motoneurones could reduce the time taken for a (smaller) corticospinal volley to depolarize the cells to threshold. Test responses could be suppressed even though their onset latency was reduced. When the conditioning intensity is raised, spinal facilitation may increase and contribute to the reduced inhibition seen with larger conditioning shocks, although additional effects at other levels in the motor pathway cannot be ruled out. Finally, subthreshold facilitation of spinal motoneurones may contribute to the facilitation of test responses at interstimulus intervals of 10 and 15 ms.

It must be emphasized that the presence of spinal excitation does not preclude excitatory influences at other levels. For example, a single shock over the motor cortex can, depending on the stimulus intensity, give rise to one or more I waves in the pyramidal tract which are generated by continuing facilitatory input to pyramidal cells over several milliseconds. Inhibition may be an important factor in 'quenching the I wave' discharge after a single surface cortical stimulus (Amassian, Stewart, Quirk & Rosenthal, 1987).

Conditioning stimuli applied over the vertex

When the conditioning stimulus was moved anterior or posterior to the hand area, the amount of cortical suppression was reduced. A small effect could still be observed, especially if the stimulus intensity was increased. However, this was probably due to physical spread of the stimulus to the hand area, since the conditioning shock itself could evoke EMG responses in active hand muscle.

The situation was different at the vertex. Here a conditioning shock could evoke suppression at intensities below threshold for eliciting any EMG responses in the muscles under test even when active. Since the time course was similar, it is possible that the effect was mediated by the same intracortical inhibitory mechanisms as activated by conditioning stimuli over the hand area. Interestingly, vertex conditioning did not produce any facilitation at interstimulus intervals of 10 and 15 ms, nor did it shorten the latency of the response to the test shock. This may have been because the vertex conditioning shock failed to evoke any activity in

corticospinal projections to hand motoneurones, so that facilitatory effects at the spinal cord level were absent.

Mechanisms of cortical suppression

In the present experiments, the data to support particular mechanisms are necessarily indirect and rely on a comparison of the effect of different types of conditioning stimuli. The arguments that follow assume that magnetic, cathodal and anodal conditioning stimuli activate predominantly overlapping populations of corticospinal tract axons. Whether this is correct, though, is unknown. Certainly the descending motor volley produced by each type of stimulation is conducted with approximately the same maximum velocity to the spinal cord, and some single unit data from the monkey have shown that the same axons (109 of 124 corticospinal neurones tested) may be activated by anodal and magnetic stimulation (Edgley, Eyre, Lemon & Miller, 1992). Nevertheless, this does not prove that most of the fibres actually are the same in each case in man. If there are substantial differences in the populations of descending axons excited by different conditioning stimuli, then the arguments below may need revision.

If the suppression that we have described takes place in the cerebral cortex, then what is the mechanism? One possibility is that the conditioning stimulus, although subthreshold for motor responses in relaxed muscle, may still excite some pyramidal tract neurones and leave them partially refractory to the test stimulus. However, this is unlikely to be the sole reason for the period of suppression. First, test responses to anodal stimulation, which are probably produced by activity in the same set of descending corticospinal axons as recruited by magnetic stimulation, were not inhibited by a conditioning shock; in fact they were facilitated. Second, magnetic or cathodal electric conditioning stimuli were much more effective in producing inhibition at low intensities than anodal conditioning stimuli. Since all three forms of stimulation activate corticospinal axons, then all three should have produced inhibition if axonal refractoriness was an important mechanism. Anodal conditioning shocks sometimes produced inhibition at very short interstimulus intervals of 1 ms or so, and this effect may indeed have been due to the refractory period of corticospinal axons and/or after-hyperpolarization in the cell body. However, at 3, 4 and 5 ms, this was not the case.

The experiments comparing the effectiveness of magnetic, anodal and cathodal conditioning stimuli also suggest that inhibitory effects from corticospinal recurrent collaterals are not an important contributor to cortical suppression. When all three forms of conditioning stimuli were matched in size to evoke similar-sized EMG responses in active muscle, cathodal and magnetic stimuli were found to be much more effective in evoking suppression at low intensities than anodal stimuli. Since all three types of stimulation probably activated corticospinal axons (either directly or indirectly), there should have been little difference in their effectiveness if collateral inhibition were important. Indeed, early experiments by Phillips (1959) suggested that inhibition from a single impulse in corticospinal collaterals was relatively weak in the monkey. We conclude that cortical suppression in the present experiments was the result of other intracortical inhibitory mechanisms.

