Latency of effects evoked by electrical and magnetic brain stimulation in lower limb motoneurones in man

J. Nielsen, N. Petersen and M. Ballegaard

Division of Neurophysiology, Department of Medical Physiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

1. The latency of effects in the tibialis anterior (TA) and soleus (Sol) muscles evoked by electrical and magnetic stimulation of the motor cortex was evaluated in human subjects by H reflex testing. Post-stimulus time histograms (PSTHs) were established for the discharge of single voluntarily activated motor units and motor-evoked potentials (MEPs) in the surface electromyogram.

2. At rest both electrical and magnetic stimulation evoked an inhibition of the Sol H reflex at the lowest intensities of stimulation. In some subjects a facilitation with an earlier onset was seen when increasing the stimulation strength. When the anode for the electrical stimulation was placed at the vertex directly above the leg motor area, the inhibition or facilitation often had the same latency as when evoked by magnetic stimulation. However, when the anode was placed 2–3 cm lateral to the vertex, effects evoked by the electrical stimulus often occurred 1–2 ms earlier.

3. Short-latency peaks in the PSTH of the discharges of single TA motor units also tended to occur earlier when evoked by electrical stimulation with the anode lateral to the vertex than when evoked by magnetic stimulation or electrical stimulation with the anode at the vertex.

4. In one subject, near-maximal electrical stimulation evoked MEPs with a latency corresponding to that seen following stimulation of the brainstem by electrodes placed bilaterally over the mastoid processes approximately 16 cm more distal. Maximal magnetic stimulation, in contrast, never resulted in responses with a latency shorter than that seen with the weakest electrical stimuli at the vertex.

5. The initial facilitation of the Sol H reflex evoked by magnetic stimulation and by electrical anodal stimulation at the vertex increased when the subject performed a voluntary plantarflexion. In contrast, the earlier facilitation evoked by electrical anodal stimulation 2–3 cm lateral to the vertex had the same size both at rest and during contraction.

6. We suggest that magnetic stimulation and electrical anodal stimulation at the vertex may preferentially activate descending cortical cells at, or close to, the cell soma. The initial responses evoked by these two stimuli may therefore be influenced by the excitability of the cortical cells. On the other hand, electrical stimulation with the anode 2–3 cm lateral to the vertex seems to often activate the axons at a deeper level. The initial responses evoked by this type of stimulation may therefore not be influenced by the excitability of the cortical cells.

Motor-evoked potentials (MEPs) in upper limb muscles and peaks in the post-stimulus time histogram (PSTH) of upper limb motor units occur 1–2 ms earlier when evoked by electrical rather than magnetic transcranial brain stimulation in man (Day *et al.* 1987, 1989). The explanation for this latency difference still remains controversial, but it is widely accepted that both types of stimulation activate the same corticospinal neurones although by different mechanisms or at different sites (Hess, Mills & Murray, 1987; Day *et al.* 1987, 1989; Amassian, Quirck & Stewart, 1990; Edgley, Eyre, Lemon & Miller, 1990). It is generally believed that electrical stimulation primarily activates the pyramidal cells directly, by depolarizing the axon and thus evoking a D-wave in the corticospinal tract (Patton & Amassian, 1954; Landau, Bishop & Clare, 1965; Kernell & Wu, 1967;

Amassian *et al.* 1990; Burke, Hicks & Stephen, 1990; Edgley *et al.* 1990; Burke, Hicks, Gandevia, Stephen, Woodforth & Crawford, 1993). When increasing the stimulation strength, later-occurring waves (I-waves) are seen. These are presumably caused by stimulation of cortical interneurons with synapses on the corticospinal neurones. One explanation of the later-occurring effects of magnetic stimulation is that this type of stimulation preferentially activates cortical interneurons and hence evokes I-waves in the corticospinal tract (Day *et al.* 1987, 1989). This possibility has received some support from the finding that the initial responses evoked by magnetic but not electrical stimulation appear to be influenced by changes in cortical excitability (Datta, Harrison & Stephens, 1989; Day, Riescher, Struppler, Rothwell & Marsden, 1991; Deuschl, Michels, Berardelli, Schenck, Inghilleri & Lücking, 1991; Maertens De Noordhout *et al.* 1992). However, an alternative possibility has been suggested. Edgley *et al.* (1990) found that in the monkey, both types of stimulation evoked D-waves in the pyramidal tract at low intensities of stimulation. When increasing the stimulation strength, however, the latency of the waves evoked by electrical stimulation occurred at a shorter interval. Similar changes in the latency of descending volleys evoked by brain stimulation have also been observed in recordings from the thoracic spinal cord in anaesthetized human subjects during surgery (Burke *et al.* 1990, 1993). The latency difference in the responses evoked by the two types of stimulation may therefore be explained simply by their different sites of activation along the corticospinal axons.

It is well accepted that latency differences are seen for upper limb motoneurones, but some controversy exists in the case of projections to lower limb motoneurones. Whereas Iles & Cummings (1992) and Priori *et al.* (1993) were unable to detect any latency difference of effects evoked by the two types of stimulation in lower limb motoneurones, Nielsen, Petersen, Deuschl & Ballegaard (1993) reported that the soleus (Sol) H reflex was inhibited at an earlier latency following electrical, rather than magnetic, stimulation. Several methodological differences, such as the location of stimulating electrodes, the type of magnetic coil and the voluntary activity of the subject, may explain this discrepancy. Clarifying these issues may help to determine the locus of activation of the cortical cells by the two types of stimulation. The present study was undertaken for this purpose.

METHODS

The experiments were performed on six subjects aged 27–49 years. The subjects gave informed consent to the experimental procedure, which was approved by the local ethics committee. Because of the pain related to the electrical stimulation of the brain the experiments were performed on a restricted number of subjects and repeated several times to ensure the reproducibility of the results.

The subjects were seated in a reclining armchair with their right foot attached to a foot plate. The hip was flexed to 120 deg, the knee was flexed to 160 deg and the ankle was in 110 deg plantarflexion. In some experiments the subject was requested to perform a voluntary plantarflexion. In these experiments the torque exerted on the foot plate was recorded by a torque meter and displayed on an oscilloscope in front of the subject for visual feedback.

The latency of the effects evoked by electrical and magnetic stimulation of the contralateral (left) motor cortex was evaluated by direct responses in the surface EMG, by H reflex testing and with post-stimulus time histograms of the discharges of single voluntarily activated motor units.

H reflex

Surface electrodes were used for both stimulation and recording. The Sol H reflex was evoked by stimulating the tibial nerve through a monopolar stimulating electrode (1 ms rectangular pulses). The indifferent electrode was placed on the anterior aspect of the thigh above the patella. The tibialis anterior (TA) H reflex was evoked by stimulating the common peroneal nerve through a bipolar stimulating electrode placed at the level of the head of the fibula. The reflex responses were measured as the peak-to-peak amplitude of the non-rectified reflex. The responses were recorded by the same disc electrodes as used for MEP measurements.

Conditioned and unconditioned reflexes were randomly alternated. At least twenty reflexes of each alternative were measured and stored on a personal computer (33 MHz 486-based PC) for later analysis. The mean and the standard error of the mean were calculated for each condition. The statistical significance of differences in the means obtained during the different tasks was tested using Student's two-tailed t test. The latency of inhibition or facilitation evoked by the conditioning stimulations was defined as the earliest conditioning–test interval at which the conditioning stimulation had a significant effect on the H reflex ($P < 0{\cdot}05$). It was ensured that the unconditioned control H reflex had a size of 15–25% of M_{max} in all situations (i.e. at rest and during contraction).

