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Comparison of activation of corticospinal neurons and spinal motor neurons by magnetic and electrical transcranial stimulation in the lumbosacral cord of the anaesthetized monkey

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Summary

To illuminate the action of non-invasive stimuli on the human cerebral cortex, responses of corticospinal axons and of plantar α-motor neurons following transcranial magnetic (TMS) and electrical stimulation (TES) were recorded in the lumbosacral cord in the anaesthetized macaque monkey. A round coil was used for TMS, and the anode was located at the vertex for TES. The responses of 175 identified corticospinal axons (conduction velocities of 24–95 m/s) were recorded from the lateral corticospinal tract at the T_{12}–L_3 spinal level. A single magnetic or electrical stimulus could evoke an early spike corresponding to the direct (D) wave in surface recorded volleys and was termed a D response. In the same axon, up to four further spikes, termed indirect (I) responses, could also be evoked. At a given intensity of stimulation, D responses had clear thresholds and fixed latencies, whereas I responses were labile in both respects. For TMS and TES, the thresholds of both D and I responses were inversely correlated with axonal conduction velocity. For TMS, fast conducting axons (>75 m/s) had lower thresholds for D responses, while more slowly conducting axons (<55 m/s) had lower thresholds for I responses. Very few of the axons with a conduction velocity of <40 m/s (three out of 23) gave a D response to TMS. For TES, the majority of axons had lower thresholds for D responses or a similar threshold for both D and I responses. At threshold, the latencies of D responses evoked by TMS and TES were consistent with activation within the cortex, while TES also excited some corticospinal axons deep to the cortex. At 2.5 times threshold for the D response, TMS still excited axons mostly within the cortex, but with TES the site of activation shifted by as much as 65 mm below the cortex (mode 20 mm). Intracellular responses were recorded in 23 plantar alpha motor neurons supplying intrinsic muscles of the foot. All showed monosynaptic excitatory post-synaptic potentials (EPSPs) to both TMS and TES with no significant differences in the rise times of the evoked EPSPs. At threshold for a surface corticospinal volley, the average EPSP to TES began 0.5 ms earlier than that to TMS, and 1.0 ms earlier at 2.5 times this threshold. The different sites of activation of corticospinal neurons by TMS and TES, as well as the different distribution of D and I responses that they evoke, may both contribute to the differences in the onset latencies of the EMG responses evoked by these methods in human subjects.

Keywords: magnetic stimulation; motor cortex; corticospinal pathway; motor neuron; monkey

Abbreviations: D = direct (response); EPSP = excitatory post-synaptic potential; I = indirect (response); L = lumbar; PSTH = post-stimulus time histogram; T = thoracic; TES = transcranial electrical stimulation; TMS = transcranial magnetic stimulation

Introduction

The discovery that transcranial electrical stimulation (TES) (Merton and Morton, 1980) and magnetic stimulation (TMS) of the brain (Barker *et al*., 1985) excite the motor cortex and evoke activity in contralateral muscles has led to the widespread use of these methods to study the physiology and pathophysiology of the corticospinal projection in man (Hess *et al*., 1987; Eyre *et al*., 1991; Rothwell *et al*., 1991; Heald *et al*., 1994*a*, *b*). An understanding of how both TES and TMS stimuli activate corticospinal neurons is fundamental to interpretation of such studies.

Direct recording from the surface of the spinal cord in man has shown that both types of stimuli can excite corticospinal neurons at short latencies consistent with direct activation of the neurons in the cortex, and at longer latencies (Boyd *et al*., 1986; Burke *et al*., 1990, 1993; Rothwell *et al*., 1994). These responses are assumed to be similar to direct (D) and indirect (I) responses evoked by surface anodal stimulation of the motor cortex in experimental primates (Patton and Amassian, 1954; Kernell and Wu, 1967; Amassian *et al*., 1987). With suprathreshold stimuli, there is evidence that TES can excite the corticospinal tract at specific sites deep to the cortex related to changes in the course of the axons through the neuraxis (Burke *et al*., 1990, 1993; Maccabee *et al*., 1992; Rothwell *et al*., 1994).

The results of these investigations have been used to interpret differences in EMG and single motor unit responses evoked by TES and TMS. In particular much attention has been focused on the longer latency of response to TMS than to TES in upper limb muscles, and the greater facilitatory effects on responses to TMS of a background voluntary contraction (Hess *et al*., 1987; Day *et al*., 1989). This difference has been explained in two ways. Either TES produces earlier responses because it is more effective than TMS in eliciting D responses (Hess *et al*., 1987; Day *et al*., 1989); the 1–2 ms difference in latency is similar to the D–I wave interval seen on direct activation of the cortex in primates (Kernell and Wu, 1967; Amassian *et al*., 1987). Alternatively, the shorter latency of responses to TES may be due to activation of the tract at sites deep to the cortex. (Burke *et al*., 1990; Edgley *et al*., 1990). Interestingly, the latency difference seen in the lower limbs is subject to the position of the anode electrode for TES: with the anode at the vertex both TES and TMS produce responses with similar latencies (Iles and Cummings, 1992; Priori *et al*., 1993; Nielsen *et al*., 1995), whereas, when placed lateral to the vertex, TES produces responses with shorter onset latencies than TMS (Nielsen *et al*., 1995).

All previous studies have inferred the action of non-invasive stimuli from results using either single motor unit or EMG recordings in conscious human subjects (Hess *et al*., 1987; Day *et al*., 1989; Nielsen *et al*., 1995), or from mass recordings of descending volleys in anaesthetized patients during surgery (Boyd *et al*., 1986; Burke *et al*., 1990, 1993; Rothwell *et al*., 1994) or in macaque monkeys (Edgley *et al*., 1990). These volleys are likely to be dominated by the fastest conducting corticospinal axons and nothing is known about the responses of the vast majority of the tract which is made up of more slowly conducting axons (Porter and Lemon, 1993). In addition, the presence of I discharges cannot be inferred from such recordings because they are non-synchronous, and are subject to cancellation (Amassian *et al*., 1987).

In this study, the responses of individual corticospinal neurons to both forms of stimulation have been examined by recording from their axons in the lateral corticospinal tract of anaesthetized macaque monkeys. In order to obtain a clearer idea of the origin of motor responses, data for a large population of axons have been compiled, and intracellular recording has been used to determine the post-synaptic responses of plantar motor neurons to this population input.

A preliminary report of this work has been published (Edgley *et al*., 1992).

Methods

This study was performed on six adult macaque monkeys (*Macaca fascicularis*) weighing 2.2–8.8 kg. Anaesthesia was induced with ketamine (10 mg/kg, i.m.) and after cannulation of a femoral vein, was maintained by continuous i.v. infusion of alfentanil (150 mg/kg/h) combined with midazolam (1 mg/kg/h). The trachea was cannulated for positive pressure ventilation. An intra-arterial cannula inserted into a femoral artery was used to monitor blood pressure throughout the experiment. Rectal temperature was maintained at 36–37.5°C.

Varnish-insulated tungsten stimulating electrodes (tip impedance 50 kΩ at 1 kHz) were positioned stereotactically 5 mm apart in the left medullary pyramid, at antero-posterior levels of A+2 and P–3. Stimuli (0.2 ms square pulses) of up to 300 μA were delivered at 1 Hz. Correct placement was verified by recording antidromic field potentials from the motor cortex which had low thresholds (20–50 μA; Lemon *et al*., 1986) and subsequently by post-mortem histological analysis (Edgley *et al*., 1990).

A laminectomy was made to expose the upper part of the lumbar enlargement (T_{12}–L_3) and in one experiment was continued caudally to expose the entire lumbosacral enlargement. The dura was opened and the exposed spinal cord was immersed in a pool of oxygenated fluoro-carbon fluid (FC75, from the 3M Co.) maintained at 36–37.5°C. The fluoro-carbon fluid is inert and an insulator, and has oxygen and carbon dioxide carrying capacity even greater than blood. It is superior to mineral oil in protecting the exposed spinal cord and, with a relative density of 1.7, it has the advantage that cerebrospinal fluid and blood float to the surface of the pool.

When surgery was complete the animals were paralysed with Flaxedil (20 mg/kg initial dose and subsequently 20 mg/kg/h) and ventilated. Deep anaesthesia was maintained throughout the experiment and was verified by ensuring that

neither heart rate nor blood pressure, which were monitored continuously, were altered by high intensity percutaneous stimulation of a forelimb peripheral nerve. At regular intervals the eyes were checked for the absence of corneal and pupillary reflexes. Arterial blood samples were taken at hourly intervals for blood-gas analysis.

