Interaction between intracortical inhibition and facilitation in human motor cortex

Ulf Ziemann, John C. Rothwell* and Michael C. Ridding

MRC Human Movement and Balance Unit, Institute of Neurology, Queen Square, London WC1N 3BG, UK

1. In seven normal subjects, subthreshold transcranial magnetic conditioning stimuli (using a figure-of-eight coil) were applied over the motor cortex in order to evoke activity in intracortical neuronal circuits. The net effect on cortical excitability was evaluated by measuring the effect on the size of EMG responses elicited in the abductor digiti minimi (ADM) muscle by a subsequent suprathreshold test stimulus.

2. A single conditioning stimulus suppressed the size of the test response at interstimulus intervals (ISIs) of 1–4 ms whereas the response was facilitated at ISIs of 6–20 ms. The facilitation could be augmented if pairs of conditioning stimuli were given.

3. Inhibition and facilitation appeared to have separate mechanisms. The threshold for inhibition (0·7 active motor threshold) was slightly lower than that for facilitation (0·8 active threshold). Similarly, the inhibitory effect was independent of the direction of current flow induced in the cortex by the conditioning shock, whereas facilitation was maximal with posterior–anterior currents and minimal with lateromedial current.

4. Direct corticospinal effects were probably not responsible for the results since facilitation of cortical test responses could be produced by conditioning stimuli which had no effect on the amplitude of H reflexes elicited in active ADM muscle.

5. Inhibition and facilitation appeared to interact in a roughly linear manner, consistent with separate inputs to a common neurone.

6. We suggest that subthreshold transcranial magnetic stimulation is capable of activating separate populations of excitatory and inhibitory interneurones in the motor cortex.

Kujirai *et al.* (1993) reported that a single subthreshold magnetic stimulus over the motor cortex could suppress the response to a later suprathreshold test stimulus. They postulated that the first stimulus produced this effect by activation of a set of intracortical inhibitory neurones. They also noted a later phase of facilitation, but did not investigate the mechanism in any detail. The present paper addresses this question and shows that the later facilitation is probably caused by the activation of a separate set of facilitatory cortical neurones. The outputs of these two sets of neurones appear to interact independently at or before the final stage of pyramidal output.

METHODS

The experiments were performed, with the approval of the joint ethical committee of the National Hospital for Neurology and Institute of Neurology, on seven normal healthy subjects (all men) aged 27–44 years. The subjects gave their informed consent and were seated in a comfortable reclining chair during the procedures. Surface electromyographic (EMG) recordings were made from the right abductor digiti minimi (ADM) muscle with the active electrode placed over the motor point and the reference electrode on the proximal interphalangeal joint of the small finger. The raw signal was amplified and filtered by Digitimer D150 amplifiers (Digitimer Ltd, Welwyn Garden City, Herts, UK) with a time constant of 10 ms and a low-pass filter of 3 kHz. Signals were then passed through a CED 401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) and fed to a personal computer (sampling rate 5 kHz), using data collection and conditional averaging software.

Transcranial magnetic stimulation (TMS) was applied over the hand area of the left motor cortex through figure-of-eight-shaped coils (outer diameter of each loop, 9 cm; peak magnetic field of 2·4 T) using high-power Magstim 200 magnetic stimulators (Magstim Co., Whitland, Dyfed, UK). In most experiments, two or three stimulators were connected to the same coil through a bi-stim or tri-stim module (Magstim). In those experiments using a sequence of four stimuli, three were given via the tri-stim module and the forth via a second coil placed on top of the other one. Experiments were conducted in a conditioning–test design. The suprathreshold test shock was set to evoke an EMG response in the right ADM of 0·5–1·5 mV of amplitude. The preceding conditioning stimuli in

* To whom correspondence should be addressed.

most experiments were set to 5% of stimulator output below motor threshold in the active ADM. Threshold intensity was defined as the intensity needed to produce a minimal EMG response (> 100 μV) in at least two of five consecutive trials. Threshold was checked throughout the experiments and usually was found to be of constant value. The interstimulus interval (ISI) was varied in order to study the time course of the conditioning effect on the test response. Several blocks of trials were performed, each consisting of four to six randomly intermixed conditions: the test shock given alone and the test shock preceded by one, or in some experiments, two or three conditioning stimuli. The minimum time between trials was 10 s. In all experiments, ten trials per condition were collected and averaged. Changes in peak-to-peak amplitude of the EMG response were expressed as a percentage of the unconditioned mean. These experiments were performed in the relaxed ADM.

In some experiments, a single conditioning stimulus was used at varying intensities. ISIs of 3 ms and of 8, 10 or 15 ms were selected as representative intervals which produced clear inhibition and facilitation of the test response, respectively. To investigate the effect of coil orientation, two coils on top of each other were used. The test shock was delivered through the bottom coil which was always held so that the junction was oriented from posterior to anterior with the induced current in the brain flowing forwards, perpendicular to the assumed direction of the central sulcus. The conditioning shock was applied through the top coil which was either oriented as the test coil or turned through a 90 deg angle so that the induced current in the brain was flowing from lateral to medial along the central sulcus.

In order to map the topographic extent of the conditioning effect, a single subthreshold conditioning stimulus was given through one coil which was moved in 1 cm steps along an anteroposterior and lateromedial axis away from the position of the lowest motor threshold, while the test shock was applied through a second coil which was fixed at this position. The interstimulus interval in the mapping studies was set individually so as to produce a maximum facilitation of the test response. In order to exclude a significant spinal contribution to the facilitation seen when the EMG response to a suprathreshold cortical magnetic shock is conditioned by a subthreshold cortical shock, H reflexes were elicited in the tonically active ADM by low-intensity electrical stimulation of the ulnar nerve at the elbow. These were conditioned at appropriate intervals by the same transcranial magnetic conditioning shocks as used above.