Conditioning stimuli

A MagStim 200 (MagStim Co. Ltd, Sheffield, UK) was used for magnetic stimulation of the brain and a Digitimer D180A (Digitimer Ltd, Welwyn Garden City, UK) was used for electrical stimulation of the brain, brainstem and spinal cord. The intensities of all stimuli were expressed as a percentage of the maximal stimulator output (2 T for the magnetic stimulator and 1500 V for the electrical stimulator). The time constant of the stimulation pulses was 100 μs. In most experiments a prototype of the figure-of-eight coil (loop diameter, 9 cm) was used for the magnetic stimulation. The coil was placed so that the current in the coil flowed in the anterior–posterior direction along a line 2 cm to the left of the vertex. For electrical stimulation of the brain the cathode was placed 4–6 cm anterior to the vertex and the anode was placed (i) 2–3 cm left of (and in some experiments in addition 2–3 cm posterior to) the vertex (denoted lateral anodal stimulation) or (ii) at the vertex (denoted vertex anodal stimulation). The brainstem stimulation was applied by electrodes placed on the posterior part of the mastoid processes bilaterally (Ugawa, Rothwell, Day, Thompson & Marsden, 1991). This corresponds to position B of Ugawa *et al.* (1991). This position was chosen because the data of Ugawa *et al.* (1991) indicate that with this electrode arrangement, activation

of the corticospinal tract takes place close to the level of the electrodes. Stimulation of the spinal cord was applied through electrodes placed over the spinous processes, either with the cathode at Th1 or C7 and the anode at C5, or with the cathode at C5 or C4 and the anode at C2.

Motor-evoked potentials

Motor-evoked potentials (MEPs) were recorded from the TA and Sol surface EMG following each of the conditioning stimulations (see above). The recording electrode was a bipolar non-polarizable Ag–AgCl disc electrode (1 cm^2 area, 1 cm distance between poles). The responses were amplified and filtered (20 000 times; 5 Hz–1 kHz) before they were sampled on a computer (software package: Infowest Inc., Winnipeg, Canada). Five to ten responses were averaged.

Post-stimulus time histograms

Post-stimulus time histograms (PSTHs) of the probability of discharge of single voluntarily activated TA motor units were constructed following the different types of brain stimulation and following stimulation of the brainstem and spinal cord. The discharges of the single motor units were recorded either by the surface electrodes (described above) or by monopolar needle electrodes (Dantec, Skovlunde, Denmark). To reduce the number of triggers, the stimuli were triggered on the previous discharge of the motor unit (Fournier, Meunier, Pierrot-Deseilligny & Shindo, 1986). By changing the delay between the trigger and the stimulus, the stimulation could always be given at an optimum time (when the unit was not refractory due to the previous discharge). The PSTH was constructed for a window between 20 and 70 ms after the stimuli, using bins of 0·1, 0·2 or 1 ms. A histogram was also constructed in a control situation without stimulation. Measurements with and without stimulation were randomly alternated. The spontaneous discharge probability of the unit could thus be subtracted from that resulting from the stimulation. The interval between each measurement was 4 s. A χ^2 test was used to detect significant changes in the firing probability of the units evoked by the stimuli. The latency of peaks was defined as the earliest bin of at least two consecutive bins in which a significant increase in firing probability was observed.

RESULTS

Latency of facilitation and inhibition of the Sol H reflex evoked by magnetic and electrical brain stimulation

Figure 1 shows time courses of the effect of magnetic and electrical stimulation of the brain on the Sol H reflex in two different subjects at rest. The intensities of the two stimulations were adjusted to be just below the threshold for evoking an MEP in the Sol muscle. At lower intensities of stimulation, an inhibition without any evidence of facilitation was seen in both subjects as described previously (Iles & Pisini, 1992; Nielsen *et al.* 1993). This was also the case at the stimulus intensity used in the experiment for the subject shown on the left, but in the other subject the inhibition was replaced by an earlier occurring facilitation (see also Nielsen *et al.* 1993). When the anode was placed 2 cm left of the vertex (lateral anodal stimulation), both the inhibition (Fig. 1*A*) and the facilitation (Fig. 1*D*) occurred 1–2 ms earlier than when the same effects were evoked by electrical stimulation with the anode placed at the vertex (vertex anodal stimulation; Fig. 1*B* and *E*). For the subject on the left, the inhibition evoked by magnetic stimulation occurred yet another 1 ms later (Fig. 1*C*), whereas the facilitation evoked by magnetic stimulation in the other subject had a similar latency to the facilitation evoked by vertex anodal stimulation (Fig. 1*F*).

The latency of inhibition/facilitation of the Sol H-reflex evoked by vertex anodal and lateral anodal stimulation was compared in seven experiments in four subjects. In six of the seven experiments the inhibition/facilitation evoked by the lateral anodal stimulation occurred 1–2 ms earlier than similar effects evoked by vertex anodal stimulation. In the remaining experiment the latencies were the same. In five of the seven experiments the effects evoked by magnetic and vertex anodal stimulation had the same latency. In the remaining two experiments (in two different subjects) the effect of the magnetic stimulation occurred 1 ms later (e.g. Fig. 1*C*).

Figure 2 first of all demonstrates that differences in the latency of effects evoked by the three stimuli were also seen at the single motor unit level. Figure 2*A–C* shows changes in the firing probability of a single TA motor unit following lateral anodal stimulation (Fig. 2*A*), vertex anodal stimulation (Fig. 2*B*) or magnetic stimulation (Fig. 2*C*). The intensities of the three stimuli were adjusted to be just below the threshold for recruiting additional nearby units and thus evoking a compound MEP. All three types of stimulation evoked significant peaks of increased firing probability in the PSTH. These peaks had a similar duration (1·0–1·4 ms) but different latencies. The peak evoked by lateral anodal stimulation had a latency of 38·8 ms (Fig. 2*A*), whereas the peaks evoked by vertex anodal stimulation and magnetic stimulation occurred 1·4 and 2·8 ms later, respectively. This type of experiment was performed in eleven motor units from four subjects (Fig. 2*D*). The peak evoked by vertex anodal stimulation occurred 1–2 ms later than the peak evoked by lateral anodal stimulation in all but two motor units. In three units the peak evoked by magnetic stimulation had the same latency as the peak evoked by vertex anodal stimulation, whereas it occurred 1–6 ms later in the remaining eight units. In addition, twenty-two experiments were performed in which only lateral anodal and magnetic stimulation were compared (Fig. 2*E*). In an additional two experiments only vertex anodal and lateral anodal stimulation were compared (Fig. 2*F*). In all but three of the thirty-three motor units studied, the peak evoked by magnetic stimulation had a longer latency than the peak evoked by lateral anodal stimulation (Fig. 2*E*). As in the study by Day *et al.* (1989), in which the latency of peaks in the PSTH of motor units in hand muscles was investigated, we found that the peaks were

clustered in four different groups. The earliest group of peaks contained those evoked by lateral anodal stimulation as well as six peaks evoked by either vertex anodal or magnetic stimulation. The groups following were seen at latencies of 1–2, 2·2–3·5 and 5–6 ms after the first group. In some cases, secondary peaks following the initial peak were seen with a latency comparable to the last group (Fig. 2*E*; open triangles).

