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THE MODE OF ACTIVATION OF PYRAMIDAL TRACT CELLS BY INTRACORTICAL STIMULI

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SUMMARY

1. Direct and indirect effects of intracortical stimulation on pyramidal tract cells were compared in the monkey and in the cat under barbiturate or chloralose anaesthesia. The hind-limb motor areas were explored, that in the monkey only within the convex part of the precentral gyrus. The intracortical stimuli were applied in the nearest vicinity of pyramidal tract cells, where antidromic spike potentials of single cells were recorded.

2. Averaged records of descending volleys in corticospinal tract fibres were taken from the surface of the lateral funiculus or from its dissected fascicles. The sensitivity of the recording was sufficient to detect responses in single fibres.

3. The latencies of the earliest descending volleys evoked by weak intracortical stimuli were compared with the latencies of the antidromic spike potentials of pyramidal tract cells evoked by stimulation of the lateral funiculus at a low lumbar level (same conduction distance). Only in about one third of cases these latencies were similar and compatible with a direct activation of pyramidal tract cells. In the remaining cases they indicated mono- or polysynaptic activation of pyramidal tract cells.

4. Latencies of the later components of the descending volleys indicated that they were due to indirect activation of pyramidal tract cells in practically all cases.

5. The components of the descending volleys attributable to the indirect activation of pyramidal tract cells were greatly increased when repetitive intracortical stimuli were applied instead of single ones.

6. The investigation leads to the conclusion that a weak intracortical

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stimulation is relatively ineffective in a direct excitation of pyramidal tract cells and that the effects of such a stimulation are mainly indirect, especially when repetitive stimuli are used.

INTRODUCTION

In studies requiring electrical stimulation of cells of origin of the pyramidal tract two methods of activation have been used: by stimuli applied to the surface of the cortex and by intracortical stimuli. The surface stimulation has the advantage of being followed by a direct activation of pyramidal tract cells when its strength is within the ranges for evoking *D*-wave of the descending pyramidal tract volley (Patton & Amassian, 1954; Phillips, 1956*a*; Hern, Landgren, Phillips & Porter, 1962; Kernell & Wu, 1967). Its disadvantage is that the thresholds for activation of pyramidal tract cells are then relatively high, due to a large distance between these cells and the stimulating electrode, and even weakest stimuli are likely to excite a functionally mixed conglomerate of cells within a considerable volume of the cortex (Phillips, 1956*b*). The technique of intracortical stimulation introduced by Landau, Bishop & Clare (1965) and Asanuma & Sakata (1967) seemed, therefore, to offer a much better opportunity to activate selectively small groups of functionally homogeneous pyramidal tract cells because the thresholds are in its case more than 100 times lower than for the surface stimulation (Stoney, Thompson & Asanuma, 1968) and the character of the responses is much more focal (Asanuma & Sakata, 1967; Asanuma & Rosén, 1972). On the basis of a number of arguments the effects of the intracortical stimulation were attributed mainly to the direct activation of pyramidal tract cells when the stimuli were applied in their proximity (Asanuma, Stoney & Abzug, 1968; Asanuma, 1973; Andersen, Hagan, Phillips & Powell, 1975), although the possibility of a concomitant synaptic activation of these cells was a well-established fact (Stoney *et al.* 1968; Asanuma & Rosén, 1973). The occurrence of a synaptic activation of pyramidal tract cells following the intracortical stimulation may, however, greatly complicate the interpretation of the results of such a stimulation. The effects of activation of pyramidal tract cells via other cells or fibres which make synaptic contacts with them would depend on a sample of the excited cells or fibres, on the radius of their projection (which may be of the order of 1–2 mm (Asanuma & Rosén, 1973)) or more (for references see Jankowska, Padel & Tanaka, 1975) and the population of pyramidal tract cells on which they terminated. It seemed thus desirable to verify the extent to which direct and indirect activation of pyramidal tract cells might contribute to the results of the intracortical stimulation, before undertaking any further study of the

motor cortex with this technique. The problem has been taken up in the present experiments which revealed a very considerable indirect activation of pyramidal tract cells by intracortical stimuli. The consequences of this finding will be further discussed in the following paper on post-synaptic effects produced in spinal motoneurones following stimulation of the motor cortex of the monkey (Jankowska, Padel & Tanaka, 1975).