Two metal ring electrodes (outer diameter 8 mm) were sewn to the scalp at the vertex (anode) and frontally close to the orbital crest (cathode) for delivery of anodal scalp stimuli (50 μs pulses; TES), using a D180 stimulator (Digitimer Ltd, Welwyn Garden City, UK). The intensity was recorded as the percentage of the maximum voltage of the stimulator output. TMS was delivered using a Magstim 200 stimulator (Magstim Co. Ltd, Dyfed, UK) with a maximum output of 2 Tesla. A circular coil (9 cm diameter) was placed tangentially just above the scalp, and it was fixed in an orientation in which corticospinal volleys recorded from the surface of the dorsolateral funiculus could be obtained at the lowest threshold. The edge of the coil was centred over the right motor cortex and the initial current in the coil flowed in a clockwise direction. The stimulus intensity was recorded as power, i.e. the square of the output voltage, and expressed as a percentage of maximum output (Eyre *et al*., 1991). Both the scalp and pyramidal electrodes were disconnected from their respective stimulators during TMS to ensure that no currents were induced in them during magnetic stimulation. Recordings from the surface of the spinal cord were made with silver ball electrodes placed on the right dorsolateral funiculus. Recordings from single axons were obtained with glass microelectrodes filled with 1 M potassium citrate and fed to an Axoprobe 1A amplifier (Axon Instruments Ltd). For both surface and depth recordings, a silver reference electrode was placed on muscles close to the cord. For surface recordings a differential amplifier was used in which the inputs could be muted for a few milliseconds during application of magnetic stimuli (Barker *et al*., 1987). This greatly reduced the size of the stimulus artifacts and allowed recording to be interpreted within 1 ms of the stimulus.

At the end of each experiment, recording electrodes were placed on the left motor cortex and antidromic conduction delays were determined from the electrodes in the pyramid. Conduction delays from the spinal recording locations were determined by replacing the recording glass microelectrodes with varnish insulated tungsten microelectrodes for stimulation.

In one monkey, intracellular recordings were made from α-motor neurons innervating intrinsic foot motor neurons. These were identified antidromically by electrical stimulation of the plantar nerves which were exposed medial to the Achilles tendon at the ankle and mounted in tunnel electrodes for stimulation.

At the end of each experiment the monkey was killed by an overdose of midazolam and alfentanil and perfused with 10% formal saline through the aorta. Conduction distances along the corticospinal pathway were measured between the motor cortex and pyramidal electrodes and between pyramidal electrodes and spinal recording and stimulation sites. The brain and spinal cord were removed for histological analysis.

All signals were recorded on magnetic tape for off-line analysis using a programmable interface (CED 1401) and its suite of analysis programs (Cambridge Electronic Design Ltd, Cambridge, UK). Latencies were measured from stimulus onset to the onset of the rising phase of the action potential in axons, to the first inflection in surface recorded volleys and to the foot of the excitatory post-synaptic potential (EPSP) for intracellular recordings. Where the data were normally distributed parametric statistic are used. The data for the conduction velocities of the axons was not normally distributed and non-parametric statistics were therefore used for analysis of these data.

These experiments were approved by the UK Home Office. The project licence was given under the Animals (Scientific Procedures) Act 1986.

Results

Recordings from single corticospinal axons

Characterization of corticospinal axons

Recordings were obtained from 175 single axons in the dorsolateral funiculus which responded with a single spike to activation of the corticospinal pathway in the contralateral pyramid (Fig. 1A–F). Responses in all these axons followed trains of three stimuli at 250 or 333 Hz (Fig. 1B). The onset latencies of the responses to pyramidal stimulation ranged from 1.74 to 6.56 ms, median 2.40 ms, corresponding to conduction velocities of 24 to 95 m/s, median 60 m/s. For each corticospinal axon, attempts were made to characterize responses to both TMS and TES using a range of stimulus intensities (Fig. 1C–F; note: the intensity of TMS is recorded as power, i.e. the square of the output voltage, and expressed as a percentage of maximum output; *see* Eyre *et al*., 1991).

Thresholds for direct (D) and indirect (I) responses to TMS and TES

A single TMS or TES stimulus could evoke an early spike, the short latency of which indicated that it arose from direct activation of the corticospinal neuron, corresponding to the D wave of Patton and Amassian (1954) and Kernell and Wu (1967). These are termed D responses. In the same axon, they could be followed by up to four further spikes, corresponding to I waves and termed I responses (Fig. 1C–F).

Figure 2 shows the post-stimulus time histograms (PSTH) of illustrative responses to TMS recorded from four axons (parts A and B with fast and C and D with slow conduction velocities). Note the high probability, sharp onset and narrow latency band of the D responses in the fast conduction axons. The D responses were remarkably consistent in onset with a latency jitter of <0.2 ms. There was a low probability of D responses in the slowly conducting axons (Fig. 2C and D)

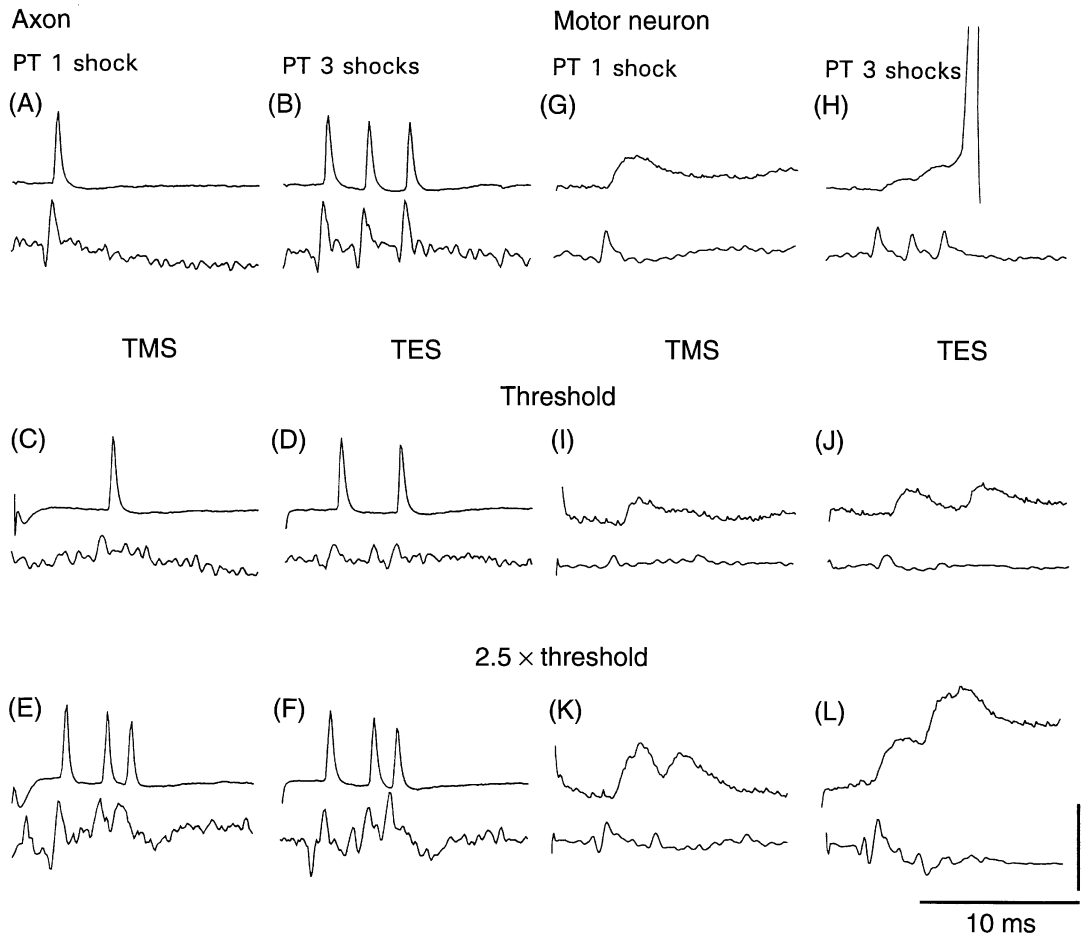

Fig. 1 (**A–F**) Responses of corticospinal axons (upper trace) and surface volleys (lower trace) recorded from the right dorsolateral funiculus at L_{2-3} (single sweeps). (**G–L**) Intracellular recordings from a right plantar α-motor neuron (upper trace) and surface volleys recorded from the right dorsolateral funiculus at L_{2-3} (single sweeps). The onset of stimulation for all traces is at the beginning of the trace. **A**, **B**, **G**, and **H** show responses to pyramidal tract stimulation (0.2 ms, 100 μA). **C** and **I** show responses to TMS, and **D** and **J** responses to TES at the threshold for a surface volley. **E** and **K** show responses to TMS, and **F** and **L** responses to TES at 2.5 times the threshold for a surface volley. The vertical calibration bar is for intracellular responses in **G–L**, and corresponds to 2.0 mV (**G**), 4.0 mV (**H**) and 0.5 mV (**I–L**).

with no D responses in the axon of Fig. 2C and only two D responses in the axon of Fig. 2D. The timing of the each axon's discharge in response to pyramid stimulation is indicated by the arrow. All four axons showed later peaks, presumably I responses. The durations of the peaks of the I responses were longer than those of the D responses, although still within 1 ms. In the whole population of axons studied the mean interval between D and the earliest I response was 2.5 ± 0.1 ms and that between the first and second I response 1.6 ± 0.08 ms (means are given ± SD throughout this paper). This mean interval was not related to conduction velocity.

