Depression of Renshaw recurrent inhibition by activation of corticospinal fibres in human upper and lower limb

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1. This study tested whether the recurrent inhibition of soleus and wrist flexor motoneurones could be modified by transcranial magnetic stimulation in human subjects.

2. Magnetic stimulation was given through a circular coil centred at the vertex. The intensity of the magnetic stimulus was subthreshold for evoking a motor response in the active soleus and wrist flexor muscles. The recurrent inhibition brought about by a conditioning H1 reflex discharge was estimated by a test H′ reflex. The modifications of the recurrent inhibition after cortical stimulation were distinguished from the motoneuronal changes by comparing H′ to a reference H reflex.

3. In the soleus motoneurones, the reference H reflex was inhibited at a minimum conditioning–test interval of −2 ms (H reflex stimulus before magnetic stimulation). In contrast, the H′ reflex was facilitated at minimum conditioning–test intervals of +1 ms. In the wrist flexor motoneurones, both H′ and reference H reflexes were facilitated. However, at lower cortical stimulus intensities, only the H′ reflex was facilitated at minimum conditioning–test intervals of +1 ms.

4. In both motoneurone pools, H′ facilitation started 3–4 ms later than the earliest changes in the reference H reflex. Also, the threshold of H′ facilitation was lower than that of reference H reflex.

5. It is concluded that facilitation of the H′ reflex is produced by corticospinal inhibition of Renshaw cells via a short interneuronal chain in both the upper and lower limb.

It is well known from animal studies that Renshaw cell activity can be influenced from various supraspinal structures (for references see Baldissera, Hultborn & Illert, 1981). In humans, characteristic changes in recurrent inhibition have been observed during various voluntary and postural contractions: (1) weak tonic contraction of triceps surae, quadriceps and pretibial flexors increases the excitability of the respective Renshaw cells more than can be explained by the input from active motor axon collaterals (Hultborn & Pierrot-Deseilligny, 1979; Rossi & Mazzocchio, 1991), whereas strong tonic contraction of triceps surae decreases the excitability of Renshaw cells which can only be explained by a simultaneous inhibitory convergence on the Renshaw cells (Hultborn & Pierrot-Deseilligny, 1979); (2) before and at the beginning of phasic ramp contractions of triceps surae there is some indication of Renshaw cell facilitation which shifts progressively to an inhibition that peaks at the end of the ramp (Katz, Pierrot-Deseilligny & Hultborn, 1982); (3) tonic or phasic ramp contractions of the pretibial flexors increase the recurrent inhibition of soleus motoneurones (Katz & Pierrot-Deseilligny, 1984); (4) postural contractions while standing unsupported (Pierrot-Deseilligny, Morin, Katz & Bussel, 1977) and after backward tilt of the head–body (Rossi, Mazzocchio & Scarpini, 1987) increase the excitability of Renshaw cells projecting to soleus motoneurones. From all these studies it appears that various supraspinal inputs can modify transmission in the recurrent pathway during natural movements. However, the descending pathways mediating these effects can only be hypothesized. In addition, although the presence and distribution of recurrent inhibition in the human upper limb has been recently reported (Rossi & Mazzocchio, 1992; Katz, Mazzocchio, Penicaud & Rossi, 1993), it is not yet known whether Renshaw cell activity in the upper limb is subjected to the same control as in the lower limb. We therefore tested whether the activity of Renshaw cells could be modified by cortical stimulation in humans at rest.

When the human motor cortex is stimulated transcranially using an electromagnetic stimulator, the initial short-latency facilitatory effects on

motoneurones–interneurones at spinal cord level have been attributed to activity in the fast-conducting component of the corticospinal pathway (Day *et al.* 1989; Brouwer & Ashby, 1992; Palmer & Ashby, 1992). We used this tool to explore the effects of the activation of a 'relatively specific' descending tract on the activity of Renshaw cells of two functionally different motor nuclei: the soleus in the lower limb and the flexors of the wrist in the upper limb. It will be shown that cortical stimulation produces depression of Renshaw cell activity in both motoneurone pools. The type of motor activity in which the descending control of Renshaw cells is involved is discussed.

METHODS

Stimulation and recording technique

Experiments were performed on twelve normal subjects (23–38 years old) with their informed consent and Poeal Ethical Committee approval. The muscles investigated were the flexors of the wrist in the upper limb (18 experiments on 6 subjects) and the soleus in the lower limb (10 experiments on 6 subjects. The subjects were seated and relaxed with the elbow semiflexed (120 deg), the forearm pronated and the hand or the foot immobilized. Surface electrodes were used for both stimulation and recording. The soleus H reflex was elicited by stimulating the posterior tibial nerve. The cathode was a half-ball (2 cm in diameter) in the popliteal fossa, while the anode was fixed to the anterior aspect of the knee. The H reflex from wrist flexors was elicited by stimulating the median nerve through bipolar electrodes placed 2 cm apart in the cubital fossa. Rectangular pulses of 0·5–1 ms duration were delivered by constant-current stimulators. The reflex responses were recorded by non-polarizable disc electrodes (0·9 cm in diameter) and measured in terms of the amplitude of muscle potential.

The brain was stimulated using a magnetic stimulator (Magstim 200; The Magstim Co. Ltd, Whitland, Dyfed, UK) with a 13 cm external diameter coil centred at the vertex with the electric current flowing clockwise in the coil. Intensities were expressed as a percentage of the maximum output of the stimulator. Subthreshold stimuli were defined as shocks below the intensity needed to produce a motor-evoked response during a small background voluntary contraction of the soleus muscle or the wrist flexor muscles (approximately 5–10% of maximum). In most subjects it was in fact difficult to evoke a clear motor-evoked potential in the relaxed soleus muscle.

Stimuli were given randomly every 6–8 s with or without subthreshold shocks to the scalp.