METHODS

The experiments were performed on twelve monkeys (*Macaca irus*) and five cats, lightly anaesthetized with chloralose (40–70 mg/kg) and/or Nembutal (3·5 mg/kg every 1–3 hr when supplementing chloralose; 30–40 mg/kg during the dissection and 3–5 mg/kg every 1–3 hr when used without chloralose).

Stimulation. The stimulation of the motor cortex was done either with spring-mounted silver ball electrodes lightly touching its surface (positive pulses 0·2–1·5 mA) or with glass micro-electrodes, filled with NaCl solution (tip 2·0–2·5 μm, resistance 0·8–1·0 MΩ), positioned near to pyramidal tract cells and first used for recording from the cortex. The micro-electrodes were inserted through pia-arachnoid under the visual control through a dissecting microscope. In the monkey the explored cortical area was confined to the convexity of the precentral gyrus and the electrode penetrations were approximately at right angles to its surface (see Jankowska *et al.* 1975). The positioning of the tip of the electrode was guided by records of antidromic spike potentials following stimulation of the lateral surface of the spinal cord. The pyramidal tract cells selected for stimulation were those with the latencies corresponding to the highest (50–70 m/sec) conduction velocities of the corticospinal tract fibres. Rectangular negative pulses of 0·2 or 0·5 msec duration with amplitudes varying between 2 and 50 μA were used for intracortical stimulation. The stimulus strength was monitored as a voltage drop across a 100Ω resistor, which was placed in the return path to ground, and recorded in parallel with other records. In view of the long stimulation-recording distances no particular precautions were taken to reduce the shock artifacts. A closed chamber (Andersson & Källström, 1971) filled with paraffin oil was attached to the skull over the exposed cortical area to prevent brain pulsations and to provide stable conditions of stimulation. The corticospinal tract fibres within the lateral funiculus were stimulated with two silver ball electrodes lightly touching the surface of the spinal cord in a paraffin oil pool.

Recording. The records of the descending pyramidal tract volleys were taken from the surface of the lateral funiculus or from the fascicles of its dorsal part dissected as described by Laporte, Lundberg & Oscarsson (1956), at a low thoracic (Th 11–Th 13) or a cervical (C3–C5) level. The records were averaged in a digital averaging computer (Hewlett-Packard, type 5480A) with time resolution of 10, 20 or 40 μsec/address.

The sensitivity of recording from the fascicles was tested in separate experiments. Their main aim was to find out if action potentials in single fibres can be detected under our routine recording conditions since weak intracortical stimulation might activate only a very limited number, or single, pyramidal tract cells. Single fibres in the lateral funiculus or in the dorsal columns were stimulated through micro-electrodes inserted into them. The fibres selected for this purpose were those having conduction velocities over 60 m/sec, i.e. of the same order as the fastest corticospinal tract fibres (cf. Kernell & Wu, 1967) and running at 0·5–1·5 mm depth, which corresponds to the location of the corticospinal tract in the lateral funiculus. The fibres were impaled at L 3– L 4 level and belonged to long descending or ascending spinal tracts, as evidenced by their activation by shocks applied at Th 10 or C 2. For

intra-axonal stimulation short rectangular positive pulses were applied through the micro-electrode. The resulting potentials were recorded simultaneously with the same micro-electrode and with the electrode that lay in contact with the lateral funiculus. The latter touched the cord close to the site from which the fibres were originally excited.

Fig. 1 shows records from two fibres that ran in the dorsal column close to its surface (*A–C*) and in the dorsal part of the lateral funiculus (*D–F*). Potentials of