Statistical procedures

Student's two-tailed t test for paired samples was applied for comparison of the mean data from the same subjects obtained under different conditions. Student's two-tailed one-sample t test was carried out for comparison of test data from a group of subjects with a 100% control value. Whenever the data did not meet the assumption of an approximately normal distribution, a logarithmic transformation was performed before using the t test.

RESULTS

Characteristics of facilitation and inhibition

Figure 1*A* shows the time course of the effect of a single (continuous lines) conditioning shock on the size of EMG responses evoked in ADM by a suprathreshold test shock. The intensity of the conditioning shock was set to 5% less than the threshold for producing any responses in active muscle. The time course is similar to that described by Kujirai *et al.* (1993) and consists of an early period of inhibition, followed by a later facilitation. We also confirmed that the threshold for inhibition was lower than that for facilitation. Intervals between conditioning and test stimuli of 3 ms (inhibition) and 8, 10 and 15 ms (facilitation) were investigated in five subjects. Figure 1*B* shows that suppression at 3 ms was significant when the intensity of the conditioning shock was only 70% of the threshold for evoking a minimal response in active muscle. In contrast, significant facilitation occurred at the slightly higher intensity of 80% threshold.

The amount of facilitation produced by a single conditioning shock in any individual can sometimes be quite small. In order to demonstrate facilitation in these subjects more clearly we sometimes used pairs of equal conditioning shocks. When two conditioning stimuli were given (so that the interval between the two conditioning stimuli was the same as the interval between the last conditioning shock and the test shock), there was significantly more facilitation at intervals at 4·5–6 ms ($P < 0{\cdot}05$, paired t test; see Fig. 1*A*, dotted lines) and significantly less suppression at 4 ms. The strong suppression at intervals of 1 and 3 ms was unchanged by the additional conditioning shock.

Spinal or cortical facilitation?

It is possible that the conditioning shock might evoke a small descending corticospinal volley insufficient to discharge spinal motoneurones, but nevertheless capable of raising excitability so that the response to a subsequent test shock is enhanced. The present very low threshold at which conditioning shocks could produce facilitation makes this seem unlikely. However, we sought more evidence for possible spinal effects using H reflex studies.

Because H reflexes are rarely observed in relaxed ADM, the next set of experiments was conducted during voluntary activation of the muscle. Figure 2*A* shows raw data from one subject using conditioning stimuli which were just subthreshold for producing any responses in active muscle. Two such conditioning stimuli given at intervals of 8 and 16 ms prior to a cortical test shock produced clear facilitation (upper pair of superimposed traces). The size of the effect was rather less than that usually observed in relaxed subjects since the amount of facilitation (and inhibition) is often less in active than in relaxed muscles (see Ridding, Taylor & Rothwell, 1995). Indeed in some subjects, facilitation may even be absent when only a single conditioning shock is given. In contrast to the facilitation of a cortical test response, conditioning stimuli at equivalent intervals (corrected for the different efferent delays of the H and cortical responses) had no effect on the H reflex (lower superimposed traces in Fig. 2*A*). The graph in Fig. 2*B* shows the mean data from three subjects at three different interstimulus intervals. At 5·5, 8 and 15 ms, a pair of conditioning stimuli produced facilitation of cortical test responses, but had no effect on H reflexes (or even suppressed them at 5·5 ms). We conclude that when pairs of

conditioning stimuli are given, their intensity may be adjusted so that they produce clear facilitation of cortical responses without observable effects on spinal cord circuitry.

Effect of coil orientation and position on amount of facilitation

Several authors have reported that the EMG responses in hand muscles following a single suprathreshold magnetic stimulation are maximal when the direction of the induced stimulus current in the brain flows from posterior to anterior approximately perpendicular to the central sulcus (Brasil-Neto, Cohen, Panizza, Nilsson, Roth & Hallett, 1992; Mills, Boniface & Schubert, 1992). In order to study whether subthreshold inhibition and facilitation are equally sensitive to coil orientation we conducted experiments with two figure-of-eight coils on top of each other. While the coil delivering the test shock remained at a constant orientation (induced current from back to front of the brain