Electrophysiological method for estimating recurrent inhibition

The method for exploring the recurrent inhibition of α-motoneurones in human subjects was that originally developed in the soleus muscle by Bussel & Pierrot-Deseilligny (1977). The discharge of the α-motoneurones by a group I afferent volley (S1) produces, through their recurrent collaterals, orthodromic activation of the Renshaw cells and an EMG response called conditioning H1 reflex. The inhibitory effect of Renshaw activation is assessed by a second H reflex called test H′ reflex elicited by a stimulation supramaximal for α-motor fibres of the same nerve after the S1 volley. The effect of giving the S1 conditioning and the supramaximal test stimuli together at adequate conditioning–test intervals is twofold: on the one hand, it eliminates by collision the descending H1 reflex discharge; on the other hand, it opens the way for the H′ reflex response produced by the orthodromic reactivation of the same motoneurones. Since the H′ reflex can only pass along the motor axons in which previous collision has taken place, only motoneurones which have already fired in the first conditioning discharge have their excitability assessed by the following test H′ volley. Thus the test H′ reflex can be at the very most equal to the H1 reflex. In the soleus motor nucleus (Bussel & Pierrot-Deseilligny, 1977), quadriceps and pretibial motor nuclei (Rossi & Mazzocchio, 1991) and flexor carpi radialis and extensor carpi radialis motor nuclei (Rossi & Mazzocchio, 1992; Katz *et al.* 1993), when the H1 reflex increases beyond a certain value, the absolute value of the test H′ reflex decreases indicating that an increasingly smaller fraction of the motoneurones which have given rise to H1 are involved in the H′ response. Provided that the conditioning–test interval is more than 9 ms, the effect of Ib fibre stimulation by the conditioning stimulus can be eliminated since firstly, it has been demonstrated that in humans the duration of Ib inhibition from triceps to soleus motoneurones is less than 10 ms (Pierrot-Deseilligny *et al.* 1977), and secondly, the amount of inhibition of the test H′ reflex is independent of the conditioning stimulus strength and is only related to the size of the conditioning H1 reflex (Pierrot-Deseilligny & Bussel, 1975; Bussel & Pierrot-Deseilligny, 1977). This reduction of the fraction of the motoneurones involved in H1 which is recruited by the supramaximal test stimulus may well be explained by recurrent inhibition, the amount of which depends upon the size of the conditioning discharge (Ryall, Piercey, Polosa & Goldfarb, 1972). However, the absolute size of the test H′ reflex also depends on the relationship between the strength of the Ia connections and the size of the after-hyperpolarization (AHP). It could be expected that the AHP would prevent the firing of the motoneurones with the weakest excitatory action from the Ia test volley (see Awiszus & Feistner, 1993). Accordingly, the larger the amplitude of H1, the greater the number of motoneurones with AHP which prevent excitation by the Ia test volley and the smaller the fraction of motoneurones involved in H1 which is activated by the supramaximal stimulus. If one disregards the recurrent inhibition it could be postulated that the slope of the curve relating the size of H′ to the size of H1 should decrease with increasing H1. However, increasing H1 results in recruiting faster conducting motoneurones which have a less pronounced AHP than the smallest motoneurones (for references see Bussel & Pierrot-Deseilligny, 1977). Therefore the AHP alone cannot give a decrease of H′ because the individual AHPs are not enhanced when H1 is increased. It follows that if AHP was acting alone, there would be a fixed limit to the number of motoneurones available for the test H′ reflex. Increasing the conditioning reflex should not decrease this number, and the variations in test reflex *versus* those in conditioning reflex

should exhibit a plateau. Since the value of the test H′ reflex is seen to decrease progressively as H1 increases beyond a specific value (as illustrated in Fig. 6), which implies a reduction in the number of motoneurones involved in the test reflex when the conditioning reflex is increased, then this decrement should be due to the recurrent inhibition elicited by the conditioning reflex. This conclusion has been recently confirmed using a pharmacological approach to test the validity of the electrophysiological method (Mazzocchio & Rossi, 1989). As a corollary, it is highly probable that the motoneurone population which is recruited by the supramaximal stimulus is restricted to the motoneurones which are the most susceptible to the Ia excitatory effects. Therefore the motoneurones available for the test H′ reflex should not be very different from each other regarding their AHP and may constitute a relatively homogeneous fraction within the various motor nuclei (see Rossi & Mazzocchio, 1991, 1992).

Experimental procedure with transcranial magnetic stimulation

Cowan, Day, Marsden & Rothwell (1986) using H reflex testing described changes in spinal cord excitability following subthreshold electrical stimulation of the scalp. In the relaxed wrist flexor muscles an initial peak facilitation of the H reflex (lasting 2·5 ms on average) was followed by a more variable and longer-lasting (from 5 to 20 ms) phase of facilitation. The same facilitatory event has been recently described also when using transcranial magnetic stimulation (Mazzocchio, Rothwell, Day & Thompson, 1994). In the soleus muscle, in contrast to the forearm flexors, Cowan *et al.* (1986) found that the initial event was mainly an inhibition of the H reflex. It follows that changes in the size of the test H′ reflex during scalp stimulation can be ascribed to variation in corticospinal control of Renshaw cell activity provided that: (a) the size of the H1 conditioning reflex is maintained constant, given that its variability would *per se* affect the amplitude of the test H′ reflex; and (b) the background excitability of the explored motoneurones is simultaneously estimated, as these are also subjected to the effect of the corticospinal volley. This can be monitored by using a reference H reflex having the same size as the H′ reflex. However, the sensitivity of these reflexes to excitation or inhibition is not the same. It has been shown that identical excitatory inputs to soleus motoneurones cause a smaller increase of the test H′ reflex than of a reference H reflex of similar size (see Hultborn & Pierrot-Deseilligny, 1979). To verify whether this was also the case for the wrist flexor motoneurones, the sensitivities of H′ and reference H reflexes of wrist flexor muscles to combined stimulation of the same nerve were compared. Conditioning stimulation to the median nerve was applied through the same electrode as the test stimulus. The intensity of this conditioning stimulus was just below the threshold of the motor response. Conditioning–test intervals between 2·5 and 5 ms were explored.

Experimental protocol

The basic experimental procedure is illustrated in Fig. 1. The sequence of responses on the left represents the control situation, i.e. in the absence of scalp stimulation. It consists of: *A*, a conditioning stimulus alone so as to elicit the H1 reflex. Its size was always made large enough to be within the range in which the corresponding H′ amplitude was decreasing (see above). The intensity of the conditioning stimulus was adjusted so that there was no, or very little, direct M wave; *B*, the supramaximal test stimulus alone which produced a maximal direct response; *C*, the combined conditioning and supramaximal test stimuli in order to elicit the test H′ reflex; *D*, a stimulus of such intensity as to evoke the reference H reflex. This was always given at the same timing as that for the test H′ reflex. In the lower limb, conditioning–test intervals of 10, 15 and 20 ms were used. It has been demonstrated that the amplitude of the soleus test H′ reflex is relatively stable when the conditioning–supramaximal stimulus interval is larger than 8 ms (Pierrot-Deseilligny, Bussel, Held & Katz, 1976). In the upper limb, the reflex arc being much shorter, interstimulus intervals of 10, 12 and 15 ms were used (see Katz *et al.* 1993). At these intervals, possible Ib effects from the conditioning S1 stimulus should be over, if the duration of Ib inhibition of soleus motoneurones is taken as a parameter (see Pierrot-Deseilligny, Katz & Morin, 1979). All phases were then repeated during subthreshold magnetic stimulation of the scalp delivered at different time intervals with respect to the supramaximal stimulus for the H′ reflex. The time of the scalp shock was considered to be 0 ms; so if the stimulus for the H′ reflex was given first, the interstimulus interval was negative. In the case illustrated in Fig. 1, it was +1 ms. The amplitude of H1 was kept constant by readjusting the stimulus strength when necessary so as to be the same as in control conditions. A differential net effect between the H′ reflex and the reference H reflex was looked for as evidence of corticospinal control of transmission in the recurrent pathway. The amplitude of the two reflexes may vary inversely or in the same direction. The amplitudes of H′ and reference H reflexes obtained during magnetic stimulation were expressed as a percentage of those recorded under control conditions. The same phases were also studied while changing the intensity of the magnetic shock which was delivered at a fixed interstimulus interval usually corresponding to the peak of the effect. At least twenty-four reflexes (with and without magnetic stimulation) were measured in every series for later statistical analysis (mean, standard error of the mean and differences between groups by Student's paired *t* test). The above protocol was performed in all subjects. In some subjects, the size of the H1 reflex was changed (while keeping the same cortical stimulus intensity and the same delay between the magnetic shock and the test reflex) so as to evoke H′ reflexes of different amplitude. In this case, both reflexes were expressed as a percentage of the maximum direct response (M_{max}).