While D responses generally had a clear and sharp threshold, I responses were more labile. The threshold of the I response was taken as the stimulus intensity required for the appearance of any I response; a given suprathreshold stimulus could evoke one or several I responses in an unpredictable fashion.

TES was significantly more effective in evoking a D response which occurred in 134 of the 135 axons investigated (99%), whereas TMS evoked a D response in 139 of 175 axons (79%) (χ^2, $P < 0.0001$; Fig. 3). There was a significant inverse linear correlation between the threshold for a D response and the conduction velocity of the axon for both forms of stimulation (Fig. 3A and C); for TMS $r = -0.45$, $P < 0.001$; for TES $r = -0.61$, $P < 0.001$. Those axons which did not respond to TMS had significantly slower conduction velocities than those which responded (Fig. 3A). The non-responding axons had a median conduction velocity

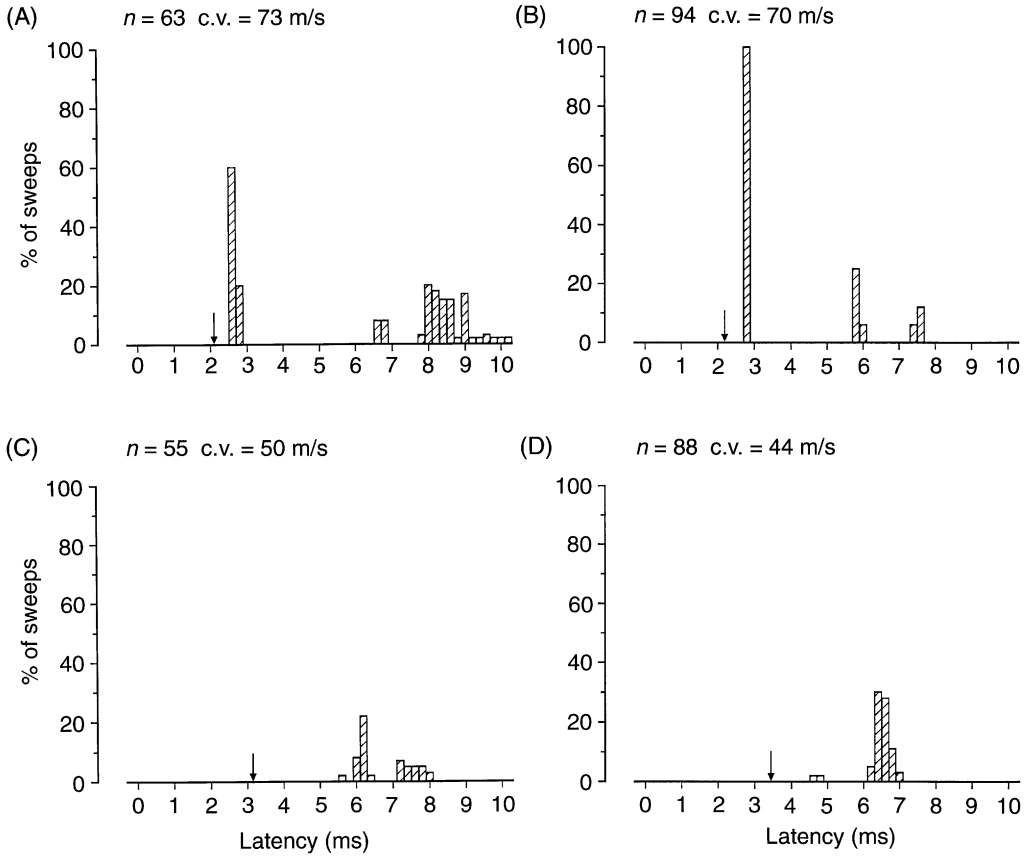

Fig. 2 Frequency distribution of responses to TMS in four axons at different latencies. The conduction velocities (c.v.) of the axons are indicated. Arrow indicates response latency to stimulation of the pyramidal tract.

of 42 m/s and in the responding axons it was 65 mm/s (Mann–Whitney U, $P < 0.00001$). Twenty-three axons with conduction velocities of <40 m/s were tested, and only three of these gave a D response to TMS at maximum stimulator output. All axons recorded with conduction velocities >80 m/s gave a D response.

In contrast to the D responses, there was no significant difference between the two forms of stimulation in their abilities to evoke I responses, (in 117 out of 157 axons after TMS, 74%; in 105 out of 126 axons after TES, 83%; χ^2, $P > 0.05$). However, there was a similar, although less marked, inverse linear correlation between the threshold for evoking I responses and the conduction velocity of the axon (Fig. 3B and D); for TMS $r = -0.19$, $P < 0.05$, and for TES $r = -0.35$, $P < 0.001$. The axons which did not respond had significantly lower conduction velocities. For TMS the median conduction velocity in non-responding axons was 53 m/s and in responding axons it was 62 m/s (Mann–Whitney U, $P < 0.004$). For TES the median conduction velocity in non-responding axons was 46 m/s and in responding axons 62 m/s (Mann–Whitney U, $P < 0.002$). The threshold with both forms of stimulation was defined in 109 axons for the D response and in 90 axons for the I response. Linear correlations were found between the thresholds to each form of stimulation in the same axon (for the D response $r = 0.39$, $P < 0.001$, and for the I response $r = 0.34$, $P < 0.01$).

Relative thresholds for D and I responses

In some axons the threshold for a D response was lower than that for an I response, but in others the converse was observed (*see* Fig. 1C and E). The relative thresholds of these responses were arbitrarily defined by subtracting the threshold of the D response from that of the I response. Thus, if the threshold of the D response was lower than the I response the relative threshold value is positive (D < I), and if it is higher the value is negative (I < D) (Fig. 4A and B).

For TMS, the relative threshold was related to the conduction velocity of the axon (Fig. 4A). All axons of low conduction velocity (<55 m/s) had thresholds for I responses which were equal to or less than those for D responses. The converse was observed for axons with high conduction velocities (>75 m/s), all of which had thresholds for I responses equal to or greater than those for D responses. Axons with conduction velocities 55–75 m/s were equally