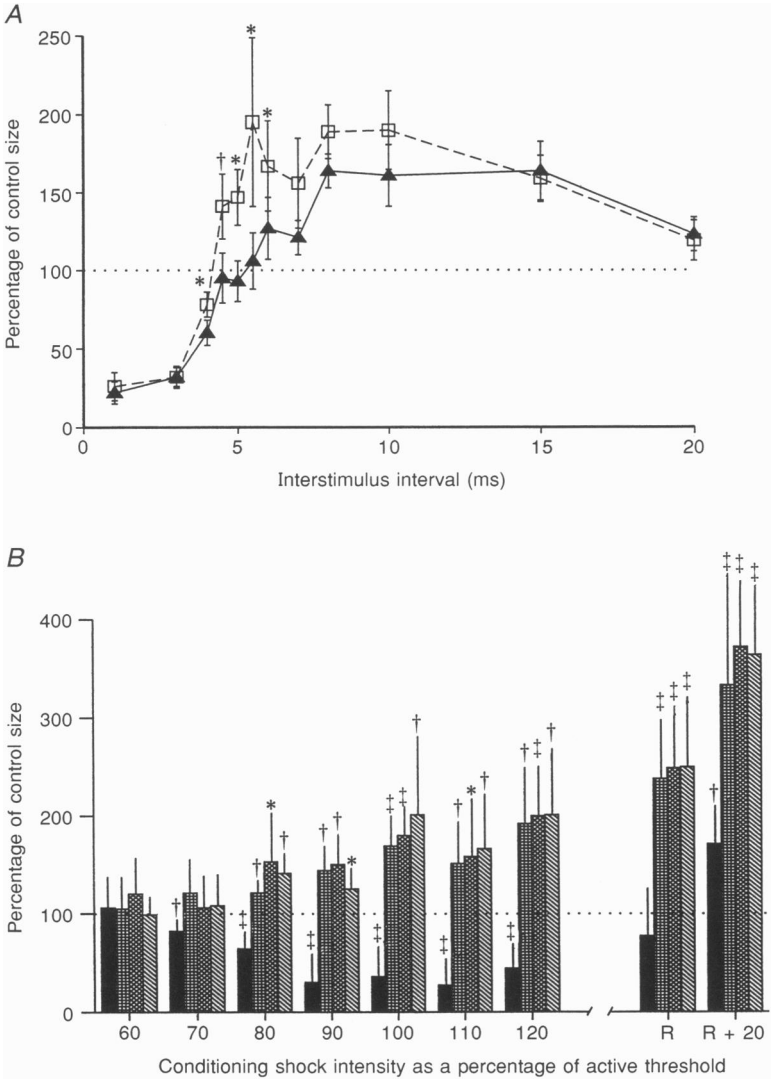

Figure 1. Time course and effect of stimulus intensity on intracortical inhibition and facilitation

A shows the mean (± S.E.M.) time course of inhibition and facilitation of the test response in the relaxed ADM of six subjects by a single (▲) or a pair of subthreshold conditioning stimuli (□). The interval between the first of the two conditioning stimuli was always the same as the interval between the second conditioning stimulus and the test shock. The ISI on the abscissa refers to the latter interval. At each interval, the size of the conditioned response is expressed as a percentage of the size of the control response. Symbols indicate significantly less suppression or extra facilitation of the test response when two instead of one conditioning shocks were given (* $P < 0{\cdot}05$; † $P < 0{\cdot}01$; two-tailed t test for paired samples, data logarithmically transformed). *B*, the effect of the intensity of conditioning shock on the amount of inhibition and facilitation at ISIs of 3 (■), 8 (▦), 10 (▩) and 15 ms (▧). Mean (+ S.E.M.) data from five subjects. R is the threshold in relaxed muscle. Symbols indicate a significant inhibitory or facilitatory effect of the conditioning shock on the size of the test response (* $P < 0{\cdot}05$; † $P < 0{\cdot}01$; ‡ $P < 0{\cdot}001$; two-tailed t test for single samples).

perpendicular to the presumed line of the central sulcus) two orientations of the conditioning coil were compared (same orientation as test coil; orientation turned through a 90 deg angle with induced current from lateral to medial along the central sulcus). The results of three subjects are shown in Fig. 3. While the inhibition remained largely unaffected by the orientation of the conditioning coil, the facilitation, which was clearly visible when the conditioning coil had the same orientation as the test coil, disappeared when the conditioning coil was turned through 90 deg (compare ▲ and □ in Fig. 3). The differential effect was significant at interstimulus intervals of 5·5, 7 and 8 ms. These findings again confirm the hypothesis that inhibition and facilitation are produced by separate mechanisms. In addition, they show that the inhibition is mediated by neural elements which have no preferred orientation in the plane parallel to the skull, whereas facilitation is mediated by neural elements which have a preferred orientation perpendicular to the line of the central sulcus.

In three subjects we mapped the best position for producing facilitation by using two coils placed on top of each other. The test coil was kept fixed at the position at the lowest motor threshold, whilst the conditioning coil was moved in 1 cm steps away from this point along the lateromedial or posterior–anterior axis. In separate sessions, both coils were oriented either perpendicularly to the assumed line of the central sulcus, or parallel to this. In each of the three subjects the interstimulus interval between conditioning and test shocks was adjusted individually to produce maximum facilitation. All three subjects showed that the maximum effect of the conditioning shock occurred when the coil was over the optimal test position (Fig. 4). Moving the coil 1 or 2 cm in any direction away from this point produced a steep fall off in the effect. In general, the decline in facilitation with distance paralleled the increase in motor threshold which occurred as the coil was moved from the optimum position.