Influence of the stimulation strength

In the previous experiments the intensities of the stimuli were always adjusted to be just below the threshold for evoking a direct MEP in the muscles investigated. Changing the intensity of stimulation may, however, have profound effects on the latencies of the observed effects (Burke *et al.* 1990; Edgley *et al.* 1990). Therefore, we also compared the effects evoked by the three types of stimulation at lower and higher intensities of stimulation than those used in the previous experiments. Using the PSTH method to investigate effects at low intensities of stimulation and the MEP to investigate effects at high intensities, we were able to use stimulation intensities ranging from below 15 to more than 70% of the maximal stimulation intensity (i.e. 225–1050 V; Higher intensities of stimulation caused profound discomfort for the subjects). Figure 3 shows PSTHs from a single TA motor unit and Fig. 4 shows MEPs from the same subject (but in two different experiments). In this subject a peak was seen in the PSTH at a lower intensity of stimulation when the anode was placed directly over the vertex than when it was placed lateral to the vertex (this was also the case in two other subjects, whereas the two stimuli evoked peaks at the same intensity of stimulation in the last subject). At an intensity of 20% the stimulus at the vertex evoked a clear peak with a maximal bin count at 41·4 ms and an onset at 40·8 ms (Fig. 5*E–F*). Lateral anodal stimulation at an intensity of 32% evoked a peak, which occurred approximately 1 ms earlier than the peak evoked by

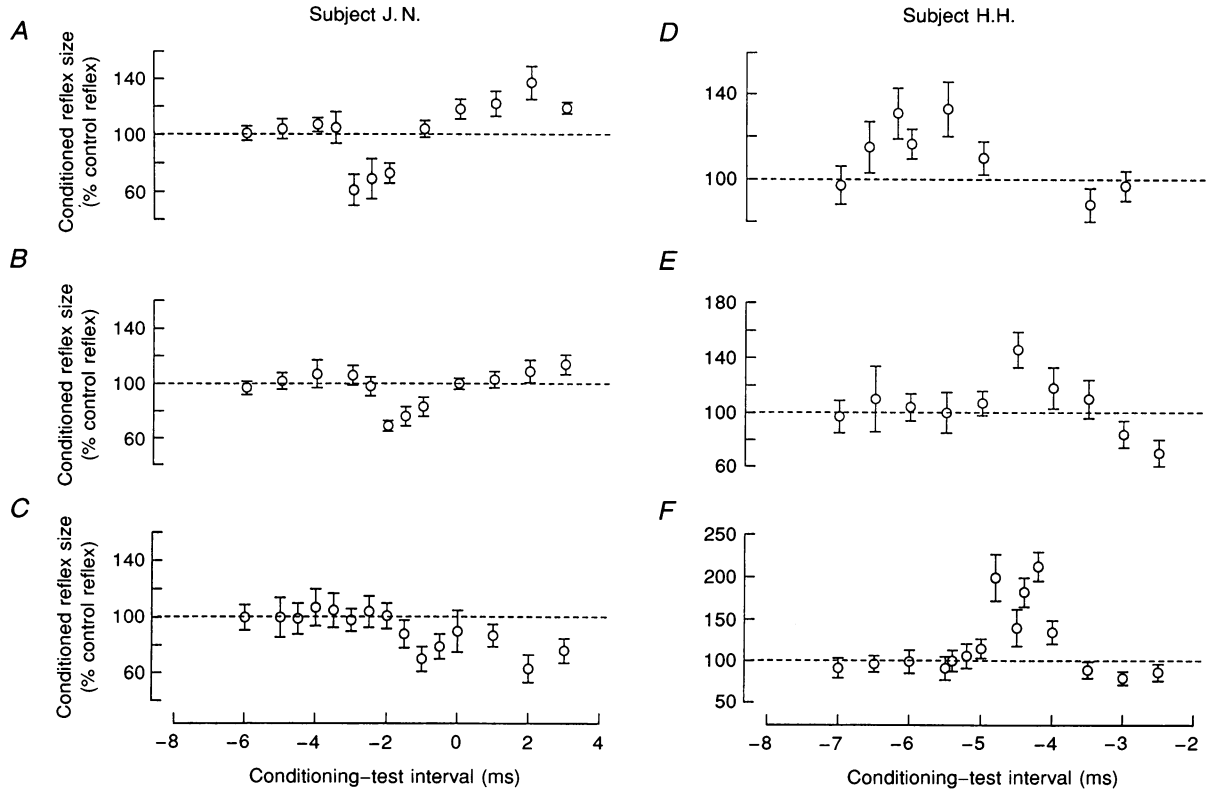

Figure 1. The effect of magnetic and electrical stimulation of the motor cortex on the Sol H-reflex in two different subjects (*A–C* and *D–F*, respectively) at rest

The size of the conditioned reflex was determined as a percentage of the size of the control reflex at a range of conditioning–test intervals (vertical bars, 1 S.E.M.). *A*, electrical stimulation, anode 2 cm lateral to vertex; *B*, electrical stimulation, anode at vertex; *C*, magnetic stimulation, figure-of-eight coil; *D*, electrical stimulation, anode 2 cm lateral to vertex; *E*, electrical stimulation, anode at vertex; *F*, magnetic stimulation, figure-of-eight coil. All stimuli were adjusted to be approximately 0·85 × MEP threshold. The intensity of the electrical stimulus was 35% of the maximal stimulator output in *A*, 37% in *B*, 45% in *D* and 40% in *E*. The intensity of the magnetic stimulus was 50% of the maximal stimulator output in *C* and 40% in *F*. The size of the unconditioned control H reflex was 22% of M_{max} in *A–C* and 18% of M_{max} in *D–F*.

vertex anodal stimulation (onset, 39·6–39·8 ms). With increasing intensities of stimulation the latencies of both peaks decreased: at intensities just below MEP threshold the peak evoked by lateral anodal stimulation had a latency of 39 ms, and that evoked by vertex anodal stimulation had a latency of 40 ms. Low-intensity magnetic stimulation evoked a peak at a latency similar to that evoked by weak vertex anodal stimulation (41·2 ms). When increasing the intensity of stimulation, however, the latency of this peak remained constant so that at strong stimulation intensities a latency difference was seen between the two peaks. In six of the seven motor units investigated from four subjects, the peak evoked by lateral anodal stimulation changed latency (mean,

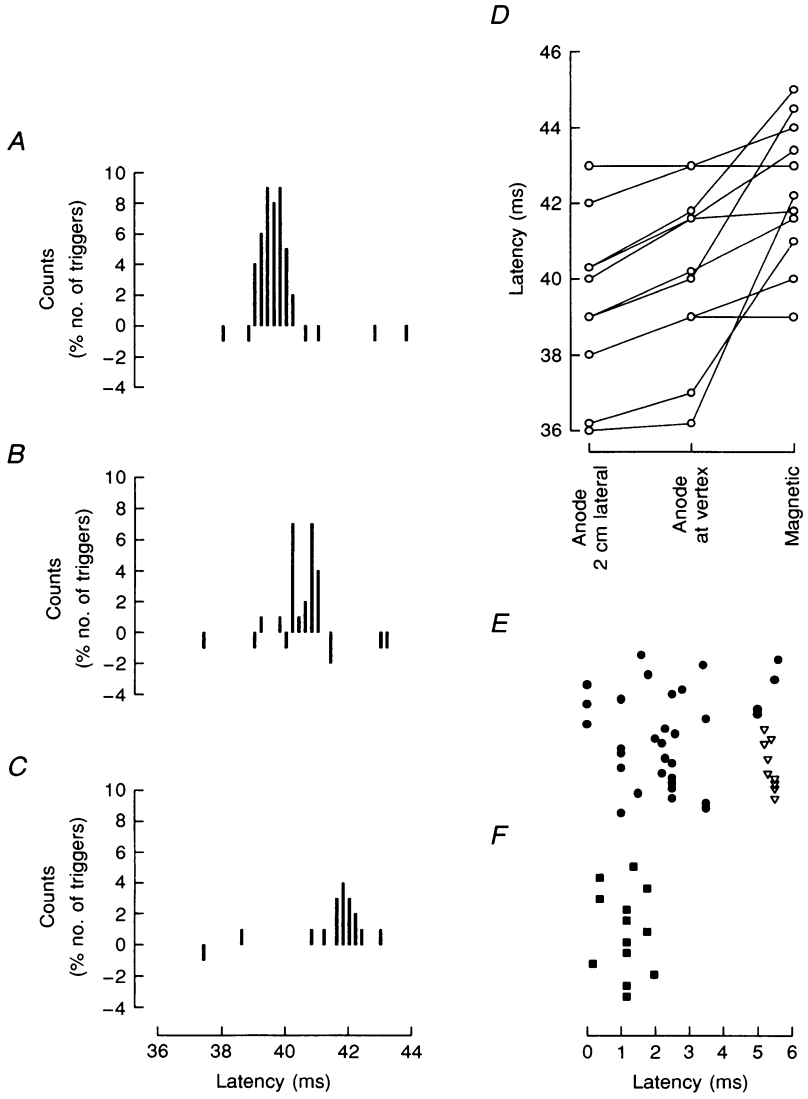

Figure 2. Latency of peaks evoked by magnetic and electrical stimulation of the brain in the post-stimulus time histogram of single voluntarily activated TA motor units