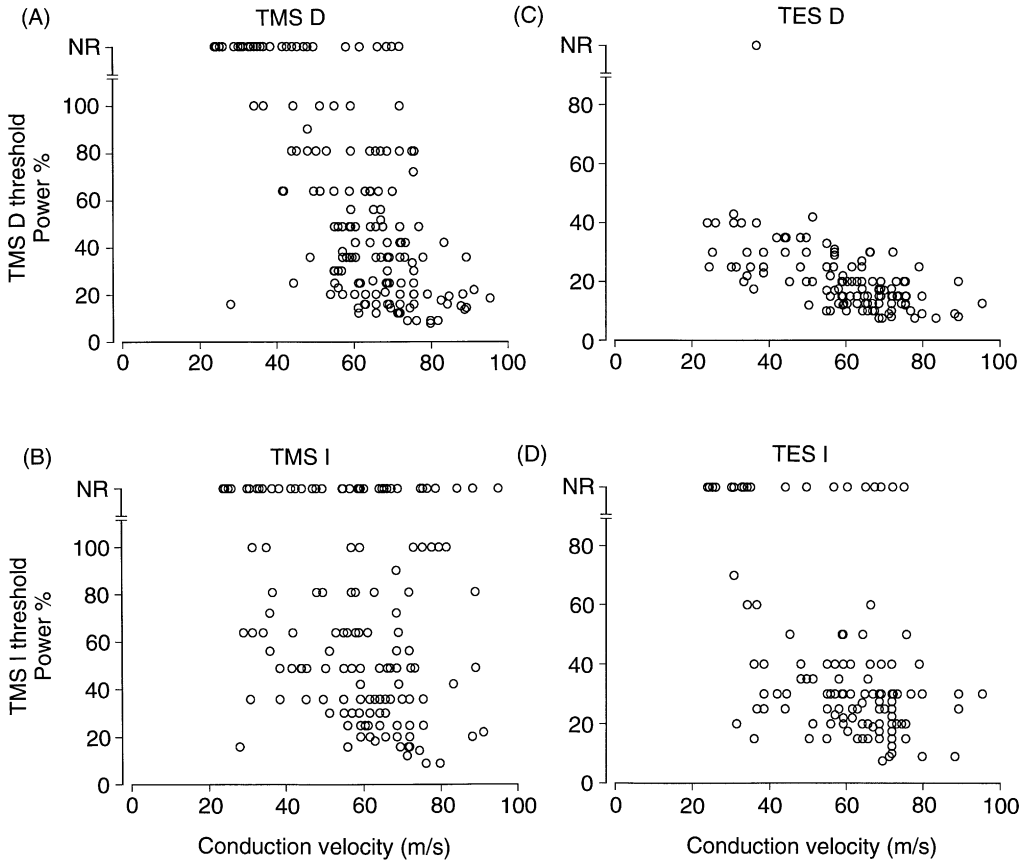

Fig. 3 Thresholds for D and I responses in single corticospinal axons in relation to their conduction velocities. The stimulus intensity for TMS is recorded as power, i.e. the square of the output voltage, and expressed as a percentage of maximum output (Eyre *et al*., 1991). The stimulus intensity for TES is expressed as the percentage of the maximum voltage output of the stimulator. (**A**) The thresholds for a D response to TMS in 179 axons, of which 36 did not respond (NR), even at maximum stimulator output. (**C**) The thresholds for a D response to TES, where only one of 135 axons investigated failed to respond. (**B**) The thresholds for an I response to TMS where 30 of 157 axons had no I response. (**D**) The thresholds for an I response to TES, where 21 of 126 axons had no I response.

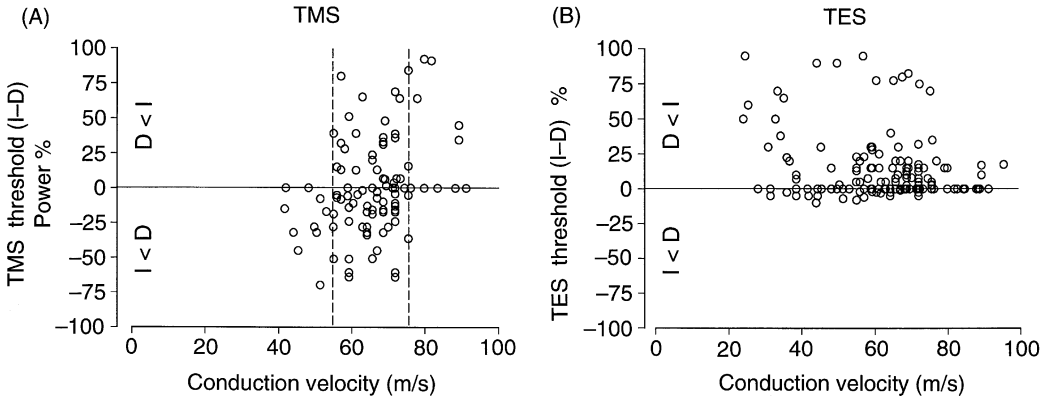

Fig. 4 Relative thresholds for D and I responses in single axons to TMS (**A**) and TES (**B**) plotted against the conduction velocities of the axons. The relative thresholds were arbitrarily defined by subtracting the threshold of the D response from that of the I response. Thus, if the threshold of the D response is lower than that of the I response (D < I), the relative threshold value is positive. If the threshold of the I response is lower than that of the D response (I < D), the value is negative and if the responses have the same threshold the value is zero, as indicated by the horizontal line. In **A** vertical dashed lines are drawn at conduction velocities of 55 m/s and 75 m/s.

distributed between those with a lower threshold for D and for I responses.

For TES, the majority of axons had thresholds for D and I responses which were equal to, or lay within, ±5% of stimulator output of each other. Where the difference was >10%, the threshold for the D response was always the lower (Fig. 4B).

Site of activation of corticospinal neurons at threshold for the D response and with increasing levels of stimulation

It was assumed that the conduction velocity of each axon, calculated from the latency of the action potential in the spinal cord following stimulation of the corticospinal pathway in the medullary pyramid, reflected the overall conduction velocity of the axon from the soma to the recording site in the dorsolateral funiculus. With this assumption, an estimate of the total latency from cortex to recording site could be made for each axon, since the conduction distance along the corticospinal pathway was measured *post mortem*. This conduction distance ranged from 206–221 mm in the different monkeys. The latencies of the D responses to TMS and TES evoked at threshold and at higher stimulus intensities could then be compared with the estimated total latency for each axon. A time interval greater than the estimated total latency would imply a delay in the cortex, likely to be due to the utilization time for activation by TMS or TES. Conversely, a shorter time interval would indicate activation of the axon along the corticospinal pathway below the level of the cortex.

In Fig. 5B and D the estimated total latency has been subtracted from the latency at threshold of the D response to each type of stimulus. A negative value indicates a site of activation of the axon below the level of the cortex. Although at threshold the modal value was zero for both forms of stimulation (Fig. 5B and D), indicating that the most frequent site of activation was within the cortex, TES excited more axons at sites below the cortex (illustrated in the axon of Fig. 5A), as shown by a significantly greater proportion of negative values than for TMS (Student's *t* test, $P < 0.001$). When the stimuli were increased to 2.5 times threshold for the D response both distributions shifted significantly towards more negative values (Fig. 5C and E; for TMS $P < 0.0005$; for TES $P < 0.00001$). For TMS the modal value shifted by –0.1 ms and the 5–95% range extended from +0.2 to –0.4 ms. A much greater negative shift was seen for TES ($P < 0.0001$) with a modal value of –0.5 ms and a 5–95% range from 0 to –1.1 ms. In the axon illustrated in Fig. 5A, shortening of latency was only 0.1 ms for TMS, but it was 0.7 ms for TES. The shortening of latency at high stimulus intensities was not significantly related to conduction velocity (for TMS $r = 0.16$, $P > 0.1$; for TES $r = 0.01$, $P >> 0.1$).

Note that for TMS and TES at both threshold and suprathreshold intensities, all but one of the latencies, which were longer than the estimated D wave latency (i.e. positive values in Fig. 5), were within 0.5 ms of the estimate, confirming that they were D responses.

For axons which had an onset latency equal to, or shorter than, the total estimated latency, the distance below the cortex of the site of activation at threshold and 2.5 times threshold could be estimated, since the conduction velocity was known for each axon (Fig. 6). TMS excited the majority of axons close to the cortex at both stimulus intensities. At 2.5 times threshold, the number of axons excited below the level of the cortex decreased monotonically with distance (Fig. 6B). For TES at both intensities, the majority of axons were excited below the cortex. At 2.5 times threshold there was a preferential site of activation 20–30 mm along the corticospinal pathway (Fig. 6D), which was also apparent at threshold (Fig. 6C).

In Fig. 7 the latencies of D responses of 11 axons, illustrating the range of conduction velocities observed (33–75 m/s), have been plotted against stimulus intensity normalized to the D response threshold for each axon. For TMS there was little change in latency with increasing stimulus intensity. For TES jumps in latency occurred, indicating preferential sites of activation. For the fastest conducting axons, a single jump in latency occurred between 1.4 and 2.0T. In slower conducting axons more than one jump occurred, indicating multiple sites for activation.

Cumulative frequency of action potentials in single axons at increasing levels of stimulation

To assess the population response of the corticospinal system, PSTHs were compiled for 29 axons recorded in one monkey (Fig. 9A–D) and cumulative frequency distributions were computed (Fig. 9E–F: cumulative sums). Each of these axons was tested with both TMS and TES, at threshold for the surface volley in the dorsolateral funiculus and also at 2.5 times this threshold. Since I responses tended to be variable in their occurrence, the most frequent pattern of I activation at each level of stimulation was chosen. The PSTHs and cumulative sums therefore show the arrival of spikes, at the lower thoracic–upper lumbar level, in the whole population of corticospinal axons sampled.