Onset latency of the inhibitory effect

The onset of inhibition was very short, whether the conditioning stimulus was applied at the hand area or the vertex. As argued in the Results, this probably underestimates the true minimum time taken for inhibition to occur within the cortex. A single test stimulus produces more than one descending volley of activity within pyramidal tract axons, which may last several milliseconds. All these volleys may contribute to the peak-to-peak size of the peripheral EMG response. Thus, inhibition of EMG responses can occur if the conditioning shock suppresses only the later portions of the descending volley, some milliseconds after the test shock was actually applied. In the present experiments it was possible to explore this possibility, at least for inhibition from vertex to arm area, by using post-stimulus time histogram techniques on single motor units. We could not use the same techniques to define the precise onset latency of inhibition from conditioning stimuli over the arm area because the conditioning stimulus itself produced effects on the post-stimulus time histogram of hand motor units. The test stimulus evoked two or more short latency peaks of excitation in the post-stimulus time histograms separated by about 2 ms. A conditioning stimulus at the vertex suppressed only the later volley at interstimulus intervals of 1 ms, but reduced the size of all volleys if the interstimulus interval was increased to 3 ms. The implication is that a conditioning stimulus at the vertex requires at least 3 ms to produce inhibition of cortical mechanisms which contribute to hand muscle EMG responses.

This estimate of the corticocortical conduction time for inhibition between leg and hand areas is shorter than the estimated conduction time for spread of excitation between the same areas calculated by Brown, Day, Rothwell, Thompson & Marsden (1991) in patients with cortical myoclonus. These authors suggested that in their patients excitation could spread from a focus in the arm to the leg area or vice versa in 7–9 ms. The much shorter estimate of 3 ms in the present experiments might be due to spread of the conditioning stimulus away from the vertex to a site nearer the hand area.

Relationship with other reports of motor cortical inhibition in man

Three types of inhibitory phenomena involving cortical mechanisms have been observed after transcranial magnetic or electric stimulation of the motor cortex. (1) Delay in the onset of voluntary reaction time movement by a suprathreshold stimulus given in the reaction period (Day, Rothwell, Thompson, Nakashima, Shannon & Marsden, 1989*c*). (2) The silent period following an EMG response to a suprathreshold stimulus (Day, Marsden, Rothwell, Thompson & Ugawa, 1989*b*; Fuhr, Agostino & Hallett, 1991; Kujirai, Rothwell, Day, Thompson & Marsden, 1992). (3) The silent period that can sometimes be produced in tonic EMG activity by submotor threshold stimuli (Calancie, Nordin, Wallin & Hagbarth, 1987; Davey, Romaiguere, Maskill & Ellaway, 1992). Of these, the latter seems most similar to the inhibition described in the present experiments because of the low stimulus intensity at which it is evoked. However, all three types of inhibitory phenomena may be related since all could be explained by cortical inhibition of a voluntary motor command.

Relationship to data from animal experiments

There are several reports of the inhibitory consequences of single cortical stimuli. In a series of papers, Krnjević *et al.* (1966*a*,*b*,*c*) showed that a single stimulus to the exposed cortex of cats, rabbits or monkeys could reduce the excitability of cortical neurones for periods of up to 300 ms, depending upon the intensity of conditioning shock. The authors concluded that the major part of the effect produced by cortical stimulation arose from activation of an intracortical inhibitory system. Later experiments suggested that this involved GABAergic activity, involving basket and probably other cells in the grey matter (see Krnjević, 1983). Work by Rosenthal, Waller & Amassian (1967) confirmed that the hyperpolarization caused by such stimuli was a true IPSP rather than a disfacilitation. Two features of the results in animals are similar to those reported here. First, inhibition was more readily observed with cathodal than with anodal stimulation (see also Phillips, 1961). Second, inhibition could be elicited by stimulation of surrounding areas of cortex up to 1 cm distant. The spread of inhibition was uneven in different directions, being, as in the present experiments, much better along the length of the motor cortex and poor under sulci.

If the inhibition in the present experiments is due to activity in small GABAergic interneurones then it is surprising that the effect occurred with such low intensities of conditioning shock. Either these inhibitory neurones are particularly excitable by magnetic stimulation because of their orientation with respect to the induced current flow, or they are activated indirectly via large diameter fibres.