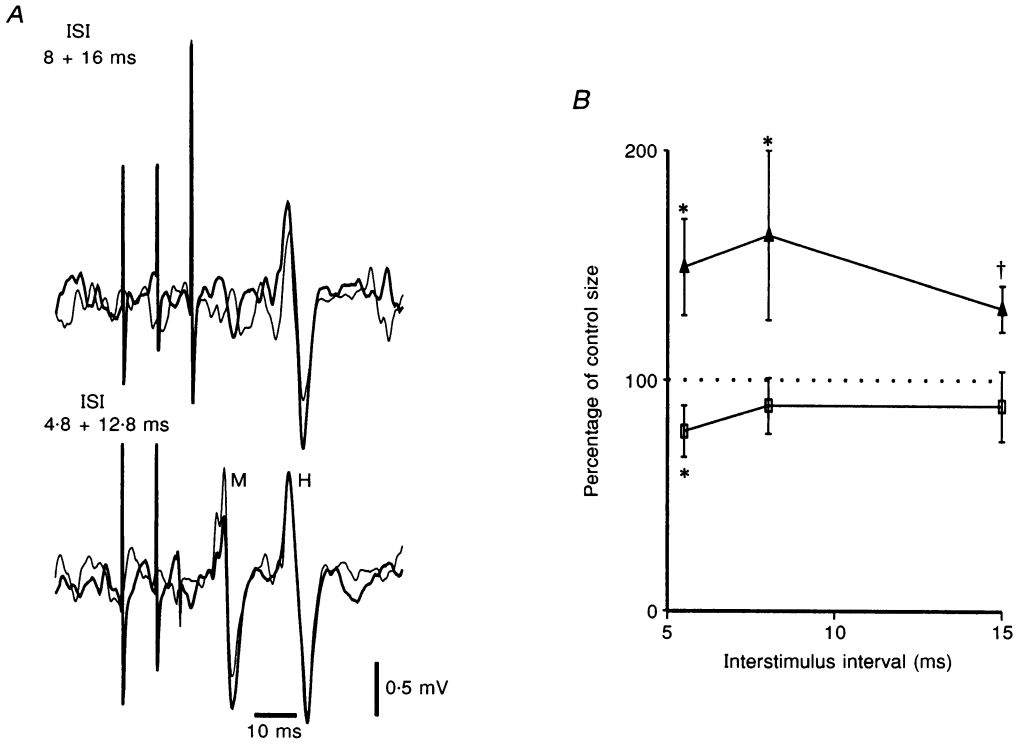

Figure 2. Comparison of the effect of a pair of cortical conditioning stimuli on the response to a cortical test shock or on the H reflex

A shows electromyographic responses in the active ADM of one representative subject: the response to a suprathreshold magnetic brain stimulus is facilitated by two subthreshold magnetic stimuli (upper trace) while the H reflex is unchanged (lower trace). The slightly smaller potential which precedes the H reflex is an M wave. The conditioning cortical shocks led to a small downward baseline shift, so that the M waves are not exactly superimposed although they are of equal amplitude in both conditions. Note that the interstimulus intervals of the conditioning brain stimuli relative to the H reflex stimulus are adjusted so as to provide a similar timing of the conditioning shocks relative to the onset of the test EMG response in the muscle. *B* shows the mean facilitation (± S.E.M.) of a cortical test shock (▲) in the active ADM of three subjects and the corresponding changes in H reflex size (□) produced by a pair of conditioning stimuli. Interstimulus intervals refer to the interval between the second of the two conditioning shocks and the cortical test shock. Symbols indicate significant changes from the control level (* $P < 0{\cdot}05$; † $P < 0{\cdot}01$; two-tailed one-sample t test).

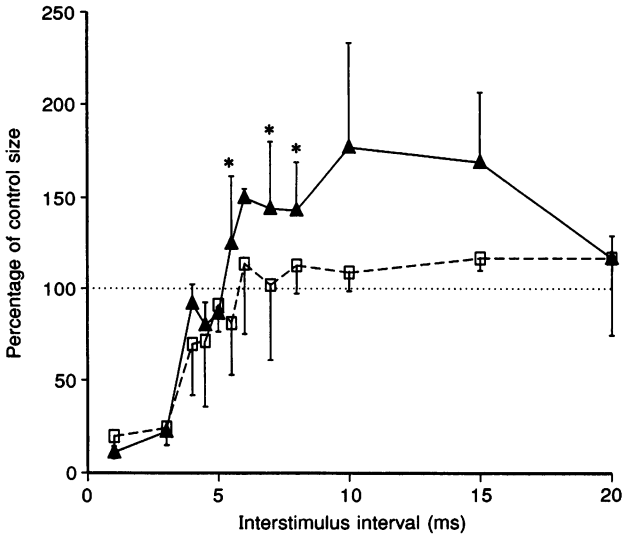

Figure 3. Effect of magnetic coil orientation on the amount of intracortical inhibition and facilitation

Mean (± S.E.M.) time course of changes in the size of the test response in the relaxed abductor digiti minimi (ADM) of three subjects for two different orientations of the conditioning coil. The test coil was held in a constant position with the current induced in the brain flowing from posterior to anterior approximately perpendicular to the assumed line of the central sulcus. The conditioning coil was held either in the same direction as the test coil (resulting excitability curve, ▲) or turned through a 90 deg angle so that the induced current flowed from lateral to medial along the central sulcus (□). Asterisks indicate a significant difference in the amount of facilitation ($*P < 0{\cdot}05$; two-tailed t test for paired samples, data logarithmically transformed). Note that a clear facilitation of the test response was visible only when the conditioning current flowed perpendicular to the central sulcus.