A–C, data from a single subject, showing the latency after differrent stimuli. *A*, electrical stimulation, anode 2 cm left of vertex; *B*, electrical stimulation, anode at vertex; *C*, magnetic stimulation, figure-of-eight coil. Spontaneous interspike interval, 105 ms; total number of triggers, 100; bin width, 0·2 ms. *D*, latency of peaks induced by electrical and magnetic brain stimulation; data from 11 motor units recorded in 4 subjects (data from two of the motor units are superimposed). Left, electrical stimulation with the anode 2 cm to the left of the vertex; centre, electrical stimulation with the anode placed directly at the vertex; and right, magnetic stimulation. Each line and symbol represents data from a single motor unit. *E–F*, the latency of peaks evoked by electrical stimulation with the anode at the vertex (*F*, ■) and magnetic stimulation (*E*, ● and ▽) in relation to the latency of peaks evoked by electrical stimulation with the anode 2 cm left of the vertex. The circles in *E* represent the latency of the first peak evoked by the stimulation and the triangles represent the latency of possible second peaks. Each symbol represents data from one motor unit.

0·88 ms; range, 0·6–1·4 ms), when the stimulus was increased from the lowest intensity at which a peak was seen to the highest intensity that could be used without evoking an MEP. The peak evoked by vertex anodal stimulation similarly decreased its latency in six of the seven units by 0·73 ms on average (range, 0·4–1·2 ms). In five of the units, the peak evoked by magnetic stimulation had the same latency as the peak evoked by vertex anodal stimulation at the weakest intensity that could evoke a peak. In these units changing the intensity of stimulation had no effect on the latency of the peak. In the remaining two units the peak evoked by magnetic stimulation occurred 5–6 ms later than the peak evoked by vertex anodal stimulation. In these units the latency of the peak decreased abruptly when increasing the stimulation intensity, so that the latency of the peak evoked by magnetic stimulation at an intensity just below MEP threshold equalled the latency of vertex anodal stimulation at the weakest intensity that could evoke a peak in one of the units, but still had a longer latency (2·5 ms) in the other.

Similar effects on the latencies of MEPs were also observed, as shown in Fig. 4. An MEP evoked by electrical stimulation of the brainstem at the level of the mastoid processes (see also Ugawa *et al.* 1991) is shown at the bottom of the figure. The latency of this MEP was 27·1 ms. With a stimulation intensity just above threshold, the magnetic stimulus evoked an MEP at a latency of 30·2 ms; i.e. 3·1 ms later than the MEP evoked by the brainstem stimulation (Fig. 4*C*). The two types of

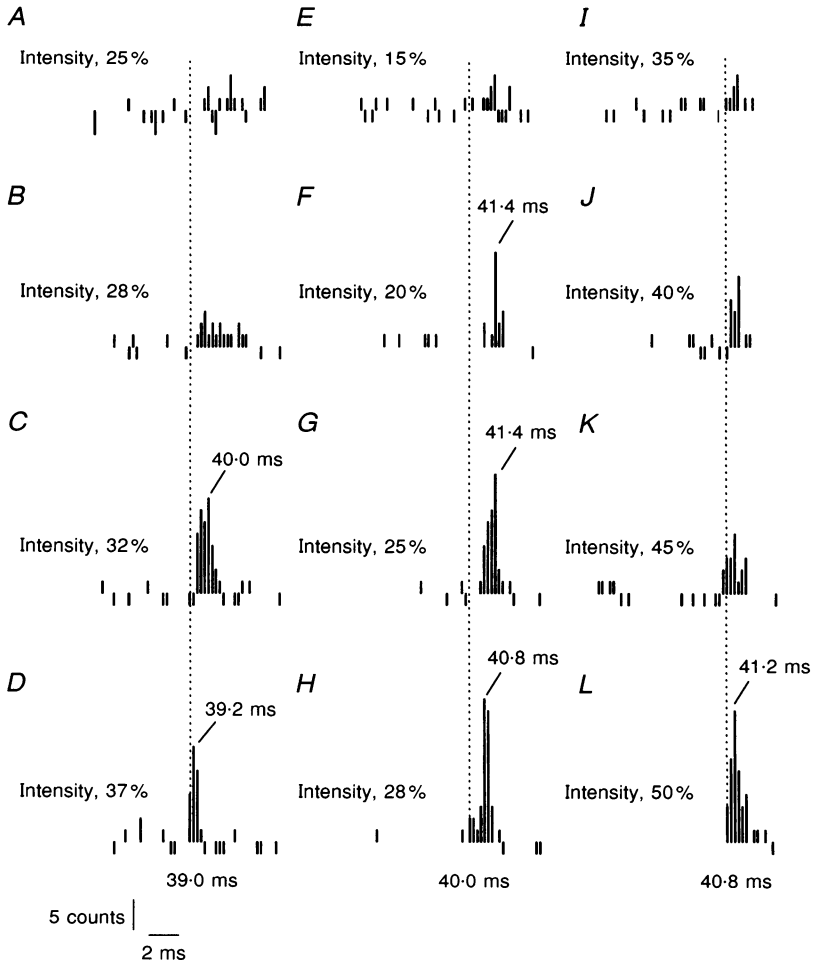

Figure 3. The influence of changes in the stimulation intensity on the latency of peaks in the PSTH of single TA motor units following magnetic and electrical stimulation of the brain

The data are from a single motor unit. *A–D*, electrical stimulation with the anode 2 cm left of the vertex. *E–H*, electrical stimulation with the anode at the vertex. *I–L*, magnetic stimulation. The intensity of the electrical stimulation was varied from 25 to 37% in *A–D* and from 15 to 28% in *E–H*; the intensity of the magnetic stimulus in *I–L* was varied from 35 to 50%. The MEP threshold was 40% in *A–D*, 30% in *E–H* and 52% in *I–L*. The vertical dotted lines represent the latency of the first bin in which a statistically significant increase in firing probablity was observed at the strongest intensity of stimulation used for each type of stimulation. Each bin is 0·2 ms.

electrical stimulation, however, evoked MEPs which, even at an intensity just above MEP threshold, had a latency 1 ms earlier than the MEP evoked by the magnetic stimulus (Fig. 4*A* and *B*). Notice that the threshold of the MEP evoked by the vertex anodal stimulus was much lower than the threshold of the MEP evoked by the lateral anodal stimulus (this was also the case in two other subjects, whereas the threshold was the same in the last subject). When the intensities of the electrical stimuli were increased, the latency of the MEP decreased dramatically. With the anode 2 cm lateral to the vertex the MEP evoked by the strongest stimulus (corresponding to 2·0 × MEP threshold) had a latency comparable to the MEP evoked by brainstem stimulation. This leads to the suggestion that the two types of stimulation, although separated by a distance of 16 cm, activated the descending corticospinal tract at the same site. With the anode at the vertex, the MEP evoked by the strongest stimulation (corresponding to 2·7 × MEP threshold) that the subject could accept occurred 1 ms later. As was seen for the single unit in Fig. 3, increasing the intensity of the magnetic stimulation had no effect on the latency of the response.