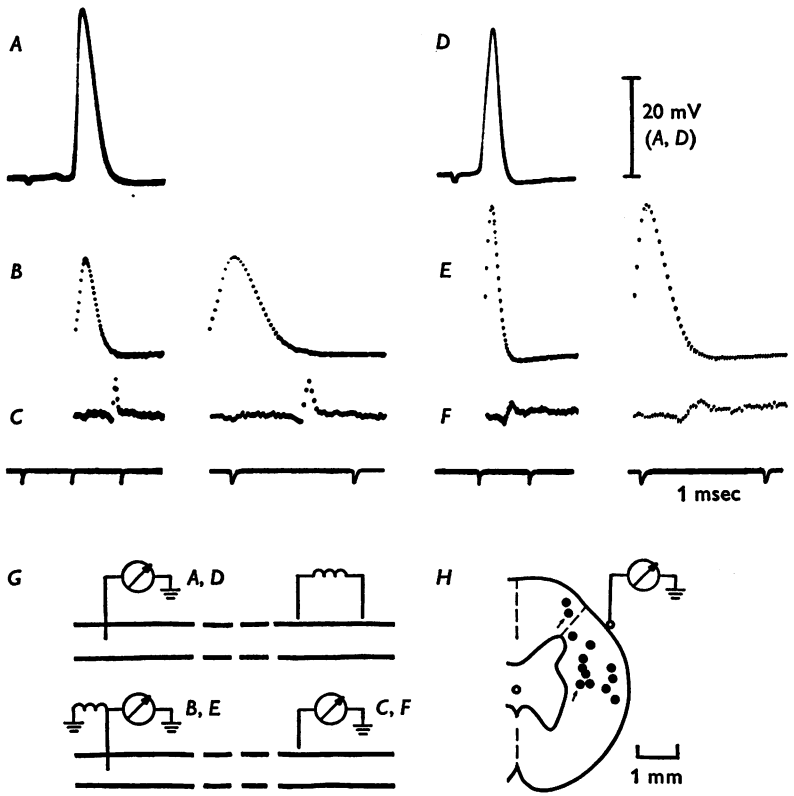

Fig. 1. Recording of action potentials in two single fibres. *A*, *D* and *B*, *E*, intra-axonal records of potentials set up by stimulation of the lateral funiculus 60 mm away from the recording point (superimposed traces) and by pulses passed through the recording micro-electrode (averaged records) respectively. *C* and *F*, averaged records from the surface of the lateral funiculus taken simultaneously with records in *B* and *E*. Right-hand traces in *B*, *C*, *E* and *F* show the same records as the left-hand traces with an expanded time scale. The averager was triggered by the spikes in *B* and *E* (128–256 averaged sweeps; 20 μsec/address). The two fibres run in the dorsal and in the lateral funiculus respectively, their position being indicated by arrows in the diagram *H*, which shows (filled circles) the location of the whole sample of analysed fibres as well. In this and the following figures the negativity is downwards in micro-electrode recording and upwards in surface recording (except in Figs. 7 and 8).

both these fibres were clearly detectable from the surface (*C* and *F*) as triphasic responses. The records are representative in that they show a lower amplitude, a longer duration and more pronounced positive phases of potentials in fibres that are more deeply located. Distinct records of unitary spikes were obtained from all tested fibres which were running 0·5–1·7 mm from the recording electrode (Fig. 1*G*).

RESULTS

A. *A comparison of the latencies of the antidromic activation of pyramidal tract cells and of the potentials set up in their axons by cortical stimulation.*

A direct excitation of a pyramidal tract cell would necessitate that the latencies of the potentials evoked in its axon by stimulation of the soma and in its soma by stimulation of the axon be identical, provided that the distances between the electrodes were the same and the conditions of the stimulation comparable. Longer latencies of the descending volleys would indicate that the pyramid tract cells were activated synaptically; i.e. that the stimuli applied to the cortex did not excite the pyramidal cells themselves but did excite them via some other cells of fibres. Depending on whether these other cells or fibres made synaptic contact with the pyramidal cells or whether they influenced them via shorter or longer neuronal chains, one or more synaptic delays in addition to the intracortical conduction times would delay the appearance of the descending volleys in the corticospinal tract fibres.

Depending on different conditions of stimulation a spread of current may result in shortening of the latencies of the recorded responses to different degrees. The latencies of the responses evoked by stimulation of the cortex and of the spinal cord as a function of stimulus strength were therefore analysed in a separate series of control experiments.

The latencies of the descending volleys evoked by surface stimulation of the cortex (*D*-waves, Patton & Amassian, 1954) were found to be practically constant within the 0·2–1·0 mA range of stimulus strengths that were used, as illustrated in Fig. 3 with records from the surface of the lateral funiculus in a monkey (*C–F*) and from a dissected fascicle in a cat (*H–J*). The latencies were only occasionally longer at the threshold (by 0·05 and 0·1 msec respectively in two of eight experiments). The constancy of the latencies of the *D*-waves was reported also by Landau *et al.* (1965, cf. their fig. 7*B*) for monopolar stimulation with positive pulses, within a range from about threshold to five times threshold.