There is one point of difference between these previous data and the present results. Previous authors emphasized the very long duration of inhibition that could be produced by a single shock, often lasting 100 ms or more. Although shorter periods of inhibition were seen when smaller conditioning shocks were used, none was quite so short as the inhibition seen here. The problem in the present experiments is that mixed excitatory and inhibitory effects may exist at both the cortical and spinal cord level. Thus the true duration of inhibition is difficult to estimate; therefore we were limited to using very small intensities of conditioning shock. Nevertheless, if the mechanisms of inhibition are similar to those described in animal experiments, this may be a useful method for investigating the effectiveness of GABAergic inhibitory systems in man.

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REFERENCES

AMASSIAN, V. E., QUIRK, K. J. & STEWART, M. (1990). A comparison of corticospinal activation by magnetic coil and electrical stimulation of monkey motor cortex. *Electroencephalography and Clinical Neurophysiology* **77**, 390–407.

AMASSIAN, V. E., STEWART, M., QUIRK, G. J. & ROSENTHAL, J. E. (1987). Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery* **20**, 74–93.

BERARDELLI, A., INGHILLERI, M., CRUCCU, G. & MANFREDI, M. (1990). Descending volley after electrical and magnetic stimulation in man. *Neuroscience Letters* **112**, 54–58.

BOYD, S. G., ROTHWELL, J. C., COWAN, J. M. A., WEBB, P. J., MORLEY, T., ASSELMAN, P. & MARSDEN, C. D. (1986). A method of monitoring motor function in corticospinal pathways during scoliosis surgery with a note on motor conduction velocities. *Journal of Neurology, Neurosurgery and Psychiatry* **49**, 251–257.

BROWN, P., DAY, B. L., ROTHWELL, J. C., THOMPSON, P. D. & MARSDEN, C. D. (1991). Intrahemispheric and interhemispheric spread of cerebral cortical myoclonic activity and its relevance to epilepsy. *Brain* **114**, 2333–2351.

BURKE, D., HICKS, R. G. & STEPHEN, J. P. H. (1990). Corticospinal volleys evoked by anodal and cathodal stimulation of the human motor cortex. *Journal of Physiology* **425**, 283–299.

CALANCIE, B., NORDIN, M., WALLIN, U. & HAGBARTH, K.-E. (1987). Motor-unit responses in human wrist flexor and extensor muscles to transcranial cortical stimuli. *Journal of Neurophysiology* **58**, 1168–1185.

COWAN, J. M. A., DAY, B. L., MARSDEN, C. D. & ROTHWELL, J. C. (1986). The effect of percutaneous motor cortex stimulation on H reflexes in muscles of the arm and leg in intact man. *Journal of Physiology* **377**, 333–348.

DATTA, A. K., HARRISON, L. M. & STEPHENS, J. A. (1989). Task-dependent changes in the size of response to magnetic brain stimulation in human first dorsal interosseous muscle. *Journal of Physiology* **418**, 13–23.

DAVEY, N. J., ROMAIGUERE, P., MASKILL, D. W. & ELLAWAY, P. M. (1992). Inhibition of voluntary contraction by transcranial magnetic stimulation of the brain subthreshold for excitation in man. *Journal of Physiology* **446**, 447*P*.

DAY, B. L., DICK, J. P. R., MARSDEN, C. D., NAKASHIMA, K., ROTHWELL, J. C., SHANNON, K. & THOMPSON, P. D. (1987). Interaction between electrical and magnetic stimulation of the human brain. *Journal of Physiology* **384**, 74*P*.

DAY, B. L., DRESSLER, D., MAERTENS DE NOORDHOUT, A., MARSDEN, C. D., NAKASHIMA, K., ROTHWELL, J. C. & THOMPSON, P. D. (1989*a*). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *Journal of Physiology* **412**, 449–473.

DAY, B. L., MARSDEN, C. D., ROTHWELL, J. C., THOMPSON, P. D. & UGAWA, Y. (1989*b*). An investigation of the EMG silent period following stimulation of the brain in normal man. *Journal of Physiology* **414**, 14*P*.

DAY, B. L., RIESCHER, H., STRUPPLER, A., ROTHWELL, J. C. & MARSDEN, C. D. (1991). Changes in the response to magnetic and electrical stimulation of the motor cortex following muscle stretch in man. *Journal of Physiology* **433**, 41–58.

DAY, B. L., ROTHWELL, J. C., THOMPSON, P. D., NAKASHIMA, K., SHANNON, K. & MARSDEN, C. D. (1989*c*). Delay in the execution of voluntary movement by electrical or magnetic brain stimulation in intact man. *Brain* **112**, 649–663.