The conditioning S1 stimulus to the median nerve always produced a local sensation without paraesthesia radiating to the fingers. The possible effects due to the activation of cutaneous fibres were therefore considered. A pure cutaneous stimulation mimicking the sensation evoked by the median nerve stimulus was provided by electrical stimulation of the skin laterally on the arm. Single shocks of 0·5 ms duration were delivered through a pair of discs (of 1 cm diameter) placed at the same level as the test electrodes. The stimulation intensity corresponded to 1·35 times the perception threshold. Sixteen to thirty-two trials each of control H reflexes and H reflexes conditioned by the cutaneous stimulation were

collected at a conditioning–test interval of 10 ms. Blocks of trials were also collected using as conditioning stimulation a sub-motor-threshold cortical shock which was delivered either at the same time as the control H reflex or 2 ms before it. The intensity of the cortical shock was such as to produce facilitation of the H reflex. The effects of the cutaneous stimulation were then tested on the amount of cortically induced facilitation.

RESULTS

The effect of magnetic stimulation of the scalp on soleus motor nucleus

Cowan *et al.* (1986) using subthreshold electrical scalp stimuli showed that the initial effect of the cortical descending volley on the soleus motoneurones was an inhibition of the H reflex. In our case the effect of cortical stimulation (subthreshold for the motor-evoked response in the active muscle) was simultaneously studied on two H reflexes of comparable size, the reference H and the H′ reflexes, the only difference between them being due to the effects elicited by the H1 discharge on the H′ reflex. Figure 2 illustrates the time course of cortical action on the reference H reflex (triangles) and on the H′ reflex (circles) of the soleus muscle from three subjects. The intensity of the magnetic stimulus was about 10% below the threshold for the evoked response in the active soleus muscles. In the subjects illustrated in Fig. 2*A* and *B*, cortical stimulation depressed the reference H reflexes at a minimum conditioning–test interval of −1 and −2 ms, respectively, as reported previously by Cowan *et al.* (1986) and by Iles & Pisini (1992). In the subject illustrated in Fig. 2*C*, there was no significant change in the size of the reference H reflex using this intensity of cortical stimulation. In contrast to its effect on the reference H reflex, cortical stimulation facilitated the H′ reflex at minimum conditioning–test intervals of +1 ms or so. There was no sign of any inhibition

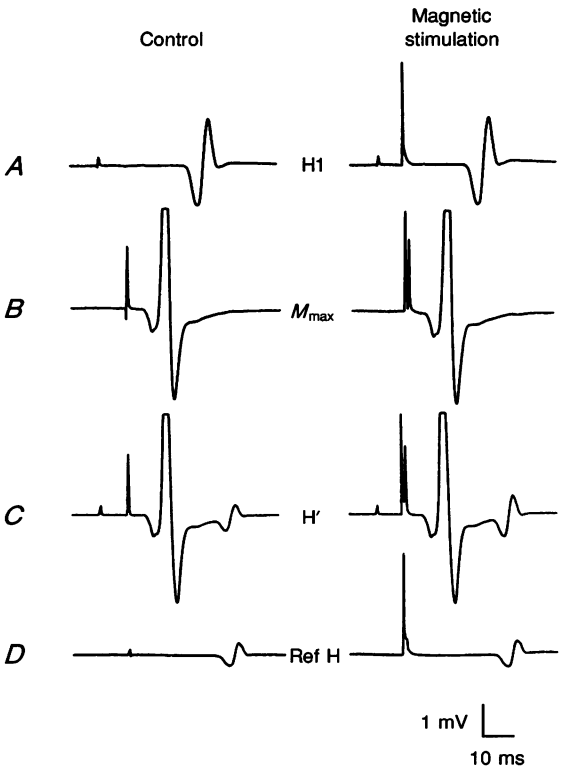

Figure 1. Comparison between soleus EMG responses without and with magnetic brain stimulation

A circular coil centred at the vertex was used to deliver the magnetic shock with a stimulus intensity of 70% of the maximum output of the stimulator (about 10% below the threshold for obtaining a motor-evoked response in the active soleus muscle in this subject). The EMG responses from the soleus muscle were obtained by electrical stimulation of the posterior tibial nerve (PTN) at the popliteal fossa with different intensities. *A*, isolated conditioning PTN stimulus producing an H1 reflex of nearly maximal size. *B*, isolated test PTN stimulus (supramaximal to the α-motor fibres) causing maximal motor (M_{max}) response. *C*, combined conditioning and test stimuli (*A* + *B*) at 10 ms interval producing an H′ reflex response. *D*, isolated PTN stimulus producing a reference H reflex (Ref H) of the same size and latency as the H′ reflex. Magnetic stimulation precedes the supramaximal test stimulus for the H′ reflex and the stimulus for the ref H reflex by 1 ms. (The time course of the cortical effect for this subject is illustrated in Fig. 2*C*.) Each trace, with the exception of those showing the M_{max} ($n = 2$), is the average of 12 responses.

of the H′ reflex at shorter intervals. In all subjects the size of both the reference H and H′ reflex had returned to baseline levels at conditioning–test intervals of +8 ms.

Results similar to those illustrated in Fig. 2*B* were obtained in three other subjects, and the mean data showing the onset of cortical effects are shown from all five subjects in Fig. 3*A*. Cortical stimulation produced its earliest effect on the reference H reflex at conditioning–test intervals of −2 ms, and thereafter at +1 and +2 ms. There was no effect on the H′ response until conditioning–test intervals of +1 ms. The cortical effect on the two responses was significantly different at conditioning–test intervals of +1 and +2 ms ($P < 0{\cdot}05$). Figure 3*B* shows the effect of different cortical stimulus intensities on the size of the reference H (open columns) and H′ (hatched columns) reflexes of the relaxed soleus muscle in all five subjects. The interstimulus interval used corresponded to the first peak of the facilitatory effect on the H′ reflex. This varied from +1 to +4 ms. At these intervals the reference H reflex was consistently inhibited in all the subjects but the one shown in Fig. 2*C*. Since motor cortical threshold varied among subjects, the cortical stimulus intensity was expressed as a percentage of the average liminal intensity (indicated as 100%) necessary to obtain a motor-evoked response in the active soleus muscle. It can be seen that the threshold of the cortical effect on the H′ reflex was lower than that on the reference H reflex. In fact, on decreasing the cortical stimulus intensity below 85%, at which the two reflexes showed opposite changes, there was still a significant facilitatory effect on the H′ reflex while the reference H reflex was no longer inhibited. Indeed, at an intensity of 70%, cortical stimulation produced a greater facilitation of the H′ response than seen at an intensity of 85%. A possible explanation for this is that the H′ response is subject to opposite influences from the cortex: an inhibition, (which is also seen by the reference H reflex), and an excitation (limited to the H′ reflex itself). Such opposing effects may also explain why the subject in Fig. 2*A* who had the largest inhibitory effect on the reference H reflex also had the smallest excitatory effect on the H′ response. The subjects in Fig. 2*B* and *C* had smaller or no inhibitory effects on the reference H reflex, and had larger excitatory effects on the H′ reflex.