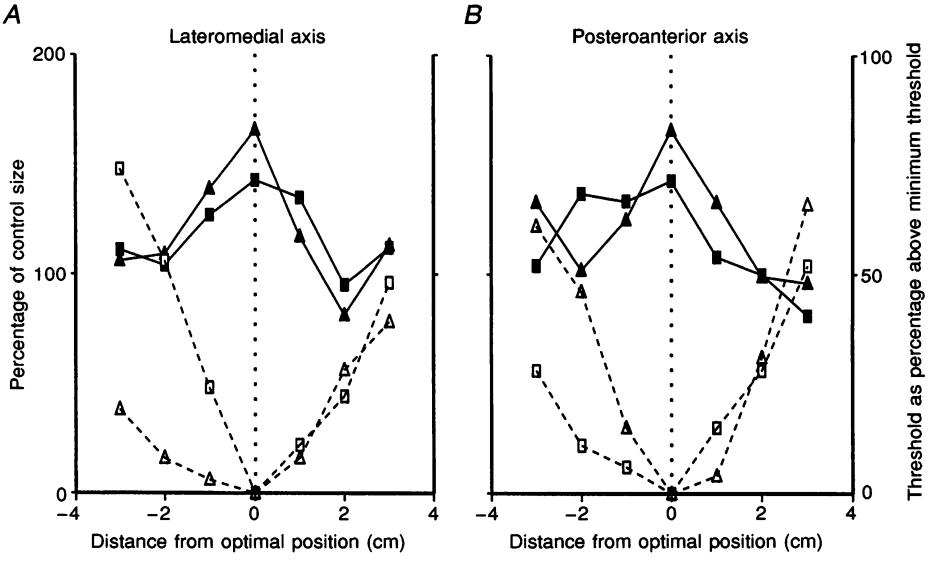

Figure 4. Mapping intracortical facilitation and motor threshold in the relaxed abductor digiti minimi

Intracortical facilitation; filled symbols, left-hand y-axis. Motor threshold; open symbols, right-hand y-axis. Mapping was performed with two coils on top of each other: the coil delivering the test shock was held fixed at the position of minimum motor threshold (indicated by the vertical dotted lines) while the coil delivering the conditioning shock was moved in 1 cm steps along the lateromedial (*A*) and posteroanterior axes (*B*). The mapping was performed with both coils oriented either at right angles (squares) or in parallel (triangles) to the central sulcus. The interstimulus interval was set individually (range, 5–8 ms) so as to produce maximal facilitation. At each stimulation site, ten test and ten conditioned trials were collected. Results are the means from three different subjects.

Interaction of facilitation and inhibition

The principal result is illustrated in Fig. 5. Two examples of average raw data from two different subjects are shown in panels *A* and *B*. The experiments were conducted in relaxed subjects and show the interaction between inhibitory and facilitatory conditioning stimuli. In the subject shown in Fig. 5*A*, a single conditioning stimulus inhibited the test response to 8% of its control value when given 3 ms beforehand. If the same stimulus was given 6 ms beforehand, then it facilitated the test response by 128%. When both conditioning stimuli were given (i.e. at intervals of 6 and 3 ms before the test shock), then the response was again