Such a pronounced change in latency was seen only in the illustrated subject. Indeed, in one of the other three subjects investigated in this manner, changing the intensity of stimulation had no effect on the latency of the MEP evoked by any of the types of stimulation. However, in the other two subjects a small decrease (0·8–1·2 ms) was seen when increasing the intensity of the electrical stimuli from 20–30 to above 50% of the maximal stimulator output. In these subjects the MEP evoked by magnetic stimulation at an intensity just above MEP threshold had a much longer latency (4–5 ms) than the MEP evoked by vertex anodal stimulation. When increasing the intensity of the magnetic stimulus the latency of the MEP decreased in both subjects so that it equalled the latency

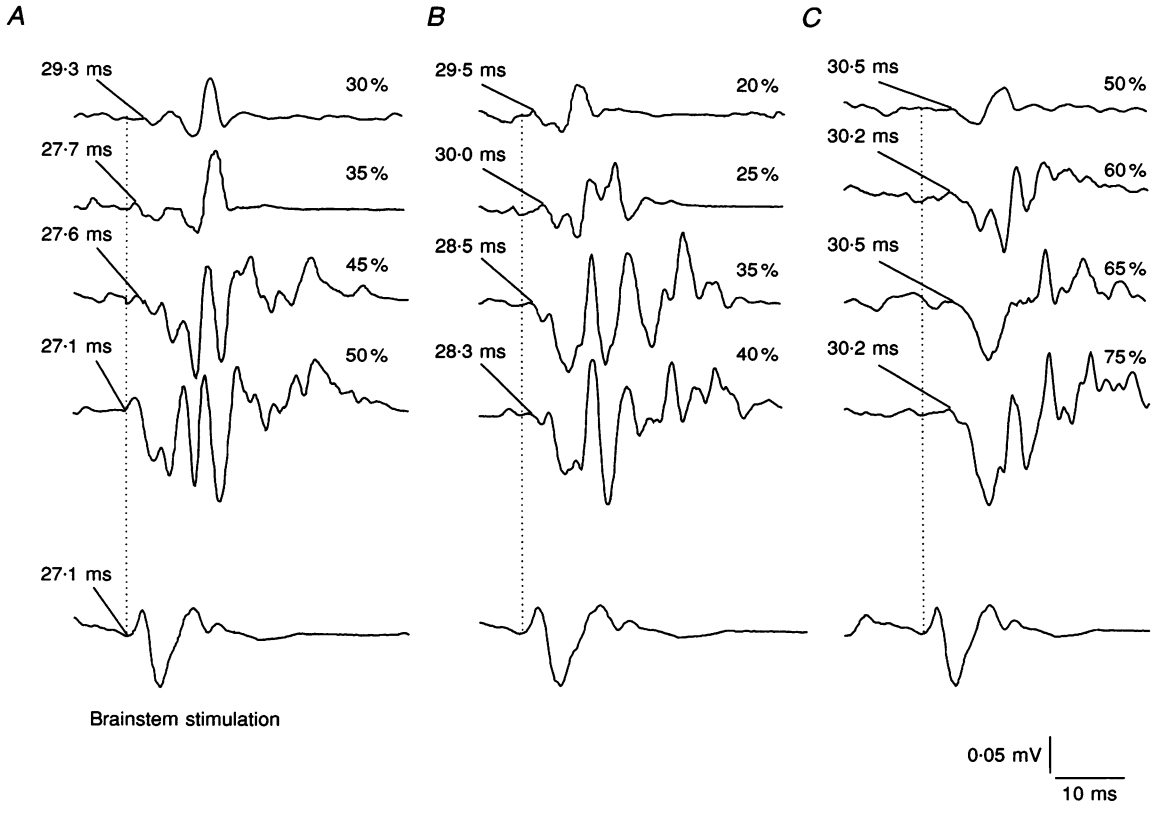

Figure 4. The influence of changes in the stimulation intensity on the latency of motor-evoked potentials (MEPs) in the TA surface EMG following stimulation of the brain

Each trace is the average of 6 sweeps. The subject performed a weak tonic voluntary dorsiflexion (5 N m) during the experiment. The lowest trace demonstrates an MEP evoked by stimulation of the brainstem at the level of the mastoid processes (intensity, 55%). *A–C*, upper 4 traces, MEPs evoked by increasing intensities of electrical stimulation with the anode 2 cm left of the vertex (*A*), by increasing intensities of electrical stimulation with the anode at the vertex (*B*) and by increasing intensities of magnetic stimulation (*C*). The stimulation intensities were varied from 30 to 50, 20 to 40 and 50 to 75% in *A*, *B* and *C*, respectively. The MEP threshold was 25% in *A*, 15% in *B* and 40% in *C*. The dotted vertical line marks the onset of the MEP evoked by brainstem stimulation. Time calibration, 10 ms; voltage calibration, 0·05 mV.

of the MEP evoked by vertex anodal stimulation at an intensity just above threshold. With intensities of stimulation just above threshold, the average latency of the MEP evoked by lateral anodal stimulation was 28·4 ± 1·1 ms as compared with 29·7 ± 1·9 and 30·5 ± 1·5 ms for the MEPs evoked by vertex anodal and magnetic stimulation, respectively (average of 7 experiments in 4 subjects). The average latency of the MEP evoked by brainstem stimulation was 26·5 ± 1·5 ms (average of 3 experiments in 3 subjects).

Comparison of latencies of the effects evoked by spinal cord, brainstem and cortical stimulation

The short interval between the responses evoked by electrical stimulation of the brain and the brainstem in Fig. 4 suggests that strong electrical stimulation of the brain may activate the corticospinal tract deep in the brain. Are the latency differences of the responses evoked by the different types of stimulation caused by activation of the corticospinal fibres at different sites along the axon (Edgley *et al.* 1990)?

Figure 5 demonstrates MEPs evoked in the TA muscle by stimulation of the brain, the brainstem and the spinal cord. The intensities of all stimuli were adjusted to 1·1 × MEP threshold. At this intensity of stimulation the initial positive component of the MEP evoked by magnetic stimulation had a much longer latency than the initial positive component of the MEP evoked by the vertex anodal stimulation (38·0 ms compared with 33·4 ms). In this subject, the initial positive component of the MEP evoked by lateral anodal stimulation occurred an additional 0·6 ms earlier. The distance from the vertex to the electrodes placed over the mastoid processes was measured as approximately 15 cm, whereas the distances from the mastoid processes to the electrode placed over C4 and from there to the electrode placed over C7 were 5·5 and 6 cm, respectively. If the conduction velocity of the cranial and spinal part of the central fibres is comparable, the interval between the MEP evoked by lateral anodal stimulation and stimulation of the brainstem should therefore be about 2·5–3 times longer than the interval