In a sixth subject, subthreshold cortical stimulation delivered between 0 and +4 ms of interstimulus interval produced facilitation rather than inhibition of reference H reflex which overlapped with the parallel facilitation of H′ reflex. Since the threshold of the cortical facilitation of

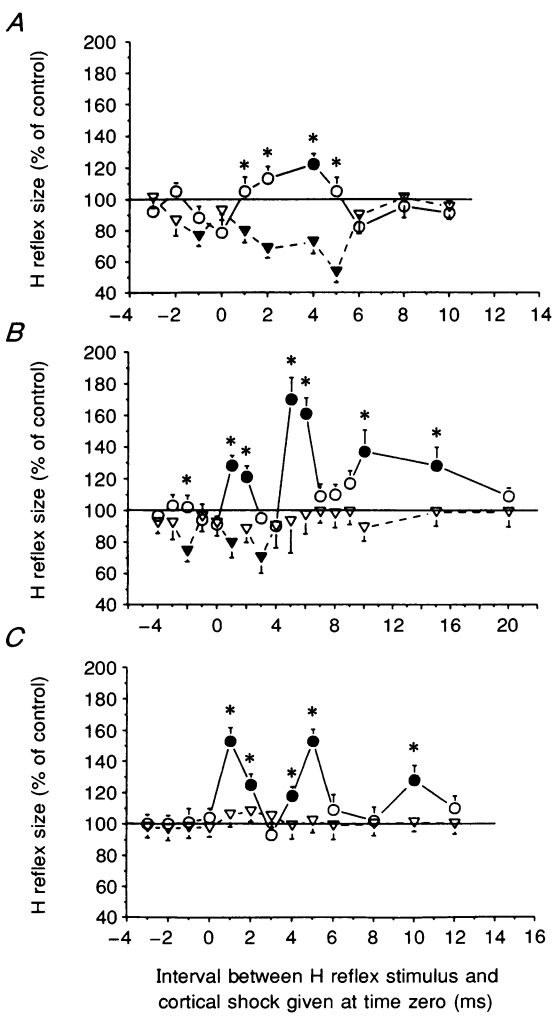

Figure 2. The effect of magnetic brain stimulation on the size of the soleus H′ and reference H reflexes at rest in three different subjects
Magnetic stimulator output was at 80% in *A*, 60% in *B* and 70% in *C*. In all three cases this corresponded to 10% below the threshold for the motor-evoked response in the active soleus muscle. The cortical shock was given at time zero. Control H reflex size is represented by the horizontal continuous line. Each point represents the mean of 12 observations; S.E.M. are expressed by the vertical bars. Open and filled symbols indicate non-significant and significant differences for the reference H and the H′ reflex *versus* the respective control values; asterisks indicate the points where there was a significative difference in the cortical effect on the reference H and the H′ reflex ($P < 0{\cdot}05$).

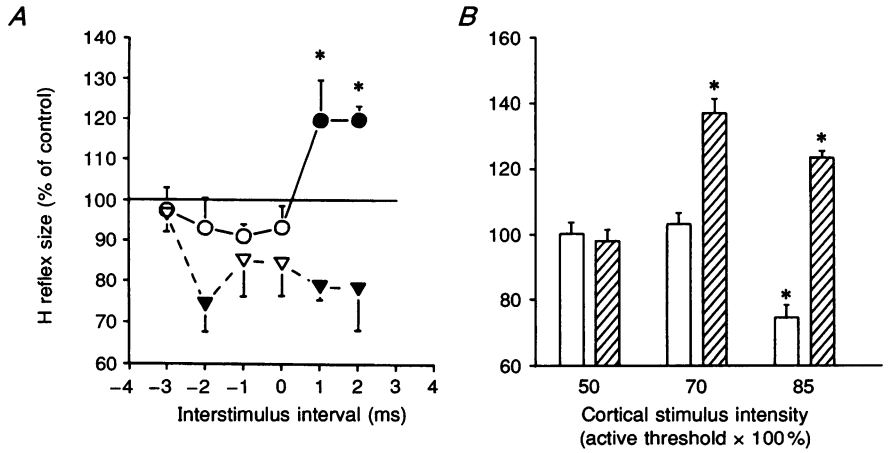

Figure 3. The effect of magnetic brain stimulation on the size of the soleus H′ and reference H reflexes in all five subjects

A, the onset of the cortical effect on the reference H (triangles). and test H′ (circles) reflexes. The magnetic shock was given at time zero. The intensity of the magnetic stimulator was on average 15% below the threshold for the active evoked response in the soleus muscle. Such a value always produced a significant inhibition of the reference H reflex at a conditioning–test interval of −2 ms in all subjects but the one shown in Fig. 2*C*. Control H reflex size is represented by the horizontal continuous line. Each point is the grand mean of the values obtained at conditioning–test intervals of −3 to +2 ms; S.E.M. are expressed by the bars. Open and filled symbols and asterisks as in Fig. 2. *B*, comparison of the mean threshold intensity of the facilitation and inhibition of test H′ (hatched columns) and reference H (open columns) reflexes at rest. To normalize cortical stimulation strength between different subjects, intensity is given as a percentage of the average intensity (indicated as 100%) needed to produce a liminal motor-evoked response in the active soleus muscle. The size of the conditioned soleus H reflex as a percentage of the control reflex size was measured at conditioning–test intervals corresponding to the first peak of H′ facilitation. The conditioning–test interval thus varied for the five subjects between +1 and +4 ms. Each vertical bar represents one standard error of the grand mean. Asterisks refer to significant differences between control and conditioned H reflexes ($P < 0{\cdot}05$).

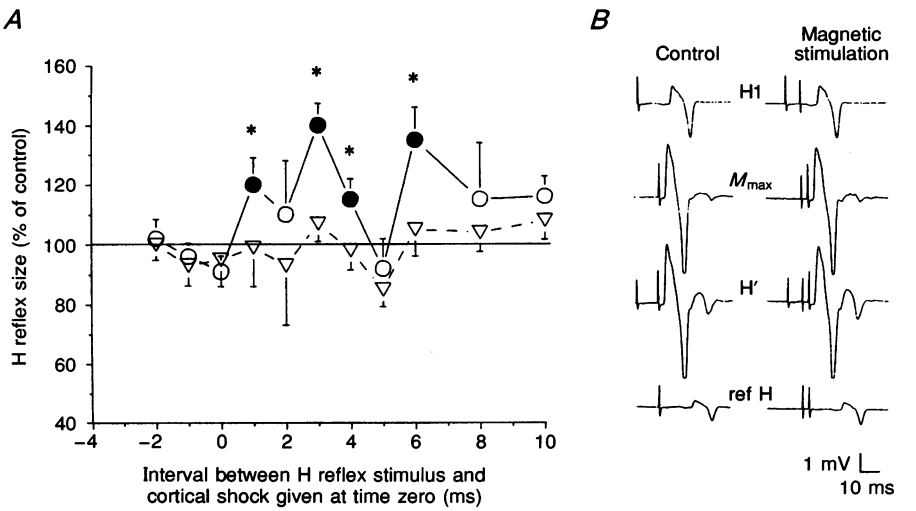

Figure 4. The effect of magnetic brain stimulation on the size of wrist flexor H′ (circles) and reference H (triangles) reflexes at rest

The cortical shock was given at time zero. The intensity of the magnetic stimulus was set at 30% (10% below the threshold for the active evoked response). Control H reflex size is represented by the horizontal continuous line. Each point represents the mean of 12 observations; S.E.M. are represented by the vertical bars. Open and filled symbols and asterisks as in Fig. 2. *B*, traces show the responses obtained from the wrist flexor muscles under control conditions and with magnetic stimulation. The latter was applied 3 ms before the stimuli for H′ and reference H reflexes. Traces are arranged as in Fig. 1.