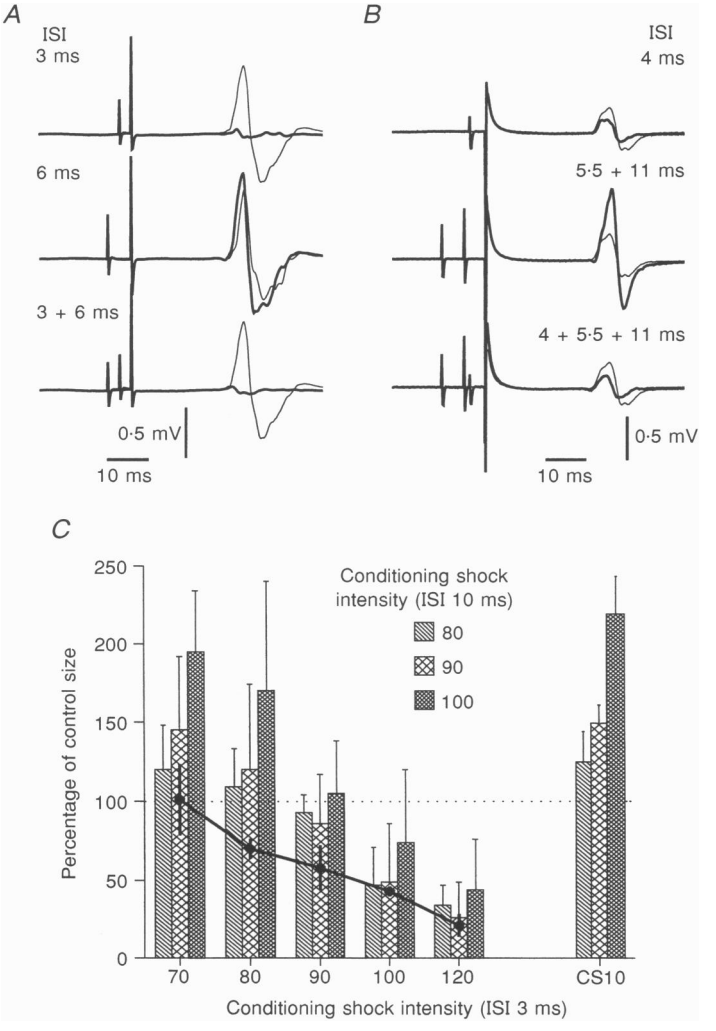

Figure 5. Interaction of inhibition and facilitation

A and *B* show mean EMG responses to focal magnetic stimulation of motor cortex in the relaxed abductor digiti minimi muscle (ADM). All records consist of two superimposed traces: the thin traces show the response to the test stimulus given alone, while the thick traces show the responses to the test stimulus conditioned by one or two prior stimuli. All traces are the means of ten trials. In *A*, a single subthreshold conditioning stimulus at ISI = 3 ms produces profound suppression of the test response, whereas the same stimulus given at an ISI = 6 ms produces facilitation. If two stimuli are given at 3 and 6 ms, then the test response is suppressed to the same extent as when a single shock is given at 3 ms. In *B*, a single subthreshold magnetic conditioning shock at ISI = 4 ms (upper trace) results in inhibition of the test response, while a pair of two subthreshold conditioning stimuli at 5·5 and 11 ms strongly facilitates the test response (middle trace). A combination of all three conditioning stimuli (lower trace) produces inhibition of the test response similar to that seen with a single conditioning pulse at 4 ms. *C* illustrates the interaction of inhibition (using a single conditioning shock at an ISI of 3 ms) and facilitation (a conditioning shock at ISI = 10 ms) in three subjects using different intensities of stimulation. The three bars on the right show the mean (+ S.E.M.) effect of three different intensities (80, 100 and 120% threshold) of conditioning shock given alone at ISI = 10 ms (CS10). The continuous line and filled circles show the effect of five different intensities (70, 80, 90, 100 and 120% threshold) of conditioning shock given alone at ISI = 3 ms. The remaining histogram triplets show the effect of giving both conditioning stimuli at each of the fifteen different combinations of intensity.

suppressed to 7% of its control value. It was as if the addition of the second conditioning shock at 6 ms had had little influence on the amount of inhibition produced by the conditioning stimulus at 3 ms.

In order for facilitation to become apparent in the subject illustrated in Fig. 5*B*, we had to use a pair of conditioning shocks at 5·5 and 11 ms before the test shock. This produced very prominent facilitation (266%). A single conditioning shock given on its own 4 ms before the test shock produced suppression of 51%. When all three conditioning stimuli were given (4, 5·5 and 11 ms), then the presence of the facilitatory pair of conditioning stimuli (5·5 and 11 ms) again had little effect on the final amount of inhibition (52%) produced by the conditioning shock at 4 ms.

The amount of inhibition in these examples was very large, and therefore the apparent predominance of inhibition could have been the result of a saturation effect. In order to address this problem, we explored in three subjects the interaction of three different intensities of facilitatory conditioning stimuli with five different intensities of inhibitory conditioning stimuli. Single conditioning shocks were used, with the inhibitory interval fixed at 3 ms, and the facilitatory interval at 10 ms. The results are shown in Fig. 5*C*. The group of bars on the right of the graph shows the effect of giving the facilitatory conditioning stimulus alone: larger stimuli produce more facilitation. The filled circles and continuous line show the effect of giving the inhibitory conditioning stimulus alone: the amount of inhibition increases with increasing stimulus intensity. The remaining bars show the effect of interacting both stimuli. When the intensity of the inhibitory conditioning shock was 90% of active motor threshold or less, then the facilitatory and inhibitory effects summed in an approximately linear fashion. For example, an inhibitory conditioning shock given at an intensity of 90% threshold suppressed test responses to approximately 60% of their control size. A facilitatory conditioning stimulus at 100% threshold intensity increased the size of the test response to about 150% control. When both were given together, the test response was about 90%, which is equal to the expected product of each effect alone (= 60% × 150%). In contrast to this, when the effect of the inhibitory conditioning stimulus given alone was very pronounced, facilitation could be swamped. For example, the effect of a facilitatory conditioning shock of 80% threshold was virtually abolished by an inhibitory shock given at 100% threshold.

DISCUSSION

The present results have confirmed that a single subthreshold conditioning shock produces inhibition and then facilitation of EMG responses elicited by a subsequent suprathreshold test shock. We argue here that the facilitation, like the inhibition (see Kujirai *et al.* 1993) may occur because of interactions within the motor cortex. Furthermore, we provide evidence that facilitation and inhibition are separate phenomena, and that they interact within the motor cortex in an approximately linear fashion.

Level at which facilitation occurs

A test shock can be facilitated at interstimulus intervals of 6–20 ms by a preceding conditioning shock. When the intensity of the conditioning stimulus is less than the threshold needed to evoke responses in relaxed muscles, but greater than the threshold needed to evoke responses in active muscles, then some part of this facilitation occurs because of changes in spinal cord excitability. Such stimuli produce effects on H reflexes which can be facilitated for up to 20 ms after a single conditioning stimulus (see for example Cowan, Day, Marsden & Rothwell, 1986). However, in the present experiments, we employed stimuli which were smaller than this. At 5% less than the threshold for evoking any EMG responses in active muscle they should evoke little or no descending corticospinal activity. Additionally, since the threshold for producing a descending corticospinal volley is higher in relaxed than in active subjects (Mazzochio, Rothwell, Day & Thompson 1994), then performing experiments whilst relaxed makes it even more likely that descending input to motoneurones will be minimal or absent. This reasoning was borne out in our H reflex experiment. Pairs of conditioning stimuli which produced clear facilitation of cortical test responses had no effect on H reflexes in the same muscles. We conclude that facilitation using very small conditioning stimuli can occur at a suprasegmental level.

From the present results, it is difficult to be completely certain whether facilitation occurs because of interaction in the motor cortex itself, or perhaps in some subcortical structure (for example the propriospinal neurones explored by Burke, Gracies, Mazevet & Pierrot-Deseilligny, 1992; Gracies, Meunier & Pierrot-Deseilligny, 1994). There is one piece of evidence, though, that favours the former possibility. If facilitation were due to interaction at, say, a propriospinal level, then it should not be evident in the initial, monosynaptic portion of the EMG response (which bypasses these subcortical relay sites). In fact, the results in Fig. 2*A* (obtained in active muscle) appear to show facilitation even at the very onset of the EMG response. (Note that preactivation of the target muscle is important for the validity of this argument since this shortens the time for spinal motoneurones to reach firing threshold (Thompson, Day, Rothwell, Dressler, Maertens de Noordhout & Marsden, 1991).) This means that propriospinal neurones or other spinal neurones at a pre-motoneuronal stage are unlikely to have time to contribute to the early part of the EMG response. In conclusion, we suggest that the facilitation in the present experiments is likely to have occurred at a cortical level.