At threshold for the surface volley, more D responses were obtained with TES than TMS. However, under this condition both forms of stimulation evoked approximately the same total number of axon discharges, since TMS evoked a higher proportion of I responses. Note that the arrival of action potentials in fibres activated by TES begins 1 ms earlier than the arrival of those activated by TMS.

At 2.5 times threshold for the surface volley, both forms of stimulation evoked similar numbers of axon discharges within the latency range of the D response (to ~3.5 ms). For TES, however, there was a marked increase in axon discharges at short latencies (2–3 ms). After 5 ms the I responses became established with a greater number of axon discharges occurring to TES than to TMS. Some segmentation of the I

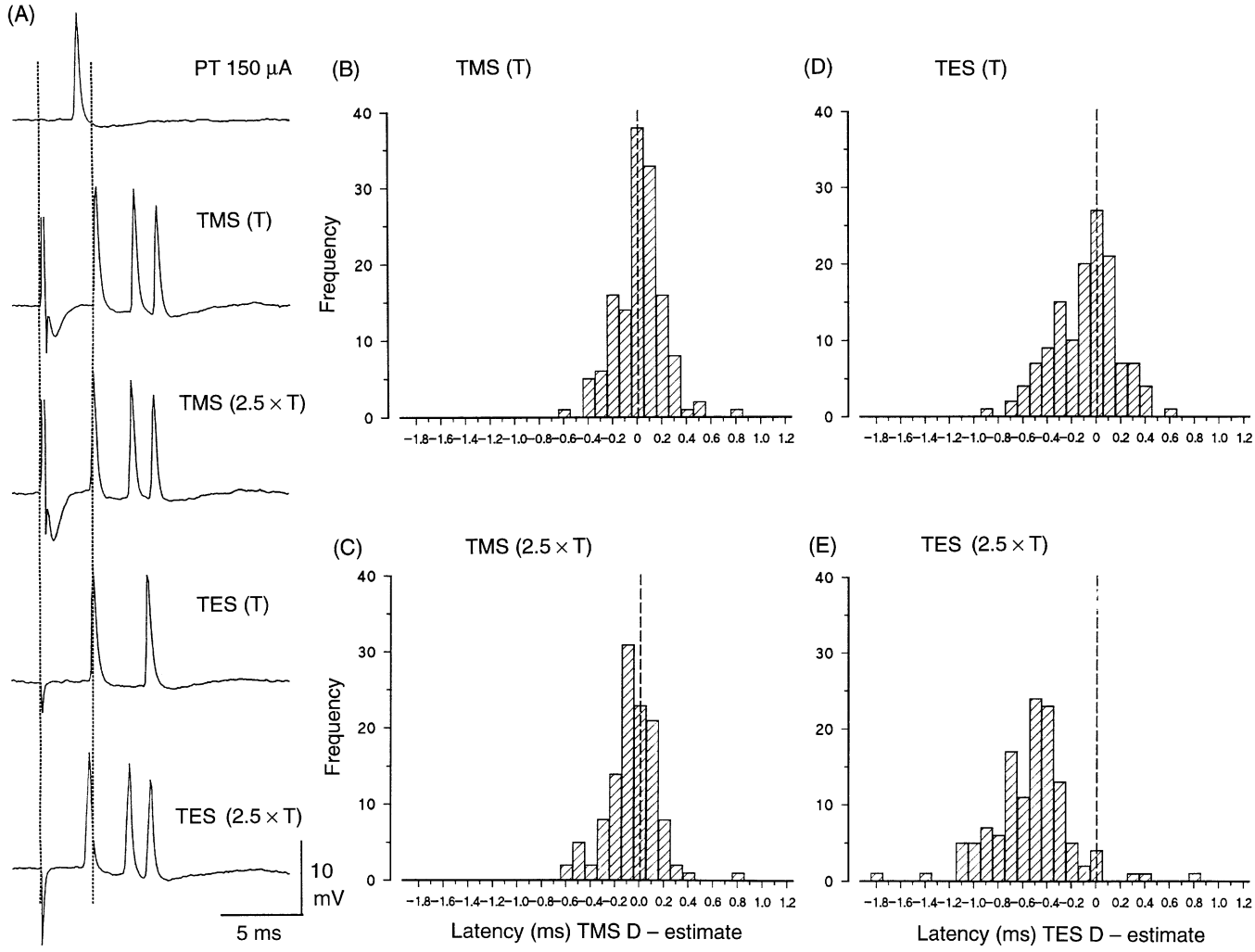

Fig. 5 (**A**) Responses of a single axon to pyramidal stimulation (PT), and to TMS and TES at threshold (T) and at ~2.5 times the threshold (2.5 × T) for a D response. The left vertical dashed line indicates the onset of the stimulus and the right vertical dashed line the estimated total conduction latency for the axon from cortex to recording site in L_{2-3} (*see* text). The D response to both TMS and TES at threshold is close to the estimated total latency. However, the D response to TES at 2.5 × T has a latency 0.7 ms shorter than the estimate, implying an activation site deep to the cortex. The estimated total latency for each axon is subtracted from the latency of the D response to TMS and TES, respectively, at threshold in **B** and **D**, and at 2.5 × T in **C** and **E**. Negative values indicate that the latency of the D response is shorter than the estimated total latency, implying a site of activation in the axon deep to the cortex. Positive values indicate the latency of the D response is longer than the estimated total latency, implying a delay at the cortex. In **B–E** the vertical dashed lines are drawn through zero to indicate those axons with a latency equal to the estimated total conduction latency.

responses, corresponding to the different I waves visible in the surface volley (*see* Fig.1E and F) was apparent.

Intracellular recording in plantar α-motor neurons innervating intrinsic foot muscles

Synaptic effects of TMS and TES

In one monkey, intracellular recordings were obtained from 23 alpha motor neurons, supplying intrinsic foot muscles, identified by antidromic invasion from stimulation of the plantar nerves. All responded with EPSPs to a pyramidal stimulus and to suprathreshold TMS and TES (Fig. 1G–L). The segmental delay of the EPSPs evoked from the pyramid was determined by measuring the latency from the arrival at the spinal segment of the earliest volley in the recording from the dorsolateral funiculus to the onset of the EPSPs (Phillips and Porter, 1964). The mean delay was 1.0 ± 0.16 ms SD; range 0.6–1.2 ms. These values are consistent with monosynaptic excitation from the corticospinal tract. The form of the earliest EPSPs produced by TMS and by TES closely resembled those excited from the pyramid (*see* Figs 1G–L and 8A). Their latencies at threshold were longer than those from the pyramid (for TMS 0.8 ± 0.12 ms; for TES 0.7 ± 0.11 ms), observations which are also consistent with monosynaptic action and can be explained by the longer conduction distance from cortex than from pyramid to the

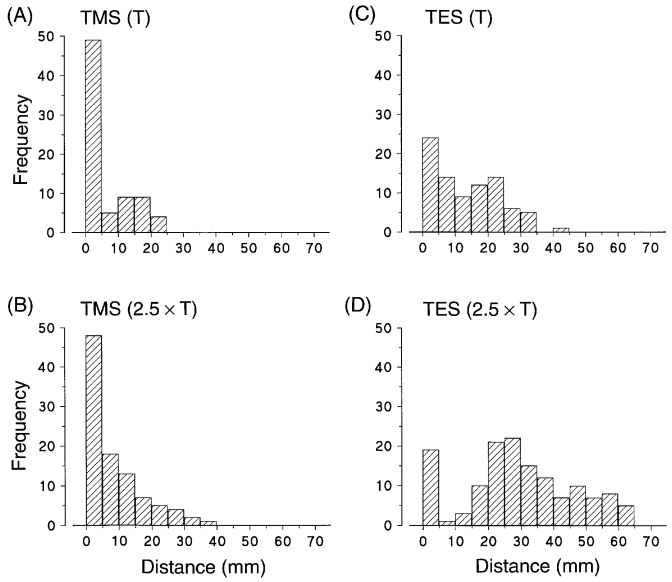

Fig. 6 Site of activation expressed as distance below cortex. For axons which had an onset latency equal to, or shorter, than the total latency, the distance below the cortex of the site of activation was estimated by multiplying the conduction velocity of the axon by the difference between the estimated total and recorded onset latency. **A** and **C**, at threshold for a D response (T); **B** and **D**, at 2.5 times threshold for a D response (2.5 × T).