H′ reflex is lower than that of reference H reflex (see Fig. 3*B*), we decreased the cortical stimulus intensity to look for a quantitative differential effect between the two reflexes. However, it was not possible to show such an effect.

The effect of magnetic stimulation on wrist flexor motor nucleus

In wrist flexor muscles, a subthreshold magnetic scalp shock delivered through a circular coil centred at the vertex produces an early facilitation of the H reflex which on average starts at an interstimulus interval of −2 ms and lasts for several milliseconds (Mazzocchio *et al.* 1994). In all the subjects studied, subthreshold cortical stimulation caused facilitation of both reference and H′ reflexes. This could be interpreted as either: (a) lack of the putative Renshaw cell effect on H′ reflex which is thus subjected to the same influence as the reference H reflex; or alternatively, (b) the effects on the H′ reflexare masked by the extent of motoneurone facilitation. In the latter case, if the threshold of the cortical effect on the H′ reflex is lower than that of reference H reflex, as observed in the soleus, then by decreasing the intensity of the magnetic stimulus it should be possible to see some difference between the two reflexes. This was the case in four of the six subjects studied. Figure 4 illustrates the time course of the effect of cortical stimulation on the reference H reflex (triangles) and on the H′ reflex (circles) of the wrist flexor muscles in one subject. This time course was obtained using a cortical stimulus intensity which was 10% below the threshold for producing any response in actively contracting muscles. At such an intensity, there was no effect on the size of the reference H reflex whereas, there was clear facilitation of the H′ response beginning at a minimum conditioning–test interval of +1 ms. Similar time courses were observed in the three other subjects and Fig. 5*A* shows the detail of the mean onset of cortical effects from all four individuals. The cortical stimulus had no effect on the reference H reflex but, produced facilitation of the H′ response at +1 ms. It is worth noting that the effects of cortical stimulation on the H′ reflexes of soleus and wrist flexor muscles were identical in spite of the fact that magnetic stimulation caused opposite changes in the two motoneurone pools. Figure 5*B* shows the effect of changing the intensity of the cortical stimulus on reference H (open columns) and H′ (hatched columns) reflexes of the relaxed wrist flexor muscles in all four subjects. The interstimulus interval used corresponded to the first peak of H′ reflex facilitation in the four subjects. It can be seen that it is possible to obtain a significant

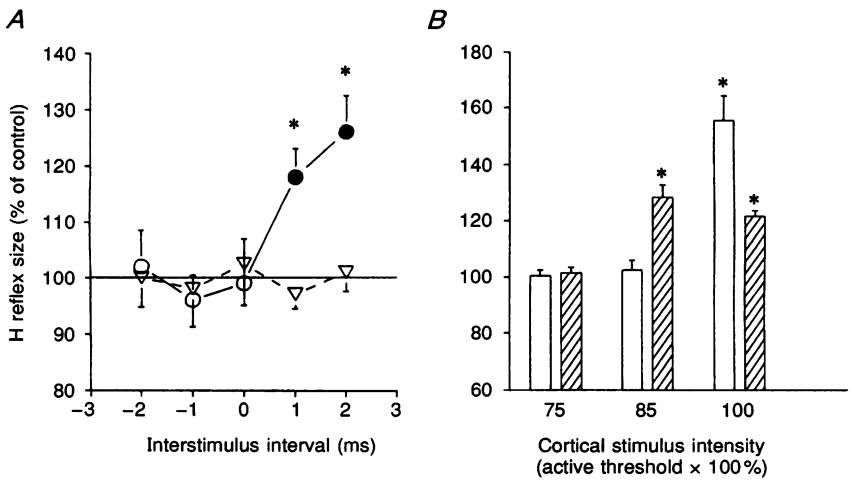

Figure 5. The effect of magnetic brain stimulation on the size of wrist flexor H′ and reference H reflexes in all four subjects

A, the onset of the cortical effect on the reference H (triangles) and H′ (circles) reflexes at rest. The magnetic shock was given at time zero. The intensity of the magnetic stimulation was such as to produce no significant effect on the reference H reflex. This was on average 15% below the threshold for the active evoked response in the wrist flexor muscles. Control H reflex size is represented by the horizontal continuous line. Each point is the grand mean of the values obtained at conditioning–test intervals of −2 to +2 ms; S.E.M. are expressed by vertical bars. Open and filled symbols and asterisks as in Fig. 2. *B*, a comparison of the mean threshold intensity of the facilitation of the H′ (hatched columns) and reference H (open columns) reflexes at rest. To normalize cortical stimulation strength between different subjects, intensity is given as a percentage of the average intensity (indicated as 100%) needed to produce a liminal motor-evoked response in the active wrist flexor muscles. The size of the conditioned wrist flexor H reflex as a percentage of the control reflex size was measured at conditioning–test intervals corresponding to the first peak of H′ facilitation. The conditioning–test interval thus varied for the 4 subjects between +1 and +4 ms. Each vertical bar represents one standard error of the grand mean. Asterisks refer to significant differences between control and conditioned H reflexes ($P < 0{\cdot}05$).

facilitation of the H′ reflex alone with cortical stimulus intensities 15% below the threshold for the motor-evoked potential in the active wrist flexor muscles. At cortical stimulus intensities corresponding to the active motor threshold, the facilitation of the reference H reflex exceeded that of the H′ reflex. Therefore, as observed in the soleus muscle, the threshold for the facilitation induced by the cortical stimulus is lower for the H′ reflex than for the reference H reflex.

In the remaining two subjects, it was impossible to demonstrate a differential cortical effect on reference and H′ reflexes. In these as well as in the other case mentioned in the lower limb findings (the sixth subject), the weaker sensitivity of the H′ reflex to excitatory inputs (see below, Fig. 7*A*) probably biased the results against finding an excitatory effect from cortical stimulation.

The effect of cortical stimulation on test H′ reflexes of different sizes

Figure 6 shows the variations of the H′ reflex seen while increasing the amplitude of the H1 reflex in control conditions (open circles) and during scalp magnetic stimulation (filled circles). The results obtained in the wrist flexor motoneurones are from the same subject as the data shown in Fig. 4. The intensity of magnetic stimulation was the same (10% below the active threshold). The time interval between the magnetic shock and the supramaximal stimulus for the H′ reflex was kept constant and corresponded to the peak of the facilitatory effect (+3 ms in this case). Under control conditions, H′ equalled H1 at low conditioning reflex amplitude. At higher conditioning H1 amplitudes, the size of the H′ reflex reached its maximum and remained constant until the largest H1 amplitudes possible were used at which the H′ reflex started rapidly to decrease. This pattern has already been described in detail in previous work (Rossi & Mazzocchio, 1992; Katz *et al.* 1993). Cortical stimulation effects were restricted to that part of the curve where an increase in H1 reflex led to a fall in H′ reflex. Within this range, the cortical action was such as to increase consistently the amplitude of the H′ reflex evoked by the same conditioning H1 discharge. The results obtained from the soleus motoneurones are from another subject whose data are shown in Fig. 2*A*. The intensity of the magnetic stimulus was the same (10% below the active threshold); the time interval between the magnetic shock and the supramaximal stimulus for the H′ reflex was +4 ms. As observed in the case of the wrist flexor muscles, the size of the H′ reflex was not significantly affected by the cortical stimulus within the ascending slope of the curve. On the other hand, the cortical facilitatory action on the H′ reflex became manifest at higher conditioning H1 amplitudes and disappeared when the size of the H1 reflex was at its maximum. Similar patterns were observed in three other subjects.