Separate mechanisms for motor cortical inhibition and facilitation

The different threshold of inhibition and facilitation seems to provide the simplest evidence that the two phenomena have separate mechanisms. However, it is possible that the

higher threshold for facilitation is due to inhibition continuing 'beneath' the facilitation. If so, then one might expect that a short latency inhibition, like that seen in the present experiments (this would be similar to the $GABA_A$ inhibition in animal experiments), would decay over 10–20 ms, and therefore that facilitation at longer intervals would have a progressively lower threshold. This was not the case, since the threshold at 8, 10 and 15 ms was the same. We conclude that the threshold difference is probably real. If so, then it is also possible to argue that the facilitation is not a rebound phenomenon from the prior inhibition since the latter can occur in isolation.

An additional piece of evidence for separate mechanisms is that facilitation depends upon the direction of the conditioning current in the brain, whereas inhibition does not. This indicates that separate populations of cortical neurones are responsible for the two effects.

We can only speculate on the nature of the neurones which might produce the facilitatory effect. They are not likely to be recurrent collaterals of corticospinal cells since the threshold for producing facilitation was less than the threshold for producing corticospinal activity. Mapping experiments suggest that the excitable portion of the neurones is likely to be located in or near the sensorimotor cortex, although their cell body may be elsewhere. Possible candidates are the numerous cortico-cortically projecting pyramidal cells and their axons which are located mainly in superficial cortical layers II and IIIa (Jones & Wise, 1977). Their axons are mainly arranged horizontally with a maximal extent in the anterior–posterior direction (Gatter & Powell, 1978). This, in addition to their superficial location, would make them readily accessible to transcranial magnetic stimulation at low threshold, especially when the induced current in the cortex flows along the anterior–posterior axis. However, their electrophysiological connectivity to the corticospinal neurones of layer V is not known in great detail. Probably, the projection is mainly polysynaptic (Asanuma & Rosén, 1973). Gosh & Porter (1988) found that closely spaced bifocal electrical stimulation of somatosensory or pre-motor cortex could elicit EPSPs in corticospinal neurones of motor cortex at latencies of 1·1–7·9 ms. They speculated that the effects were due to stimulation of layer III cells which had been preferentially activated by bifocal stimulation.

Relationship to other reports on motor cortical facilitation in man

In some recent reports, paired magnetic stimuli with subthreshold conditioning shocks were used to test the intracortical excitability of patients with various motor disorders. Patients with Parkinson's disease (Ridding, Inzelberg & Rothwell, 1995), focal task-specific dystonia (Ridding, Sheean, Rothwell, Inzelberg & Kurjirai, 1995) or cortical myoclonus (Brown, Ridding, Werhahn, Rothwell & Marsden, 1996) had reduced cortical inhibition whilst the later phase of facilitation was relatively normal. In contrast, paired-stimulation studies in normal subjects showed that single oral doses of anti-epileptic drugs which enhance the gain of the inhibitory neurotransmitter γ-aminobutyric acid suppress facilitation with little effect on the earlier inhibition (Ziemann, Lönnecker, Steinhoff & Paulus, 1996*a*, *b*). This differential effect of disease or drug therapy on the inhibition and facilitation is further evidence that they are produced by separate neuronal mechanisms.

Two other groups of authors have reported facilitation of test responses at interstimulus intervals of 10–30 ms (Claus, Weis, Jahnke, Plewe & Brunhölzl, 1992) and 25–50 ms (Valls-Solé, Pascual-Leone, Wassermann & Hallett, 1992). However, the effects were observed with conditioning stimuli that were suprathreshold for evoking motor responses (and which were usually equal in intensity to that of the test shock itself). These larger conditioning stimuli may produce effects at both cortical and subcortical levels so that it is unclear how they relate to the probable cortical effects described here.

Interaction of inhibition and facilitation

A single conditioning shock can produce strong suppression of a test response at ISIs of 5 ms or less. Kujirai *et al.* (1993) suggested that much of this effect was due to activation of intracortical inhibitory synapses. At very short intervals (< 2 ms) neuronal refractoriness may also play a part, but this is likely to be minor at intervals of 2 ms or more given that, for example, corticospinal neurones in humans can follow stimulation frequencies of up to 500 Hz (Katayama, Tsubokawa, Maejima, Hirayama & Yamamoto, 1988), and that the so called indirect (I) waves, which are thought to represent trans-synaptic activation of corticospinal neurones, follow or are even facilitated at a stimulation frequency of 300 Hz in the cat motor cortex (Amassian, Stewart, Quirk & Rosenthal, 1987).

If the inhibition is synaptic, then the approximately linear interaction with later facilitation is most simply explained by convergence of two independent inputs onto the same region of a common target cell. A likely candidate is the pyramidal cell itself, perhaps acting as suggested by many authors (e.g. Phillips, 1969; Evarts & Fromm, 1980) as an important summing point in the motor system. The very strong inhibition that can sometimes dominate facilitation is also of interest. It may be one factor that tends to terminate the repetitive series of facilitatory I-wave inputs to pyramidal neurones when single large stimuli are given to motor cortex.