EDGLEY, S. A., EYRE, J. A., LEMON, R. N. & MILLER, S. (1990). Excitation of the corticospinal tract by electromagnetic and electrical stimulation of the scalp in the macaque monkey. *Journal of Physiology* **425**, 301–320.

EDGLEY, S. A., EYRE, J. A., LEMON, R. N. & MILLER, S. (1992). Direct and indirect activation of corticospinal neurones by electrical and magnetic stimulation in the anaesthetized macaque monkey. *Journal of Physiology* **446**, 224*P*.

FERBERT, A., PRIORI, A., ROTHWELL, J. C., DAY, B. L., COLEBATCH, J. G. & MARSDEN, C. D. (1992). Interhemispheric inhibition of the human motor cortex. *Journal of Physiology* **453**, 525–546.

FUHR, P., AGOSTINO, R. & HALLETT, M. (1991). Spinal motoneurone excitability during the silent period after cortical stimulation. *Electroencephalography and Clinical Neurophysiology* **81**, 257–262.

HICKS, R., BURKE, D., STEPHEN, J., WOODFORTH, I. & CRAWFORD, M. (1992). Corticospinal volleys evoked by electrical stimulation of human motor cortex after withdrawal of volatile anaesthetics. *Journal of Physiology* **456**, 393–404.

KRNJEVIĆ, K. (1983). GABA-mediated inhibitory mechanisms in relation to epileptic discharges. In *Basic Mechanisms of Neuronal Hyperexcitability*, pp. 249–280. Alan R. Liss, New York.

KRNJEVIĆ, K., RANDIĆ, M. & STRAUGHAN, D. W. (1966*a*). An inhibitory process in the cerebral cortex. *Journal of Physiology* **184**, 16–48.

KRNJEVIĆ, K., RANDIĆ, M. & STRAUGHAN, D. W. (1966*b*). Nature of a cortical inhibitory process. *Journal of Physiology* **184**, 49–77.

KRNJEVIĆ, K., RANDIĆ, M. & STRAUGHAN, D. W. (1966*c*). Pharmacology of cortical inhibition. *Journal of Physiology* **184**, 78–105.

KUJIRAI, T., ROTHWELL, J. C., DAY, B. L., THOMPSON, P. D. & MARSDEN, C. D. (1992). An investigation of cortical excitability during silent period induced by magnetic stimulation in man. *Movement Disorders* **7**, suppl. 1, 153.

KUJIRAI, T., ROTHWELL, J. C., FONG, J., THOMPSON, P. D. & DAY, B. L. (1993). Local intracortical inhibition in human sensorimotor cortex. *Journal of Phsiology* **459**, 63*P*.

KUJIRAI, T., SATO, M., ROTHWELL, J. C., DAY, B. L., THOMPSON, P. D., WROE, S., ASSELMAN, P. T. & MARSDEN, C. D. (1991). Inhibitory interactions between transcranial brain stimuli applied over the motor cortex in man. *Society for Neuroscience Abstracts* **17**, 1023.

PHILLIPS, C. G. (1959). Actions of antidromic pyramidal volleys on single Betz cells in the cat. *Quarterly Journal of Experimental Physiology* **44**, 1–125.

PHILLIPS, C. G. (1961). Some properties of pyramidal neurones of the motor cortex. In *The Nature of Sleep*, ed. WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 4–29. Churchill, London.

ROSENTHAL, J., WALLER, H. J. & AMASSIAN, V. E. (1967). An analysis of the activation of motor cortical neurons by surface stimulation. *Journal of Neurophysiology* **30**, 844–858.

ROTHWELL, J. C., FERBERT, A., CARAMIA, M. D., KUJIRAI, T., DAY, B. L. & THOMPSON, P. D. (1991). Intracortical inhibitory circuits studied in humans. *Neurology* **41**, 192.

THOMPSON, P. D., DAY, B. L., CROCKARD, H. A., CALDER, J., MURRAY, N. M. F., ROTHWELL, J. C. & MARSDEN, C. D. (1991). Intra-operative recording of motor tract potentials at the cervico-medullary junction following scalp electrical and magnetic stimulation of the motor cortex. *Journal of Neurology, Neurosurgery and Psychiatry* **54**, 618–623.

UGAWA, Y., DAY, B. L., ROTHWELL, J. C., THOMPSON, P. D., MERTON, P. A. & MARSDEN, C. D. (1991). Modulation of motor cortical excitability by electrical stimulation over the cerebellum in intact man. *Journal of Physiology* **441**, 57–72.