Control experiments

Facilitation of an H reflex by a weak stimulation of the homonymous nerve has been used to show that identical excitatory inputs to soleus motoneurones cause a smaller increase of the test H′ reflex than of a reference H reflex of similar size (see Hultborn & Pierrot-Deseilligny, 1979). We

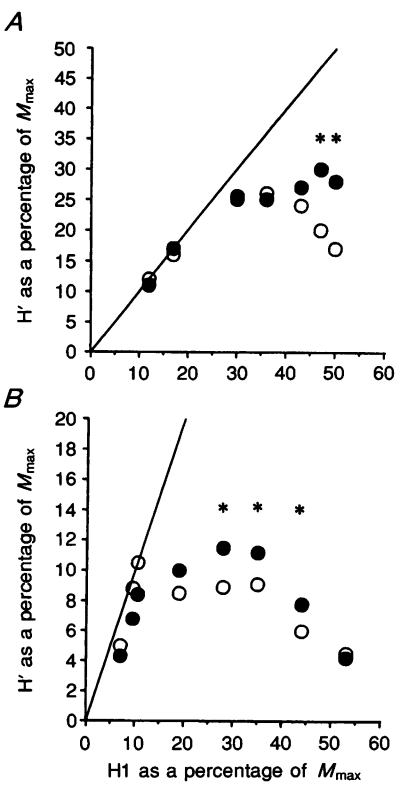

Figure 6. The effect of magnetic brain stimulation on the H′ reflex of different size

Results from *A*, wrist flexors and *B*, soleus. Control and conditioned H′ reflexes are expressed by open and filled circles, respectively. The amplitude of the H′ reflex is plotted against that of the H1 reflex, both expressed as a percentage of the maximal motor response (M_{max}). The identity line represents the theoretical curve which would be obtained if H′ equalled H1. Each point is the mean of five measurements; standard deviations ranged from approximately 5 to 15% of mean values. Asterisks indicate significant differences between control and conditioned H′ reflexes ($P < 0{\cdot}05$).

used the same experimental protocol to verify whether there was a similar difference in the sensitivity of the two reflexes in the upper limb. Figure 7*A* shows the mean data obtained in four subjects. It can be seen that there was a significant difference in the amount of facilitation of the two reflexes, H′ being smaller than the reference H reflex. This finding further supports the notion that the motoneurones giving origin to the H′ reflex may have similar characteristics even though they belong to different motor nuclei (see Methods).

There is evidence that conditioning digit stimulation may reduce the amplitude of a control H reflex from wrist flexor muscles at conditioning–test intervals of 9–10 ms (Sabatino, Ferraro, Caravaglios, Sardo, Delwaide & La Grutta, 1992). Since, in the upper limb, we used interstimulus intervals of 10–15 ms between the conditioning H1 reflex and the test H′ reflex, it could be argued that a reduction in cutaneous inhibition may be responsible for the corticospinal facilitation of the H′ reflex. Figure 7*B* shows the results obtained from two subjects. As shown previously by Malmgren & Pierrot-Deseilligny (1988), a pure conditioning cutaneous stimulation applied to the skin of the arm, mimicking the sensation evoked by the median nerve stimulus for the H reflex, produced no significant change in this H reflex at an interstimulus interval of 10 ms (column 1 in Fig. 7*B*). Column 2 shows the facilitatory effect of a cortical shock preceding the reference H reflex by 2 ms (conditioning–test interval of +2 ms). Such an interstimulus interval produced a significant facilitation of the H′ reflex in both subjects. The effect of the conditioning cutaneous stimulation on the amount of cortically induced facilitation of the reference H reflex is shown by column 3 in Fig. 7*B*. It can be seen that there was no extra facilitation of the reference H reflex when the cutaneous input was added. On the contrary, there was a small depression of the facilitation of a degree similar to the amount of inhibition evoked by cutaneous stimulation alone.

DISCUSSION

We have investigated how transcranial magnetic stimulation of the motor cortex changes the amount of recurrent inhibition elicited by a conditioning discharge of soleus and wrist flexor motoneurones in humans.

In our experiments a conditioning monosynaptic reflex (H1) was used to activate Renshaw cells orthodromically. The resulting recurrent inhibition was then estimated indirectly by a second test H′ reflex produced through a collision technique by the same motoneurones that have given rise to the conditioning discharge. It follows that the relationship between the amplitudes of the H1 conditioning and H′ test reflex (as shown in Fig. 6) is governed not only by the amount of recurrent inhibition set up by the conditioning reflex but also by the degree of after-hyperpolarization of the motoneurones (Bussel & Pierrot-Deseilligny, 1977). There is evidence, however, that only recurrent inhibition is responsible for the increasing depression of the H′ reflex with increasing H1 discharge, provided that the excitability of motoneurones is constant (Bussel & Pierrot-Deseilligny, 1977; Mazzocchio & Rossi, 1989).

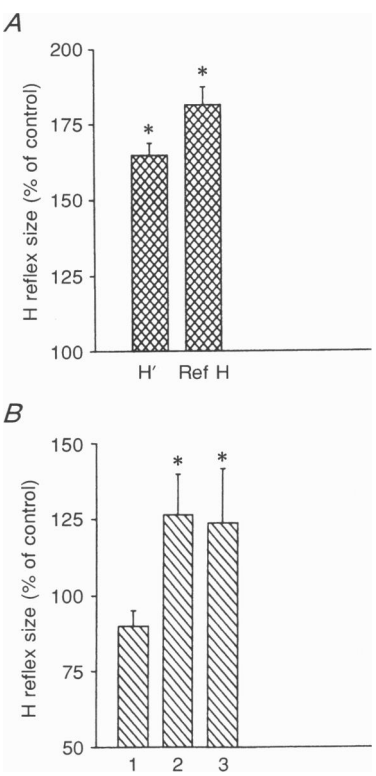

Figure 7. Effect of different conditioning stimuli on the wrist flexor H reflex

A, comparison of the facilitation of the H′ and reference H reflexes by a preceding (3 ms) weak stimulation of the median nerve (subliminal for evoking the H reflex). Mean (+ 1 S.E.M.) data from 4 subjects. Asterisks refer to significant differences between control and conditioned H reflexes ($P < 0{\cdot}05$). The difference in the degree of facilitation between the conditioned H′ and reference H reflex was also significant ($P < 0{\cdot}05$). *B*, conditioning stimuli were cutaneous stimulation mimicking the sensation produced by median nerve stimulation (1; conditioning–test interval 10 ms), magnetic brain stimulation (2; conditioning–test interval 2 ms; intensity 10% below the threshold for the active evoked response), and combined cutaneous and magnetic brain stimulation (3). Asterisks refer to significant differences between control and conditioned H reflexes ($P < 0{\cdot}05$).