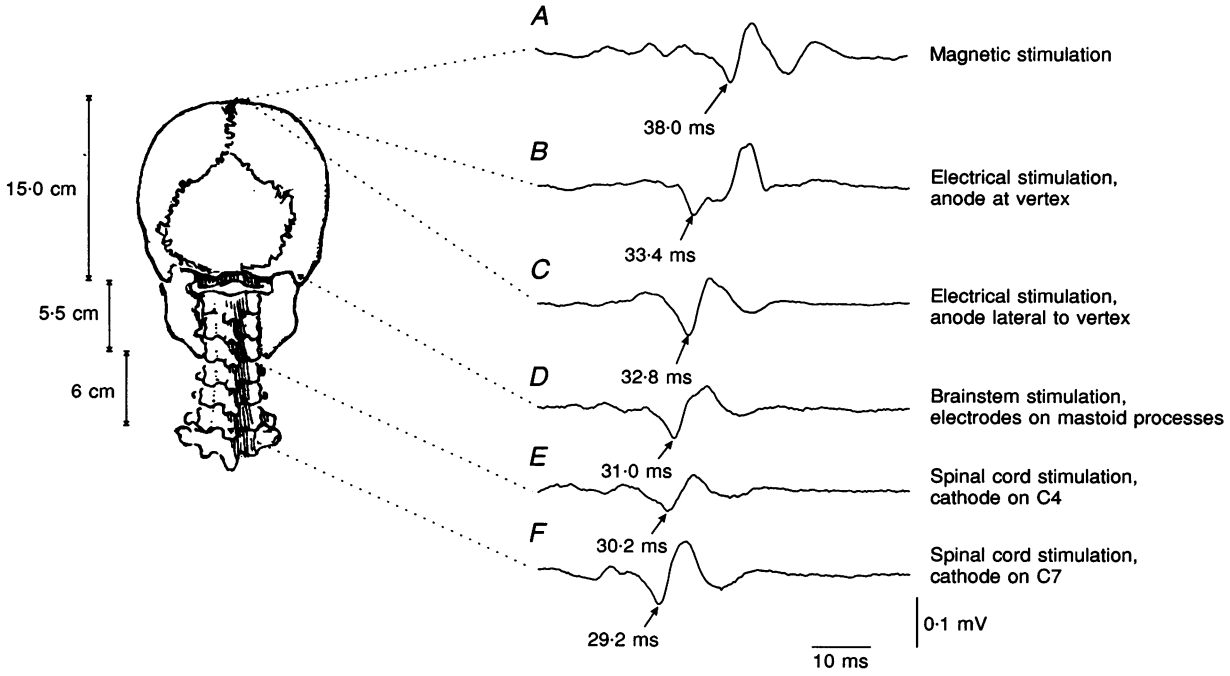

Figure 5. Latency of MEPs in the TA surface EMG following stimulation of the brain, brainstem or spinal cord

Each trace is the average of 10 sweeps. The subject performed a weak tonic voluntary dorsiflexion (5 N m) during the experiment. The intensity of all stimuli was adjusted to 1·1 × MEP threshold. *A*, magnetic stimulation; intensity, 60%. *B*, electrical stimulation with anode at the vertex; intensity, 65%. *C*, electrical stimulation with anode lateral to the vertex; intensity, 65%. *D*, brainstem stimulation with electrodes placed bilaterally on the mastoid processes; intensity, 22%. *E*, stimulation of the spinal cord with cathode placed over the spinous proces of C4 and the anode over C2; intensity, 40%. *F*, stimulation of the spinal cord with the cathode placed over the spinous proces of C7 and the anode over C5; intensity, 35%. The drawing on the left in the figure illustrates the approximate distance between the site of the different stimulations, i.e. 15 cm between the vertex and the mastoid processes, 5·5 cm from there to C4 and 6 cm from there to C7. Time calibration, 10 ms; voltage calibration, 0·1 mV.

between the MEPs evoked by the two spinal stimulations. The observed latency difference between the MEP evoked by lateral anodal stimulation and brainstem stimulation was, however, somewhat shorter than this (i.e. 1·8 ms instead of approximately 2·5–3 ms), suggesting that the stimulation already at intensities just above threshold for the MEP had activated the axons of the cortical cells at some distance below the surface of the skull. The latency of the MEP evoked by the vertex anodal stimulation on the other hand was comparable to that calculated from the latency of the spinal MEPs, suggesting activation of the cortical cells close to the surface. These data were confirmed from similar experiments in two other subjects and from single unit data in one subject.

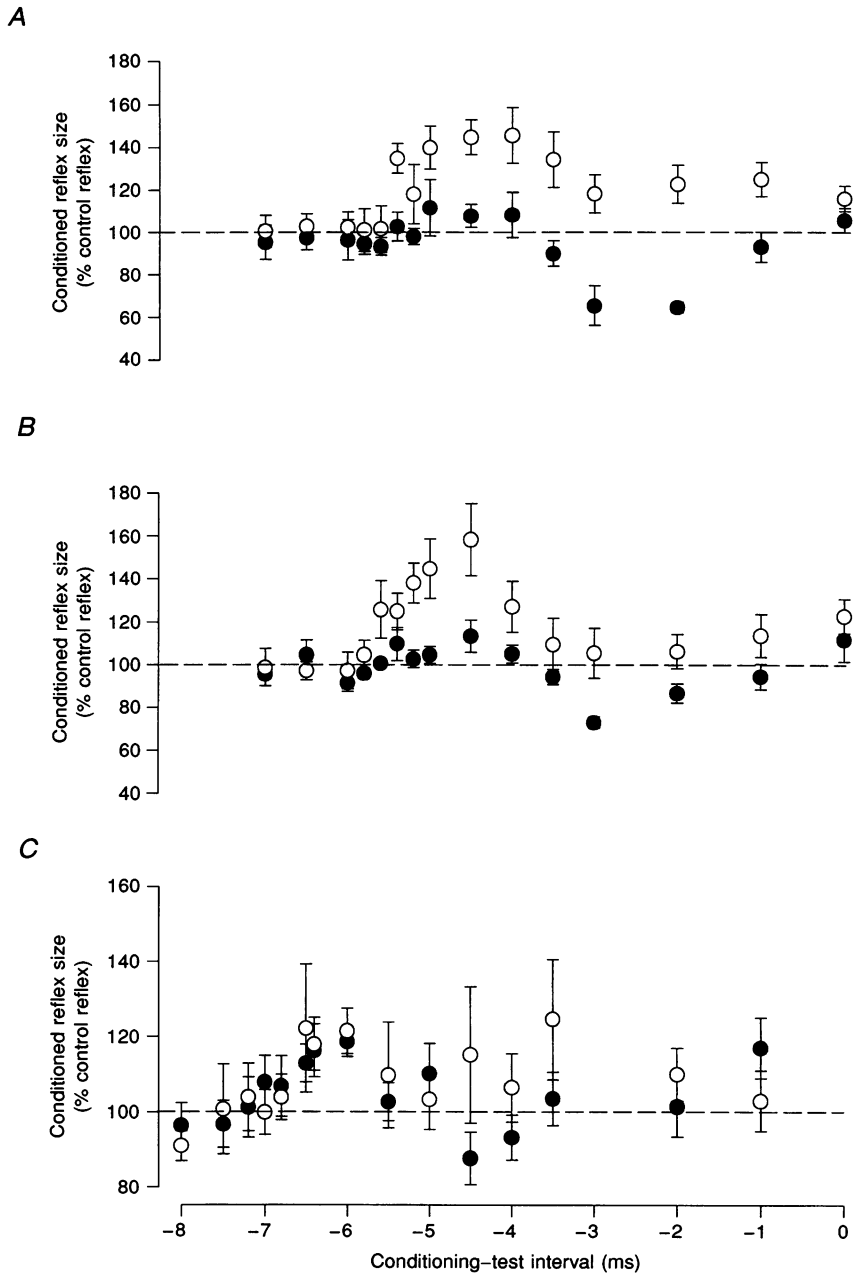

Figure 6. The effect of magnetic and electrical brain stimulation on the Sol H reflex at rest (●) and during voluntary plantarflexion (○)

A, magnetic stimulation of the brain. *B–C*, electrical stimulation of the brain. The anode was placed at the vertex in *B* and 3 cm left of the vertex in *C*. The control H reflex was 20% of M_{max}. The intensities of the stimuli were adjusted to be just below MEP threshold during voluntary plantar flexion (45% in *A*, 27% in *B*, 30% in *C*). Same ordinate and abscissa as in Fig. 1*A*. Each bar represents 1 S.E.M.