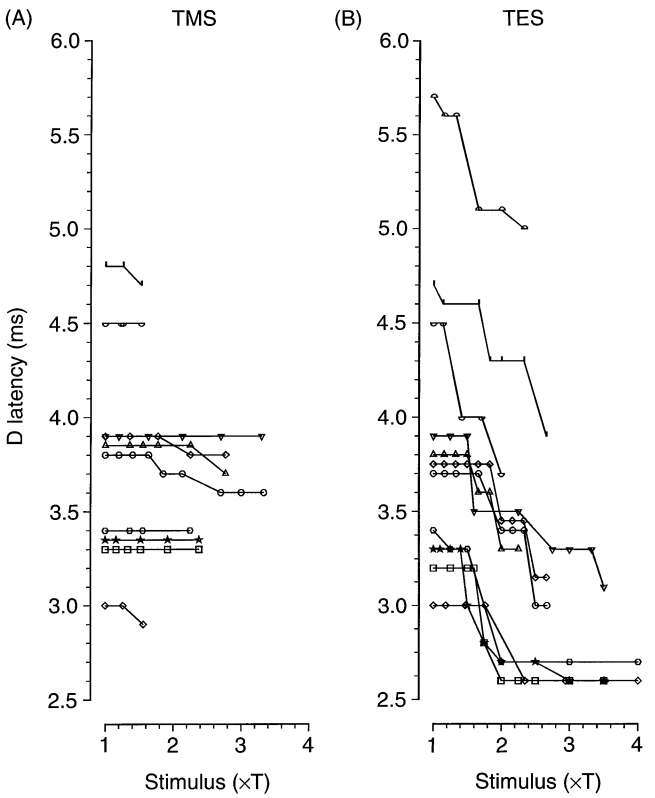

Fig. 7 Latencies of D responses in 11 single axons, conducting over the range 24–95 m/s, as a function of stimulus intensity normalized with respect to the threshold for the D response (T). The most slowly conducting axon (uppermost curve in **B**) showed no response to TMS even at maximum stimulator output.

motor neuron. The rise times of the EPSPs produced by pyramid stimulation (0.9 ± 0.2 ms, $n = 539$), TMS (1.1 ± 0.4 ms, $n = 404$) and TES (1.1 ± 0.4 ms, $n = 328$) were not significantly different. At stronger stimulation intensities both TMS and especially TES evoked later EPSPs (Figs 1K and L and 8A), presumably reflecting I wave activity in the corticospinal tract.

Onset latencies of the EPSPs

The mean onset latencies of EPSPs evoked by TMS and TES both at threshold for the surface volley and at 2.5× this intensity were measured for 19 motor neurons and are plotted in Fig. 8B–D. At threshold, the mean onset latencies of the EPSPs following TES (4.2 ± 0.16 ms; Fig. 8C) were slightly shorter than those following TMS (4.4 ± 0.14 ms; Fig. 8B), and this difference was significant ($P < 0.001$).

The mean latency of EPSPs evoked by TMS at 2.5 times threshold was identical to that at threshold (4.4 ± 0.15 ms; Fig. 8D). In contrast there was significant shortening of the onset latencies of the EPSPs to TES at 2.5 times threshold (3.8 ± 0.1 ms; Fig. 8E), both relative to TMS at this intensity ($P < 0.00001$) and to TES at threshold ($P < 0.0001$).

The differences between the mean onset latencies of EPSPs evoked in each motor neuron at threshold stimulation for the surface volley and those at 2.5 times threshold are shown in Fig. 8F and G, for TMS and TES, respectively. A negative value indicates that the EPSPs at 2.5 times the threshold were shorter than those evoked at threshold. For TMS at 2.5 times threshold, some of the evoked EPSPs had latencies 0.3 ms shorter than at threshold, which closely approximates to the maximum degree of shortening (0.4 ms), observed for similar stimuli in corticospinal axons in the other monkeys of the study (*see* Fig. 5B and C). All 19 motor neurons showed shortening of the onset latencies of the EPSPs evoked by TES at 2.5 times threshold compared with those evoked at threshold (*see* Fig. 8A). The degree of shortening ranged from 0.2 to 0.8 ms, slightly less than the maximum degree of shortening observed in the onset of axonal firing (1.1 ms; *see* Fig. 5D and E).

To provide an estimate of the response of the motor neuron pool to TMS and TES, averages of the EPSPs evoked by these stimuli at the two levels of stimulation were compared (Fig. 9G and H). At threshold for the surface volley, the averaged EPSP to TES began 0.5 ms earlier than that evoked by TMS. At 2.5 times threshold the averaged EPSP to TES began 0.9 ms earlier than that following TMS and 1.1 ms earlier than the onset of the EPSP to TMS at threshold. The EPSPs evoked by TMS and TES at 2.5 times threshold had similar initial peak amplitudes. TES at 2.5 times threshold also produced a later secondary peak in the averaged EPSP which began at ~6.5 ms; this was not observed in the averaged EPSP following TMS at 2.5 times threshold, and

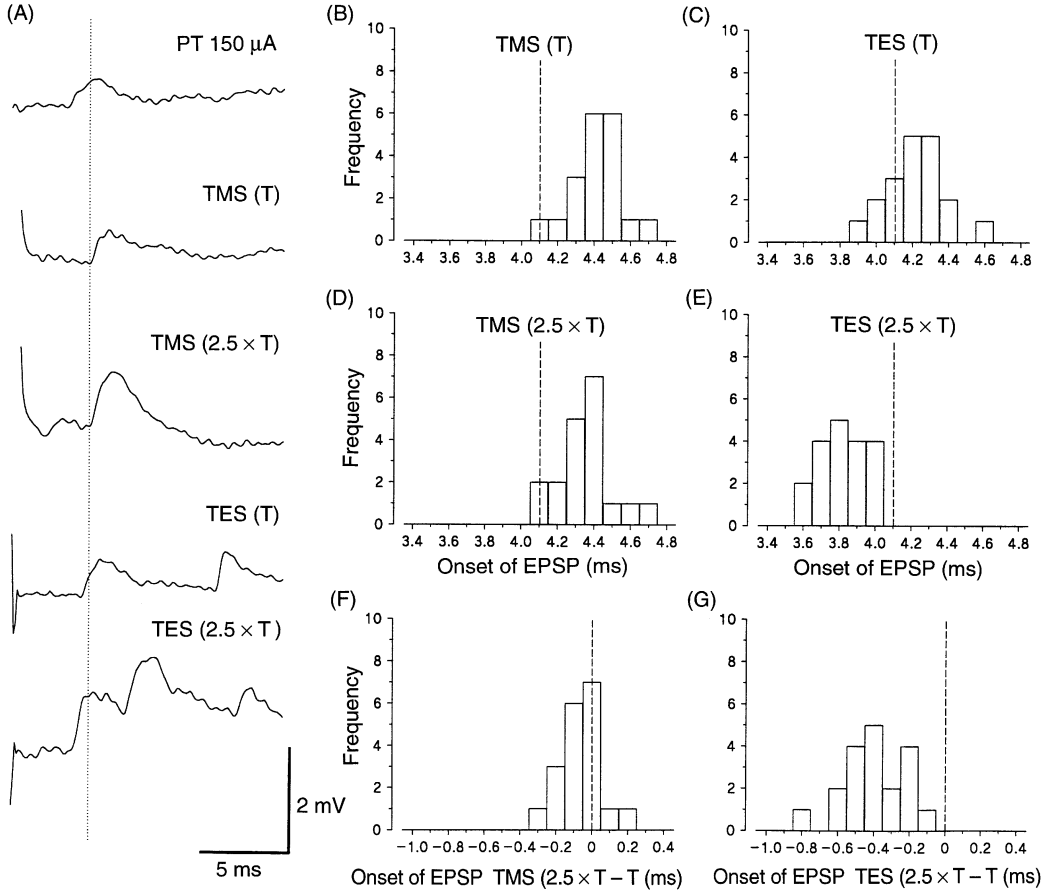

Fig. 8 Onset latencies of EPSPs in 19 motor neurons to TMS and TES at threshold (T) and at 2.5 times threshold (2.5 × T). (**A**) Response of a single plantar alpha motor neuron to pyramidal stimulation (PT) and to TMS and TES at threshold (T) for a surface volley in the right dorsolateral funiculus and at 2.5 times this threshold value. The vertical dotted line indicates the onset of the EPSP following TMS at threshold for a surface volley. The EPSP following TES occurs 0.2 ms earlier than that following TMS at threshold. At 2.5 × T, the onset of the EPSP following TES is 0.7 ms shorter than that observed to TES at threshold and 0.9 ms shorter that the EPSP evoked by TMS at threshold. (**B–E**) Histograms of onset latencies of EPSPs in the group of 19 motor neurons. The vertical dashed line indicates the shortest onset latency for TMS at threshold. (**F** and **G**) Differences in onset latency at 1 times and 2.5 times threshold. Negative values indicate shortening of latencies at 2.5 times threshold.

is likely to reflect a greater number of I waves evoked by TES at higher intensities.