Single electrical or magnetic stimuli applied to the scalp, which are below the threshold intensity needed to produce direct muscle activation at rest, produce significant changes in spinal cord excitability as monitored by H reflex testing. In the wrist flexor muscles, the initial effect is a short-latency facilitation, whereas in the soleus, it is inhibition (Cowan *et al.* 1986; Iles & Pisini, 1992; Mazzocchio *et al.* 1994). When combined, as in the present experiments, with tests for recurrent inhibition, this input will therefore be capable of influencing the size of the test H′ reflex by way of its influence on α-motoneurones, regardless of any additional effect it may have on Renshaw cells. The direct effect on motoneuronal excitability was quantified by studying the effect of cortical stimulation on the amplitude of a reference H reflex, i.e. an H reflex having the same size as the H′ reflex. Apart from the effects elicited by the H1 discharge on the H′ reflex (see Methods), both reference and H′ reflexes should be subjected to the same type of influences after cortical stimulation.

The principal findings of this study can be summarized as follows. Firstly, cortical magnetic stimulation produces facilitation of the H′ reflex in both soleus and wrist flexor motor nuclei. In the former this facilitation is superimposed on the background inhibition of the reference H reflex while in the latter it summates with a background facilitation. Secondly the threshold for producing facilitation of the H′ reflex is lower than that needed to produce changes in the size of the reference H reflex in both soleus and wrist flexor motor nuclei. Thirdly, the onset of H′ facilitation follows the cortically induced changes in the reference H reflex by 3–4 ms in both motor nuclei. The most straightforward explanation for these results is that the facilitation of the H′ reflex after cortical magnetic stimulation is due to a decrease in the amount of recurrent inhibition elicited by the H1 reflex discharge. In particular, the cortically induced inhibition of Renshaw cells would allow a larger number of motoneurones, in which collision has taken place, to be recruited by the test stimulus. However, there are two other possibilities. The first is that the magnetic stimulus might somehow reduce the amount of after-hyperpolarization of the motoneurones which have discharged in the H1 reflex. This would facilitate the H′ reflex in the absence of any changes in Renshaw inhibition. Data from animal experiments have shown that the activation of descending monoaminergic pathways can reduce the after-hyperpolarization of target motoneurones (Hultborn & Toth, 1989; see also Kiehn, 1991). However, depression of motoneurone after-hyperpolarization occurred 50–100 ms after repetitive electrical stimulation of the raphe nuclei (Hultborn & Toth, 1989). Moreover, careful inspection of the curves in Fig. 6 makes it unlikely that cortical stimulation in the present experiments could have reduced the after-hyperpolarization of soleus and wrist flexor motoneurones via a much shorter route. It is reasonable to assume on the basis of previous studies (Bussel & Pierrot-Deseilligny, 1977; Mazzocchio & Rossi 1989) that after-hyperpolarization of motoneurones is the main factor which prevents the H′ reflex from continuing to grow in parallel with the H1 reflex along the identity line (ascending slope of the curve). When the H1 reflex is small, the amount of after-hyperpolarization which is set up can be overcome by the supramaximal stimulus for the H′ reflex. As H1 becomes larger, this is no longer possible and H′ is smaller than H1 (see Methods). If cortical stimulation induced a reduction in the after-hyperpolarization of the motoneurones, one would expect the H′ reflex to follow the conditioning H1 reflex at high amplitudes since more motoneurones would become available for H′. This behaviour was not observed. The cortical shock only facilitated the H′ reflex (filled circles) on the descending limb of the H1–H′ curve. The second possibility as suggested by Burke, Gandevia & McKeon (1984) is that the amplitude of H reflexes may be affected by group Ib activity elicited by the test stimulus itself. It could therefore be argued that the H′ facilitation may be due to a corticospinal reduction of Ib inhibition. However, if this were so one would expect the reference H reflex and the H′ reflex to be affected in the same way. This did not occur. Significant quantitative and qualitative differences (i.e. opposite changes) were observed between the two reflexes in the upper and lower limb, respectively.

Inhibitory influences on Renshaw cells have been elicited in the cat by electrical stimulation of several supraspinal loci (for references see Fung, Pompeiano & Barnes, 1987). In particular, activation of the pyramidal tract either by stimulation of the pericruciate cortex (MacLean & Leffman, 1967) or of the capsula interna (Koehler, Windhorst, Schmidt, Meyer-Lohmann & Henatsch, 1978) can reduce Renshaw cell discharge produced by an antidromic motor nerve volley. In both studies, depression of Renshaw cell activity lasts for a considerable time after electrical stimulation (between 25 and 35 ms). We observed a similar long-lasting change after magnetic stimulation (see Fig. 2*B*). Such depression may be caused either by direct supraspinal inhibition of Renshaw cells or by suppression of a tonic facilitatory drive. The existence of tonic supraspinal facilitation of Renshaw cells in humans has been recently hypothesized (Mazzocchio & Rossi, 1992). This could be one of the reasons for the threshold for the cortically induced recurrent effects being lower than the threshold for the corticospinal effects on the H reflex. Whatever the mechanism, the 3–4 ms difference in the onset latency of the recurrent effects with respect to that of the earlier motoneuronal

changes suggests that a short interneuronal chain is responsible for the observed cortically induced inhibition of Renshaw cells.

Functional considerations

The responses elicited by low intensity cortical magnetic stimulation probably are transmitted via fast-conducting corticospinal fibres (Brouwer & Ashby, 1992; Palmer & Ashby, 1992). These fibres are likely to be responsible primarily for the phasic element of pyramidal control (see Clough, Kernell & Phillips, 1968; Johansson, Lemon & Westling, 1993). We have found that in humans at rest activation of such a system is accompanied by inhibition of Renshaw cell activity in two functionally different motoneurone pools such as the soleus and the flexors of the wrist. Similarly, experiments designed to study the control of Renshaw cells during various natural movements (Hultborn & Pierrot-Deseilligny, 1979) have shown that Renshaw activity is strongly diminished during phasic as compared with tonic muscle contraction. It could, therefore, be suggested that recurrent inhibition is reduced during movements mediated by large corticospinal neurones. This is consistent with the absence or weakness of recurrent inhibition in motor nuclei subserving the more distal muscles of the human limbs (Rossi & Mazzocchio, 1991, 1992; Katz *et al.* 1993) which are known to receive the larger and faster corticospinal fibres and to be under a high degree of pyramidal control (Phillips & Porter, 1964; Palmer & Ashby, 1992). The implication is that Renshaw cell activity may be more important in tonic rather than phasic muscle contraction. Interestingly, an increase in recurrent inhibition of soleus motoneurones has been observed during the maintenance of unsupported upright posture (Pierrot-Deseilligny *et al.* 1977) and during tonic backward tilt (Rossi *et al.* 1987). Also, enhancement of Renshaw cell activity during a weak tonic voluntary effort (Hultborn & Pierrot-Deseilligny, 1979) appears to be a general strategy in the lower limb (Rossi & Mazzocchio, 1991). Although the frontier between posture and movement is not quite clear, it is tempting to suggest that recurrent inhibition may be mainly concerned with the organizational processes underlying adaptation of postural responses to voluntary movements (Rossi, Decchi & Vecchione, 1992).