Task-dependency of effects evoked at different latencies

Nielsen *et al.* (1993) demonstrated that the facilitation of the Sol H reflex produced by magnetic brain stimulation increased during tonic plantar flexion in relation to rest, whereas this was not the case with the earlier occurring facilitation evoked by lateral anodal stimulation. We have since confirmed this finding in eleven experiments on five subjects. If weak anodal and magnetic stimulation both activate the cortical cells at the same level, which seems to be indicated from the similar latency of their effects, the facilitation evoked by vertex anodal stimulation should also increase with plantar flexion. As seen from Fig. 6. this was also the case. The intensity of the three types of stimulation (magnetic, Fig. 6*A*; vertex anodal, Fig. 6*B*; lateral anodal, Fig. 6*C*) was adjusted to be just below MEP threshold during a moderate voluntary plantarflexion (30% for lateral anodal, 27% for vertex anodal and 45% for magnetic stimulation). Adjusted in this way lateral anodal stimulation evoked a facilitation of the Sol H reflex at rest (Fig. 6*C*, ●; conditioning–test interval, −6·5 ms) followed by an inhibition at a conditioning–test interval of −4·5 ms, whereas the other two stimulations evoked an inhibition at a conditioning–test interval of −3·0 ms. However, when the subject performed a weak voluntary plantarflexion (5 N m) both of the latter stimuli evoked a facilitation at a conditioning–test interval of −5·5 ms (Fig. 6*A* and *B*, ○). In contrast, the earlier facilitation evoked by the lateral anodal stimulation had the same size as at rest (Fig. 6*C*; compare open and closed circles). To ensure that the lack of increase of this facilitation was not due to saturation the stimulation intensity was decreased (to 25%) so that the stimulus had almost no effect on the reflex at rest. The facilitation still did not increase (or appear) during contraction. These findings were reproducible in all four subjects investigated in this manner.

DISCUSSION

In the present study we firstly confirmed the findings of Nielsen *et al.* (1993) that in man, electrical stimulation of the brain with the anode placed lateral to the vertex mostly has effects on lower limb muscles at a shorter latency than those evoked by magnetic stimulation. However, to our surprise we also found that changing the position of the anode so that it was centred over the vertex changed the latency of the effects. With the anode at the vertex, facilitatory and inhibitory effects on the Sol and TA H reflexes, peaks in the PSTH of TA motor units and TA MEPs often had a longer latency than when the anode was placed laterally. In most experiments we failed to see a latency difference between effects evoked by magnetic stimulation and those evoked by electrical stimulation with the anode centred on the vertex. This latter finding is similar to that reported by Iles & Cummings (1992) and Priori *et al.* (1993). We therefore conclude that the discrepancy between these previous reports and that of Nielsen *et al.* (1993) is likely to be due to the difference in location of the stimulating anode.

Mechanism of activation by electrical stimulation

What is the reason for the difference in latency of the effects evoked by the two types of electrical stimulation? We cannot fully disregard the possibility that the two types of stimulation activated different populations of corticospinal cells with different conduction velocities. Such subpopulations are known to exist (Lemon, Werner, Bennet & Flament, 1993), but in all likelihood, they are located in more or less the same area of the motor cortex as the fast, large units and would therefore be expected to be activated equally by the two types of stimulation. Furthermore, considerably changing the position of the magnetic coil, which could have resulted in activation of different populations of cortical cells, had no influence on the latencies of the responses.

It is possible that the vertex anodal stimulation preferentially activated the cortical cells indirectly. However, in monkey as well as in man, anodal stimulation has been shown to activate the corticospinal neurones directly, thus resulting in a D-wave in the corticospinal tract (Patton & Amassian, 1954; Landau *et al.* 1965; Kernell & Wu, 1967; Amassian *et al.* 1990; Burke *et al.* 1990; Edgley *et al.* 1990). Only when the stimulation intensity is increased do I-waves, which are probably caused by trans-synaptic activation of the cells, appear. It may be that we overlooked the effect of the initial D-wave evoked by the vertex anodal stimulation, but not by the lateral anodal stimulation. This would happen if the D-wave evoked by the vertex anodal stimulation was too small to have a significant effect on the discharge of the motoneurones. Direct measurement of the descending volley evoked by the two types of stimulation in anaesthetized patients during surgery could disclose whether this is the case or not.

The final possibility is that both stimuli activated the same corticospinal cells directly, but at different sites along the axons. Indeed, by comparing the latency of MEPs evoked by the cortical stimulations with the latency of MEPs evoked by stimulation of the brainstem and spinal cord, evidence was obtained that the vertex anodal stimulation mostly activated the corticospinal cells at a superficial site, whereas the lateral anodal stimulation activated the descending axons at some distance below the cortical surface. The tendency of the electrical stimulation to activate the corticospinal fibres below the cortex was strikingly demonstrated by the finding that strong lateral anodal stimulation could evoke an MEP with a similar latency to MEPs evoked by stimulation of the brainstem, approximately 16 cm more distal. This interpretation of the results is supported by the findings of Burke *et al.* (1990, 1993) and Edgley *et al.* (1990). Burke

et al. (1990) found that by increasing the intensity of electrical stimulation at the vertex, D-waves recorded from the thoracic spinal cord in anaesthetized subjects were evoked at earlier latencies. They suggested that the stimulation activated the corticospinal neurones at sites either 5 or 10–11 cm below the cortex, which seems to fit well with the observations in the present study (cf. Fig. 5). In the monkey, Edgley *et al.* (1990) also found that electrically evoked responses occurred at earlier latencies with a higher intensity of stimulation and suggested that in some cases the stimulation might have activated the corticospinal tract at the level of the cervico-medullary junction.

At present, we have no definitive answer as to why electrical stimulation at the vertex should preferentially activate the corticospinal neurones superficially, whereas electrical stimulation with the anode placed laterally should be more prone to activate them at distal sites even at just above threshold intensities of stimulation. Again it could be that the lateral anodal stimulation did activate the corticospinal cells superficially, but that the resulting D-wave was too small to be demonstrated with the indirect methods used in this study. Alternatively, there is increasing evidence demonstrating the importance of the direction of the current flow in the cortex induced by stimulation. Magnetic stimulation of the cortex with the current in a single coil flowing in a clockwise direction thus produces responses at a longer latency than when the current is flowing in a counterclockwise direction (Day *et al.* 1989; Priori *et al.* 1989). The importance of the direction of the induced current has also been suggested by Amassian *et al.* (1990) who found that magnetic stimulation of the motor cortex in monkeys readily evoked D-waves in the pyramidal tract when the coil was placed on the side of the head, whereas only I-waves were elicited when the coil was placed over the vertex. Day *et al.* (1989) also found evidence that cathodal stimulation (i.e. with the cathode placed over the motor cortex) evoked responses at longer latencies than anodal stimulation (anode placed over the cortex), although later studies have failed to confirm this finding (Burke *et al.* 1990). Finally, in direct relation to the present study, Priori *et al.* (1993) found that with the anode at the vertex MEPs in TA had a shorter latency (by 0·75 ms) when the cathode was placed on the hand area of the motor cortex than when it was placed 6 cm in front of the vertex.

It is thus possible that changing the position of the electrodes changes the flow of current and thus the site at which the stimulation most easily activates the cells. Iles & Cummings (1992) suggested that the interhemispheric fissure might have a relatively low resistance and that the current from the anode at the vertex might therefore flow down the fissure and excite the pyramidal cells close to their soma. When the anode is placed more laterally (as in our study or when stimulating the hand area; Day *et al.* 1989) the current may preferentially flow through the white matter and activate the cortical cells at a distance from the cell soma. This would also be the case for the stimulation with the electrode at the vertex, but only when increasing the intensity.