Discussion

The present study of the responses evoked by TMS and TES in single corticospinal axons, and of intracellular recordings of the evoked EPSPs in α-motor neurons, confirms and extends the previous observations of corticospinal volleys recorded from the surface of the dorsolateral funiculus of the spinal cord in the monkey (Edgley *et al.*, 1990).

Activation of corticospinal axons by TMS and TES

The single axons excited by either TMS or TES showed a range of conduction velocities very similar to those discussed by Phillips and Porter (1977). In agreement with previous authors (Hern *et al.*, 1962; Phillips and Porter, 1964; Kernell and Wu, 1967), D and I responses to a single cortical stimulus were recorded from single axons; the interspike intervals between these responses were comparable with those in the baboon (Phillips and Porter, 1964; Kernell and Wu, 1967).

Both forms of stimulation could evoke D and I responses, as shown in our previous study using surface recordings of corticospinal volleys (Edgley *et al.*, 1990). The present study extends these observations by demonstrating that a single axon could respond with both D and I responses to either form of stimulation, as observed by Phillips and Porter (1964) and Kernell and Wu (1967) using surface anodal stimulation of the exposed pia. This provides direct confirmation of repetitive discharge in corticospinal axons to TMS and TES, which has been inferred from many previous studies in man (Day *et al.*, 1987; Burke *et al.*, 1990, 1993; Rothwell *et al.*,

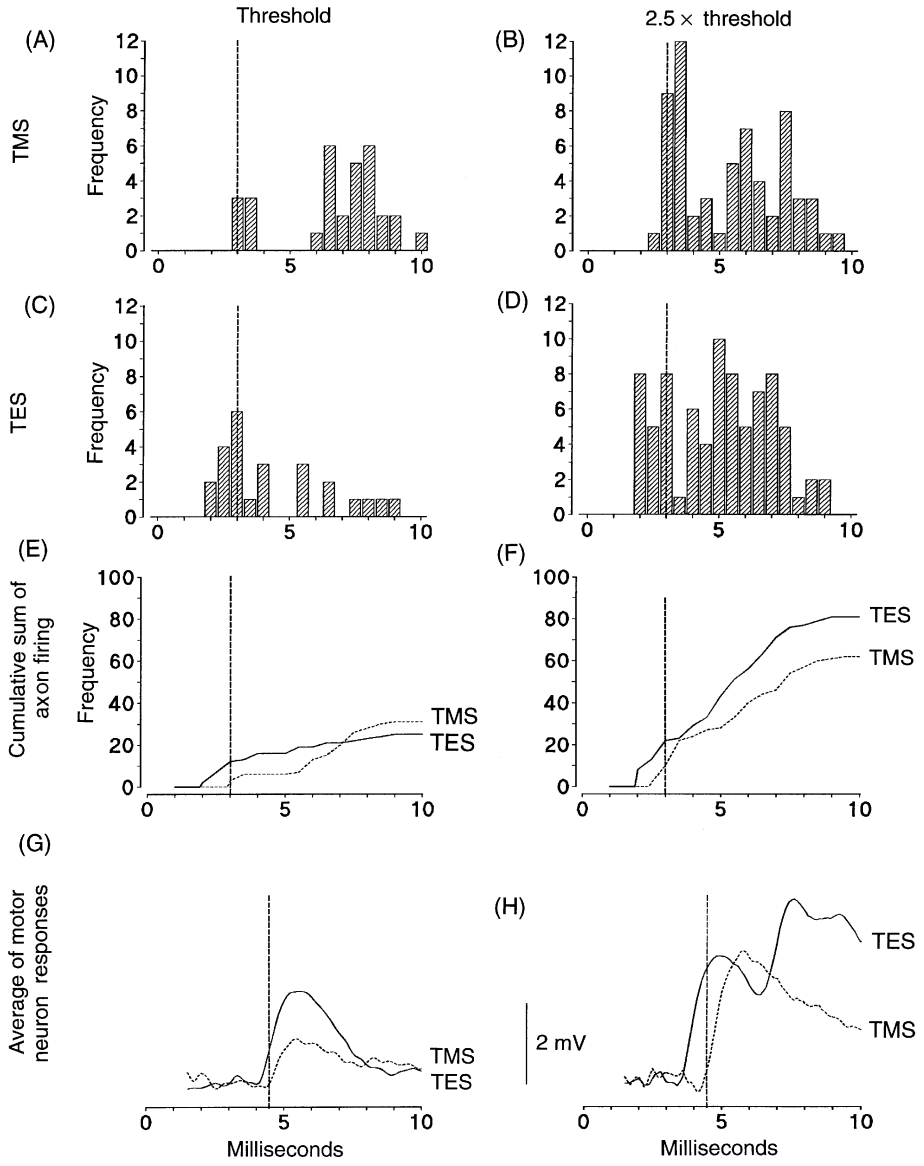

Fig. 9 (**A–F**) Timing of firing in response to TMS and TES for 29 axons recorded at L_{2-3} in one monkey when the stimulus for the surface volley was at threshold and at 2.5 times this threshold. The vertical hashed line indicates the earliest D response to TMS. (**A–D**) PSTHs of axon firing for TMS and TES, respectively, at threshold for the surface volley (**A** and **C**) and at 2.5 times this threshold (**B** and **D**). (**E**) Cumulative sum of axon discharges at threshold for the surface volley for TMS (broken line) and TES (solid line). (**F**) Cumulative sum of axon firing at 2.5 times the threshold for the surface volley for TMS (broken line) and TES (solid line). (**G** and **H**) Averages of the EPSPs recorded in 19 plantar motor neurons in one monkey to TMS (broken line) and TES (solid line) at threshold for the surface volley (**G**) and at 2.5 times the threshold for the surface volley (**H**). The vertical dashed line indicates the onset of the averaged EPSP for TMS at threshold for a surface volley.

1991). The present study also demonstrated the lability of the I responses relative to the D and also shows that the surface volley does not necessarily reflect the population response; both D responses in slower conducting axons and many I responses were not demonstrable in the surface volley.

TES was more effective in evoking both D and I responses at all levels of stimulus intensity, but this must be interpreted with caution, since there are no quantitative values for the charge density transferred by each type of stimulus. The thresholds for both D and I responses were inversely related to the conduction velocity of the axon. The D response has been shown to arise from activation of the corticospinal neuron at the level of the axon hillock, the initial segment or nodes of Ranvier (Hern *et al*., 1962; Landau *et al*., 1965), where the threshold for activation is likely to be inversely related to axon diameter and thus to axon conduction velocity

(Hursh, 1939). Under the conditions of deep anaesthesia of the present experiments it would appear that neurons with slowly conducting axons (<40 m/s) cannot be excited directly by TMS. When the D response originates from the initial segment of the corticospinal neuron, the probability of both D and I responses will depend on the level of excitatory input to the neuron. It can therefore be predicted that D and I responses to both stimuli will increase once the depressive action of anaesthesia is removed. This effect will be more marked for D responses to TMS than to TES, since the majority of D responses to TMS arise close to the cortex at all levels of stimulus intensity. Indeed, a large D wave has been recorded from the pyramid in the conscious monkey following TMS (Edgley *et al*., 1990; Baker *et al*., 1994,1995).

It is still unclear whether I responses reflect intrinsic generators of the corticospinal neuron, or are due to trans-synaptic activation. Whatever the mechanism, given the inverse relationship between their threshold and axon conduction velocity, it is clearly more susceptible to both TMS and TES in neurons with fast-conducting axons.

EPSPs evoked in plantar motor neurons by TMS and TES

The synaptic delays and the rise times of the EPSPs in plantar α-motor neurons, evoked from the pyramid, and by TMS and TES closely resemble those reported previously in hindlimb motor neurons of the monkey following cortical stimulation and suggest that they are mediated by a predominantly monosynaptic corticospinal projection (Porter and Hore, 1969; Jankowska *et al*., 1975).