REFERENCES

Awiszus, F. & Feistner, H. (1993). The relationship between estimates of Ia-EPSP amplitude and conduction velocity in human soleus motoneurones. *Experimental Brain Research* **95**, 365–370.

Baldissera, F., Hultborn, H. & Illert, M. (1981). Integration in spinal neuronal systems. In *Handbook of Physiology*, section 1, *The Nervous System*, vol. 2, *Motor Control*, ed. Brooks, V. B., pp. 509–595. American Physiological Society, Bethesda, MD, USA.

Brouwer, B. & Ashby, P. (1992). Corticospinal projections to lower limb motoneurons in man. *Experimental Brain Research* **89**, 649–654.

Burke, D., Gandevia, S. C. & McKeon, B. (1984). Monosynaptic and oligosynaptic contributions to human ankle jerk and H reflex. *Journal of Neurophysiology* **52**, 435–448.

Bussel, B. & Pierrot-Deseilligny, E. (1977). Inhibition of human motoneurones, probably of Renshaw origin, elicited by an orthodromic motor discharge. *Journal of Physiology* **269**, 319–339.

Clough, J. F. M., Kernell, D. & Phillips, C. G. (1968). The distribution of monosynaptic excitation from the pyramidal tract and from primary spindle afferents to motoneurones of the baboon's hand and forearm. *Journal of Physiology* **198**, 145–166.

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 the muscles of the arm and leg in man. *Journal of Physiology* **377**, 333–347.

Day, B. L., Dressler, D., Maertens de Noordhout, A., Marsden, C. D., Nakashima, K., Rothwell, J. C. & Thompson, P. D. (1989). Electric and magnetic stimulation of the human motor cortex: surface EMG and single motor unit responses. *Journal of Physiology* **412**, 449–473.

Fung, S. J., Pompeiano, O. & Barnes, C. D. (1987). Suppression of the recurrent inhibitory pathway in lumbar cord segments during locus coeruleus stimulation in cats. *Brain Research* **402**, 351–354.

Hultborn, H. & Pierrot-Deseilligny, E. (1979). Changes in recurrent inhibition during voluntary soleus contractions in man studied by an H reflex technique. *Journal of Physiology* **297**, 229–251.

Hultborn, H. & Toth, T. (1989). Raphe-spinal depression of motoneurone after-hyperpolarization. *Acta Physiologica Scandinavica* **36**, 35.

Iles, J. F. & Pisini, J. V. (1992). Cortical modulation of transmission in spinal reflex pathways of man. *Journal of Physiology* **455**, 425–446.

Johansson, R. S., Lemon, R. N. & Westling, G. (1993). Cortical influence over precision grip in man is strongly modulated during different phases of the task. *Journal of Physiology* **459**, 469*P*.

Katz, R., Mazzocchio, R., Penicaud, A. & Rossi, A. (1993). Distribution of homonymous and heteronymous recurrent inhibition in the human upper limb. *Acta Physiologica Scandinavica* **149**, 183–198.

Katz, R. & Pierrot-Deseilligny, E. (1984). Facilitation of soleus-coupled Renshaw cells during voluntary contraction of pretibial flexor muscles in man. *Journal of Physiology* **355**, 587–603.

Katz, R., Pierrot-Deseilligny, E. & Hultborn, H. (1982). Recurrent inhibition of motoneurones prior to and during ramp and ballistic movements. *Neuroscience Letters* **31**, 141–145.

Kiehn, O. (1991). Plateau potentials and active integration in the 'final common pathway' for motor behaviour. *Trends in Neurosciences* **14**, 68–73.

Koehler, W., Windhorst, U., Schmidt, J., Meyer-Lohmann, J. & Henatsch, H.-D. (1978). Diverging influences on Renshaw cells responses and monosynaptic reflexes from stimulation of capsula interna. *Neuroscience Letters* **8**, 35–39.

MacLean, J. B. & Leffman, H. (1967). Supraspinal control of Renshaw cells. *Experimental Neurology* **18**, 94–104.

Malmgren, K. & Pierrot-Deseilligny, E. (1988). Evidence for non-monosynaptic Ia excitation of human wrist flexor motoneurones, possibly via propriospinal neurones. *Journal of Physiology* **405**, 747–764.

Mazzocchio, R. & Rossi, A. (1989). Further evidence for Renshaw inhibition in man: a combined electrophysiological and pharmacological approach. *Neuroscience Letters* **106**, 131–136.

Mazzocchio, R. & Rossi, A. (1992). Are Renshaw cells tonically active in humans? *Journal of Physiology* **452**, 110*P*

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**, 261–267.

Palmer, E. & Ashby, P. (1992). Corticospinal projections to upper limb motoneurones in humans. *Journal of Physiology* **448**, 397–412.

Phillips, C. G. & Porter, R. (1964). The pyramidal projection to motoneurones of some muscle groups of the baboon's forelimb. *Progress in Brain Research* **12**, 222–242.

Pierrot-Deseilligny, E. & Bussel, B. (1975). Evidence for recurrent inhibition by motoneurones in human subjects. *Brain Research* **88**, 105–108.

Pierrot-Deseilligny, E., Bussel, B., Held, J. P. & Katz, R. (1976). Excitability of human motoneurones after discharge in a conditioning reflex. *Electroencephalography and Clinical Neurophysiology* **40**, 279–287.

Pierrot-Deseilligny, E., Katz, R. & Morin, C. (1979). Evidence for Ib inhibition in human subjects. *Brain Research* **166**, 176–179.

Pierrot-Deseilligny, E., Morin, C., Katz, R. & Bussel, B. (1977). Influence of voluntary movement and posture on recurrent inhibition in human subjects. *Brain Research* **124**, 427–436.

Rossi, A., Decchi, B. & Vecchione, V. (1992). Supraspinal influences on recurrent inhibition in humans. Paralysis of descending control of Renshaw cells in patients with mental retardation. *Electroencephalography and Clinical Neurophysiology* **85**, 419–424.

Rossi, A. & Mazzocchio, R. (1991). Presence of homonymous recurrent inhibition in motoneurones supplying different lower limb muscles in humans. *Experimental Brain Research* **84**, 367–373.

Rossi, A. & Mazzocchio, R. (1992). Renshaw recurrent inhibition to motoneurones innervating proximal and distal muscles of the human upper and lower limbs. In *Muscle Afferents and Spinal Control of Movement, IBRO series*, ed. Jami, L., Pierrot-Deseilligny, E. & Zytnicki, D., pp 313–319. Pergamon Press, Oxford, UK.

Rossi, A., Mazzocchio, R. & Scarpini, C. (1987). Evidence for Renshaw cell–motoneuron decoupling during tonic vestibular stimulation in man. *Experimental Neurology* **98**, 1–12.

Ryall, R. W., Piercey, M. F., Polosa, C. & Goldfarb, J. (1972). Excitation of Renshaw cells in relation to orthodromic and antidromic excitation of motoneurons. *Journal of Neurophysiology* **35**, 137–148.

Sabatino, M., Ferraro, G., Caravaglios, G., Sardo, P., Delwaide, P. G. & La Grutta, V. (1992). Evidence of a controlateral motor influence on reciprocal inhibition in man. *Journal of Neural Transmission* **4**, 257–266.

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