Mechanism of activation by magnetic stimulation

In several experiments, effects evoked by magnetic stimulation had latencies that were clearly too long to be explained by direct activation of the cortical cells ; at least one synaptic delay must have been involved. With sufficiently strong stimulation, however, the responses evoked by magnetic stimulation occurred at the same latency as the responses evoked by electrical stimulation at the vertex. We therefore suggest, in accordance with several previous studies (Edgley *et al.* 1990; Iles and Cummings, 1992; Priori *et al.* 1993), that magnetic stimulation of the cortex with an optimal flow of current is capable of activating the cortical cells directly. However, in contrast to electrical stimulation, magnetic stimulation never produced responses with latencies suggestive of penetration of the stimulus to deeper sites in the brain. Edgley *et al.* (1990) also found that magnetic stimulation of the monkey brain always activated the corticospinal cells at a superficial site. This is not surprising as the magnetic field, in contrast to an electrical field, readily penetrates bony structures (Barker, 1991; Saypol, Roth, Cohen & Hallet, 1991). Furthermore, Branston & Tofts (1990) have demonstrated that the induced field lies parallel to the surface of the skull and that the field density is very low even at a short distance from the centre of the coil (the field diminishes initially with the square and then the cube of the distance from the coil; see also Tofts, 1990).

Influence of changes in cortical excitability

The observation of task-related differences in the initial responses evoked by magnetic and electrical stimulation at the vertex, but not in the initial responses evoked by electrical stimulation lateral to the vertex, seems most easily explained by a difference in the susceptibility of the responses to changes in cortical excitability. This interpretation is easy to accept in the case of the magnetically induced effects, which judged from their latency were evidently caused by trans-synaptic activation of the corticospinal cells. However, in some experiments the magnetic stimulation evoked effects which had the same latency as effects evoked by the vertex anodal stimulation. As already discussed, it was most likely that these effects were caused by direct activation of the cortical cells, but they were, nevertheless, also demonstrated to be task-dependent. We therefore support the suggestion by Edgley *et al.* (1990) that responses evoked by activation of the cortical cells close to or at the cell soma, may be influenced by changes in cortical excitability. This hypothesis is strengthened from the finding that D-waves evoked by activation at this site are

influenced by changes in the level of anaesthesia in monkey (Baker, Olivier & Lemon, 1994) as well as in man (Burke *et al.* 1993). In contrast, the initial responses evoked by electrical stimulation with the anode lateral to the vertex would not be influenced by cortical excitability as this stimulation seemed to often activate the corticospinal axons at some distance from the cell soma

Amassian, V. E., Quirk, G. J. & Stewart, M. (1990). A comparison of corticospinal activation by magnetic coil and electrical stimulation of monkey motor cortex. *Electrophysiology and Clinical Neurophysiology* **77**, 390–401.

Baker, S. N., Olivier, E. & Lemon, R. N. (1994). Recording an identified pyramidal volley evoked by transcranial magnetic stimulation in a conscious macaque monkey. *Experimental Brain Research* **99**, 529–532.

Barker, A. T. (1991). An introduction to the basic principles of magnetic nerve stimulation. *Journal of Clinical Neurophysiology* **8**, 26–37

Branston, N. M & Toft, P. S. (1990). Magnetic stimulation of a volume conductor produces a negligble component of induced current perpendicular to the surface. *Journal of Physiology* **423**, 67*P*.

Burke, D., Hicks, R., Gandevia, S. C., Stephen, J., Woodforth, I & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *Journal of Physiology* **470**, 383–393.

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

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

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 human motor cortex: Surface EMG and single motor unit responses. *Journal of Physiology* **412**, 449–473.

Day, B. L., Riescher, H., Struppler, A., Rothwell, J. C. & Marsden, C. D. (1991). Changes in the response to magnetic and electric stimulation of the motor cortex following muscle stretch in man. *Journal of Physiology* **433**, 41–57.

Day, B. L., Rothwell, J. C., Thompson, P. D., Dick, J. P., Cowan, J. M., Berardelli, A. & Marsden, C. D. (1987). Motor cortex stimulation in intact man. 2. Multiple descending volleys. *Brain* **110**, 1191–1209.

Deuschl, G., Michels, R., Berardelli, A., Schenck, E., Inghilleri, M. & Lücking, C. H. (1991). Effects of electric and magnetic transcranial stimulation on long latency reflexes. *Experimental Brain Research* **83**, 403–410.

Edgley, S. A., Eyre, J. A., Lemon, R. N. & Miller, S. (1990). Excitation of the corticospinal tract by electromagntic and electric stimulation of the scalp in the macaque monkey. *Journal of Physiology* **425**, 301–320.

Fournier, E., Meunier, S., Pierrot-Deseilligny, E. & Shindo, M. (1986). Evidence for interneuronally mediated Ia excitatory effects on human quadriceps motoneurones. *Journal of Physiology* **377**, 143–169

Hess, C. W., Mills, K. R. & Murray, N. M. F. (1987). Responses in small hand muscles from magnetic brain stimulation of the human brain. *Journal of Physiology* **388**, 397–419.

Iles, J. F. & Cummings, R. (1992). Electrical and magnetic stimulation of motor cortex in man. *Journal of Physiology* **446**, 223*P*.

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

Kernell, D. & Wu, C.-P. (1967). Responses of the pyramidal tract to stimulation of the baboons motor cortex. *Journal of Physiology* **191**, 653–672.

Landau, W. M., Bishop, G. H. & Clare, M. H. (1965). Site of excitation in stimulation of the motor cortex. *Journal of Physiology* **28**, 1206–1222.

Lemon, R. N., Werner, W., Bennett, K. M. B. & Flament, D. (1993). The proportion of slow and fast pyramidal tract neurones producing post-spike facilitation of hand muscles in the conscious monkey. *Journal of Physiology* **459**, 166*P*.

Maertens De Noordhout, A, Rothwell, J. C., Day, B. L., Dressler, D., Nakashima, K., Thompson, P. D. & Marsden, C. D. (1992) Effect of digital nerve stimuli on responses to electrical or magnetic stimulation of the human brain. *Journal of Physiology* **447**, 535–548

Nielsen, J., Petersen, N., Deuschl, G. & Ballegaard, M. (1993). Task-related changes in the effect of magnetic brain stimulation on spinal neurones in man. *Journal of Physiology* **471**, 223–243.

Patton, H. D. & Amassian, V. E. (1954). Single- and multi-unit analysis of cortical stage of pyramidal tract activation. *Journal of Neurophysiology* **17**, 345–363.

Priori, A., Bertolasi, L., Dressler, D., Rothwell, J. C., Day, B. L., Thompson, P. D. & Marsden, C. D. (1993). Transcranial electric and magnetic stimulation of the leg area of the human motor cortex: single motor units and surface EMG responses in the tibialis anterior muscle. *Electroencephalography and Clinical Neurophysiology* **89**, 131–137.

Saypol, J. M., Roth, B. J., Cohen, L. G. & Hallet, M. (1991). A theoretical comparison of electric and magnetic stimulation of the brain. *Annals of Biomedical Engineering* **19**, 317–328.

Tofts, P. S. (1990). The distribution of induced currents in magnetic stimulation of the nervous system. *Physics, Medicine and Biology* **35**, 1119–1128

Ugawa, Y., Rothwell, J. C., Day, B. D., Thompson, P. D. & Marsden, C. D. (1991). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. *Annals of Neurology* **29**, 418–427.

Acknowledgements

We wish to thank Professor David Burke and Dr John Rothwell for valuable comments on an early version of the manuscript. We are grateful to Professor Hans Hultborn for many inspiring suggestions throughout the study. We would also like to thank Dr Robert Brownstone for scrutinizing the English manuscript. This work was supported by grants from the Danish Medical Research Council, the Danish Multiple Sclerosis Society, the Lundbeck Foundation and the Lauridtz Petersen Foundation.

Received 20 December 1993; accepted 7 October 1994.