Corticospinal activation and the evoked EPSPs at threshold

Using near-threshold values of stimulation, Hess *et al*. (1986, 1987) were the first to describe the 2–3 ms longer latency of motor evoked potentials following TMS compared with TES in relaxed muscle. Together with other authors (Rothwell *et al*., 1987) they also noted the marked shortening of latency of TMS evoked responses during voluntary contraction. The differential patterns and sites of activation of corticospinal axons at threshold by TMS and TES, described in the present paper, provide a potential explanation of these observations.

First, it is clear that, even at threshold, TES excites more axons below the level of the cortex than TMS (Figs 5B and D and 6A and C), thus the volley at the spinal cord evoked by TES will arrive earlier; this can be observed in the histograms of axons responses, in the onsets of the cumulative frequency curves of axon firing and in the averaged EPSPs at threshold (Fig. 9A, C, E and G). This observation is consistent with earlier findings in man of Burke *et al*., (1990, 1993) and with those of Nielsen *et al*. (1995), who showed that the site of activation by TES depended upon the precise location of the anode: when it was just lateral to vertex, it was possible, at threshold, to evoke EMG responses in lower limb muscles that were 1–2 ms briefer than those obtained with TMS (cf. Hess *et al*., 1987 for the upper limb), suggesting deep activation of the corticospinal axons (*see* Fig. 6C). In contrast, responses evoked from a vertex anode had, at threshold, a latency identical to those evoked by TMS. They were probably due to direct activation of corticospinal neurons within the cortex (cf. Iles and Cummings, 1992; Priori *et al*., 1993). This latter finding could reflect the fact that some axons were activated by TES at threshold within the cortex (i.e. those axons lying on the far left of the distribution plotted in Fig. 6C). Because of the complexity of the cortical architecture, it is not surprising that the site of activation of a particular corticospinal neuron is sensitive to the direction of induced currents.

A second factor influencing the latency of EMG responses at threshold is that, at threshold for a response in a corticospinal axon, TES usually evoked a D response in the majority of axons irrespective of the conduction velocity and the D response was usually accompanied by I responses (Figs 1 and 4B). In contrast, with TMS a D response was only consistently observed in axons conducting at >75 m/s, whereas I responses alone were progressively more frequent in axons conducting at <75 m/s (Fig. 4A). This differential pattern of activation is reflected in the EPSPs evoked by both forms of stimulation at threshold. Thus, for TES at threshold for a surface volley the averaged EPSP of the 19 alpha motor neurons (Fig. 9G) shows a somewhat faster rate of rise and achieves twice the initial amplitude than the averaged EPSP following TMS. This difference may reflect the greater efficacy with which TES generates the D responses in corticospinal neurons which give rise to the earliest component of the EPSP. For TMS, at threshold for a surface volley, the lower initial amplitude of the EPSP could lead to a longer latency of evoked EMG responses in relaxed muscle. Indeed, the number of axons activated directly by TMS may be insufficient to bring motor neurons in relaxed muscles to discharge at a D wave latency and are likely to require the temporal summation of the succeeding I wave activation. The axon discharges evoked by TES at threshold are predominantly D responses and are likely to bring motor neurons of relaxed muscles to discharge at short D-wave latencies. Thus, on the basis of the findings at threshold in relaxed muscles, the difference in onset of the motor evoked potentials in relaxed muscles following TES and TMS (2–3 ms; Hess *et al*., 1986, 1987) could be predicted to be similar to the difference in the latency of D and I responses found in this study (2.5 ms).

The differences in the latencies of the responses evoked by TMS and TES at threshold will be magnified because the axons excited by TES will show more temporal summation (Phillips and Porter, 1964) because both D and I responses occur in the majority of axons at threshold (Fig. 4B). In contrast, axons activated by TMS respond at threshold with either a D response alone in the faster conducting axons or an I response alone in the more slowly conducting axons

(*see* Fig. 4A). These predictions were confirmed by the motor neuron recordings, where secondary EPSPs were more commonly seen with TES than with TMS.

The disproportionately greater shortening of the latency of evoked motor responses by voluntary contraction with TMS compared with TES (Hess *et al*., 1986, 1987; Rothwell *et al*., 1987; Day *et al*., 1989) probably reflects the fact that, with voluntary activation, the earliest discharge of the more excitable motor neuron pool corresponds to the arrival of the D component of the TMS evoked volley, rather than to later arriving I components.

Corticospinal activation and the evoked EPSPs at suprathreshold intensities

In previous studies using surface recording from the spinal cord in man and in monkey, TES has been observed to excite the corticospinal pathway at some distance below the cortex, with a preferential site of activation in man at the cerebral peduncle (Burke *et al*., 1990, 1993; Rothwell *et al*., 1994; Nielsen *et al*., 1995) and in the monkey at sites as far distant as the medullary pyramid (Edgley *et al*., 1990). In all these studies a stepwise decrease in latency was observed with increasing stimulus intensity. The present study of single corticospinal axons has confirmed these findings and has added three further features. (i) Although some axons are activated within the cortex and others as far as 65 mm deep, the distribution is not unimodal nor related to the conduction velocity of the axon (Fig. 6C and D). There is a stepwise shift of the site of activation to a modal value 20–30 mm below the cortex, corresponding in the macaque to the level of the cerebral peduncle. Thus in the monkey, as has previously been observed in man (Burke *et al*., 1990, 1993), this region of the corticospinal pathway represents a preferential site for suprathreshold axonal activation following TES. (This may also be the case at threshold, though the effect is less marked; *see* Fig. 6C.) In the cerebral peduncle corticospinal fibres turn more dorsally as they enter the brainstem and are likely to cut the electrical field vectors in a different orientation (Maccabee *et al*., 1992). (ii) It was possible in the present study to compare the orthodromic latency for both TMS and TES with the latency of the response following stimulation of the medullary pyramid. In 15 axons the latency of the response to TES at suprathreshold intensities was less than that from the cathodal electrode in the pyramid, indicating sites of stimulation up to 14.5 mm below the level of the medullary pyramid. (iii) With suprathreshold intensity TMS also activates a small proportion of axons deep to the cortex as far as 40 mm (Fig. 6B), although in contrast to TES the probability falls off monotonically with distance.

The deeper activation of corticospinal axons following suprathreshold TES caused an earlier onset in axon firing (Fig. 9F), 0.5 ms before TMS discharges began, and the averaged EPSP evoked by TES began 1 ms earlier than that from TMS. At suprathreshold stimulus intensities TES and TMS were equally effective in evoking D responses.

Segmentation of axonal and motor neuronal responses

The cumulative histograms of axon discharges did show segmentation of the axonal population response to both TES and TMS (*see* Fig. 9A–D). This was evident despite the wide variety of conduction velocities within the sample. The actions of the population D and successive I discharges were presumably reflected at the motor neuron by the succession of EPSPs that could be recorded (*see* Figs 1J, K and L, 8A and 9H). The second EPSP usually followed the first (D) EPSP by ~2 ms, and was particularly marked with TES. This second depolarization may be responsible for the secondary peak observed in the response of single motor units to TMS and TES in man (Day *et al*., 1989; Boniface *et al*., 1991; Olivier *et al*., 1995). Given the relatively broad distribution of corticospinal action potential arrival times (Fig. 9A and C), it is perhaps surprising that both the EPSPs and PSTHs of individual motor units are so well segmented. This suggests that other mechanisms may be involved, including postsynaptic inhibition and intrinsic motor neuron properties, and, possibly, specific projections of corticospinal fibres to motor neurons which are based upon axonal conduction velocity.

Conclusion

The results presented here show that non-invasive stimulation activates corticospinal axons projecting to the lumbosacral segments in a complex fashion that is influenced by cell body size, conduction velocity and axon trajectory, as well as by the location of the neurons and their level of excitability. This makes it difficult, in man, to be certain whether EMG responses are brought about as a result of direct or indirect activation of the corticospinal system. However, if the mode of activation of corticospinal neurons in man follows that described in the present study, the differential timing of motor evoked potentials to TMS and TES may be explained both by deep subcortical activation by TES of the corticospinal tract (Edgley *et al*., 1990; Burke *et al*., 1990, 1993) and by the observation that, at threshold, TES evokes mainly D responses while TMS evokes mainly I responses (Hess *et al*., 1987; Day *et al*., 1989).

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