In conclusion, we have shown that subthreshold magnetic stimulation of motor cortex activates separate populations of inhibitory and excitatory interneurones within the cortex, and that these interact in a roughly linear manner before or at the pyramidal output stage.

Amassian, V. E., Stewart, M., Quirk, G. J. & Rosenthal, J. L. (1987). Physiological basis of motor effects of a transient stimulus to cerebral cortex. *Neurosurgery* **20**, 74–93.

Asanuma, H. & Rosén, I. (1973). Spread of mono- and polysynaptic connections within cat motor cortex. *Experimental Brain Research* **16**, 507–520.

Brasil-Neto, J. P., Cohen, L. G., Panizza, M., Nilsson, J., Roth, B. J. & Hallett, M. (1992). Optimal focal transcranial magnetic activation of the human motor cortex: Effects of coil orientation, shape of the induced current pulse, and stimulus intensity. *Journal of Clinical Neurophysiology* **9**, 132–136.

Brown, P., Ridding, M. C., Werhahn, K., Rothwell, J. C. & Marsden, C. D. (1996). Abnormalities in the balance between inhibition and excitation in the motor cortex of patients with cortical myoclonus. *Brain* **119**, 309–317.

Burke, D., Gracies, J. M., Mazevet, D., Meunier, S. & Pierrot-Deseilligny, E. (1992). Convergence of descending and various peripheral inputs onto common propriospinal-like neurones in man. *Journal of Physiology* **449**, 655–671.

Claus, D., Weis, M., Jahnke, U., Plewe, A. & Brunhölzl, C. (1992). Corticospinal conduction studied with magnetic double stimulation in the intact human. *Journal of the Neurological Sciences* **111**, 180–188.

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**, 333–347.

Evarts, E. V. & Fromm, C. (1980). Information processing in the sensorimotor cortex during voluntary movement. *Progress in Brain Research* **54**, 143–155.

Gatter, K. C. & Powell, T. P. S. (1978). The intrinsic connections of the cortex of area 4 of the monkey. *Brain* **101**, 513–541.

Gosh, S. & Porter, R. (1988). Corticocortical synaptic influences on morphologically identified pyramidal neurones in the motor cortex of the monkey. *Journal of Physiology* **400**, 617–629.

Gracies, J. M., Meunier, S. & Pierrot-Deseilligny, E. (1994). Evidence for corticospinal excitation of presumed propriospinal neurones in man. *Journal of Physiology* **475**, 509–518.

Jones, E. G. & Wise, S. P. (1977). Size, laminar and columnar distribution of efferent cells in the sensory-motor cortex of monkeys. *Journal of Comparative Neurology* **175**, 391–438.

Katayama, Y., Tsubokowa, T., Maejima, S., Hirayama, T. & Yamamoto, T. (1988). Corticospinal direct response in humans: identification of the motor cortex during intracranial surgery under general anaesthesia. *Journal of Neurology, Neurosurgery and Psychiatry* **51**, 50–59.

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., Wroe, S., Asselmam, P. & Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. *Journal of Physiology* **471**, 501–519.

Mazzocchio, R., Rothwell, J. C., Day, B. L., Thompson, P. D. (1994). Effect of tonic voluntary activity on the excitability of human motor cortex. *Journal of Physiology* **474**, 261–270.

Mills, K. R., Boniface, S. J. & Schubert, M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. *Electroencephalography and Clinical Neurophysiology* **85**, 17–21.

Phillips, C. G. (1969). Motor apparatus of the baboon's hand. *Proceedings of the Royal Society* B **173**, 141–174.

Ridding, M. C., Inzelberg, R. & Rothwell, J. C. (1995). Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. *Annals of Neurology* **37**, 181–188.

Ridding, M. C., Sheean, G., Rothwell, J. C., Inzelberg, R. & Kujirai, T. (1995). Changes in the balance between motor cortical excitation and inhibition in focal task-specific dystonia. *Journal of Neurology, Neurosurgery and Psychiatry* **590**, 493–498.

Ridding, M. C., Taylor, J. L. & Rothwell, J. C. (1995). The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. *Journal of Physiology* **487**, 541–548.

Thompson, P. D., Day, B. L., Rothwell, J. C., Dressler, D., Maertens de Noordhout, A. & Marsden, C. D. (1991). Further observations on the facilitation of muscle responses to cortical stimulation by voluntary contraction. *Electroencephalography and Clinical Neurophysiology* **81**, 397–402.

Valls-Solé, J., Pascual-Leone, A., Wassermann, E. M. & Hallett, M. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalography and Clinical Neurophysiology* **85**, 355–364.

Ziemann, U., Lönnecker, S., Steinhoff, B. J. & Paulus, W. (1996*a*). The effect of lorazepam on the motor cortical excitability in man. *Experimental Brain Research* **109**, 127–135.

Ziemann, U., Lönnecker, S., Steinhoff, B. J. & Paulus, W. (1996*b*). Differential effects of various anti-epileptic drugs on motor cortex excitability in man. A transcranial magnetic stimulation study. *Annals of Neurology* (in the Press).

Acknowledgements

We should like to thank Mr R. Bedlington for building and maintaining much of the equipment used in these experiments.

Author's permanent address

U. Ziemann: Department of Clinical Neurophysiology, Georg-August-Universität, Robert Koch Strasse 40, 37075 Göttingen, Germany.

Author's email address

J. C. Rothwell: j.rothwell@ion.bpmf.ac.uk

Received 22 August 1995; accepted 16 July 1996.