The dependence of the latencies of the antidromic invasion of pyramidal tract cells on varying strength of stimulation of their axons within the lateral funiculus is illustrated in Fig. 2*B* and *C*. It appeared that these latencies decreased by up to 0·2 ± 0·09 msec (mean ± S.D.) when the strength of stimulation was increased from near its threshold value to the two to

five times threshold strength which was most often used. The histogram in *D* summarizes the results from thirty-eight pyramidal tract cells pooled from the experiments in three monkeys and in three cats.

In view of these results the latencies of the antidromic responses shorter by 0·3 msec or less than the latencies of the descending responses will be considered as possibly due to the spread of current along the stimulated pyramidal tract fibres within the lateral funiculus.

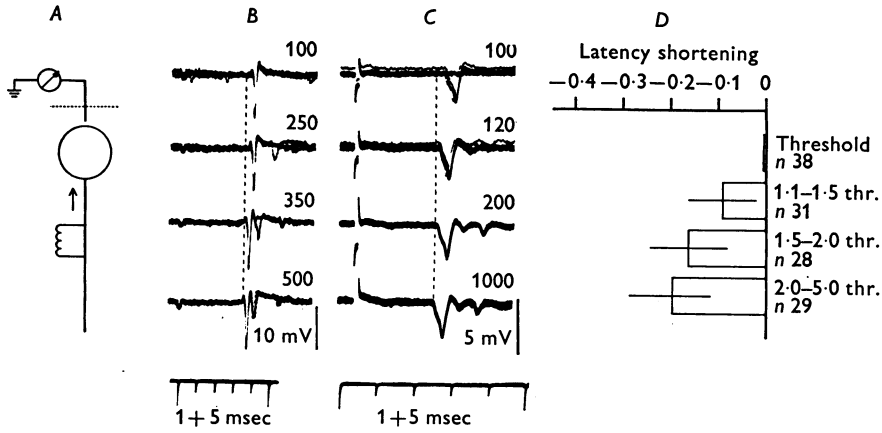

Fig. 2. Relation between the strength of stimulation of the lateral funiculus and the latency of antidromic activation of pyramidal tract cells. *A*, diagram of experimental arrangement. Large open circle represents the pyramidal tract cell and the dashed line the surface of the cortex. Stimulation and recording sites as well as the direction of the propagation of impulses are indicated. *B* and *C*, extracellular records of antidromic spike potentials of pyramidal tract cells, in a monkey and in a cat respectively. The corresponding stimulus strengths in μA are indicated above the records, the topmost being at a threshold for activating the cells. Vertical lines indicate latencies for the strongest stimuli. Stimulation was at L 1–2 segmental level for *B* and at C 4–5 level for *C* (note faster sweep speed). Diagram in *D* gives means and S.D. for the reductions in latencies at threshold for responses evoked at a strength 1·1–1·5, 1·5–2·0 and 2·0–5·0 times threshold.

1. *Latencies of antidromic activation of pyramidal tract cells and of descending volleys evoked by surface stimulation*

In Fig. 3 are examples of descending volleys evoked by surface stimulation of the motor cortex (*C–F* and *H–J*) and of antidromic spike potentials of two pyramidal tract cells (*B*, *G*). The latter were evoked by stimuli applied through the same electrodes that were used to record the descending volleys or through another electrode at a distance of 2–5 mm. The latencies of the two illustrated antidromic spikes were 0·25 and 1·7 msec longer than the latencies of the descending volleys. The distribution of the

differences between the latencies of the antidromic responses of the whole sample of analysed pyramidal tract cells and the onset of the respective descending volleys is shown in *K*. Greatly enlarged (to the same scale), a typical descending volley has been superimposed to allow a comparison of the distribution of these latencies with its time course. There is a very good correspondence between them, although in individual preparations a considerable range of the latencies of both ortho- and antidromic responses may occur. The responses were usually more synchronous in the monkey than in the cat (cf. descending volleys evoked by weak stimuli in *C* and *H*).

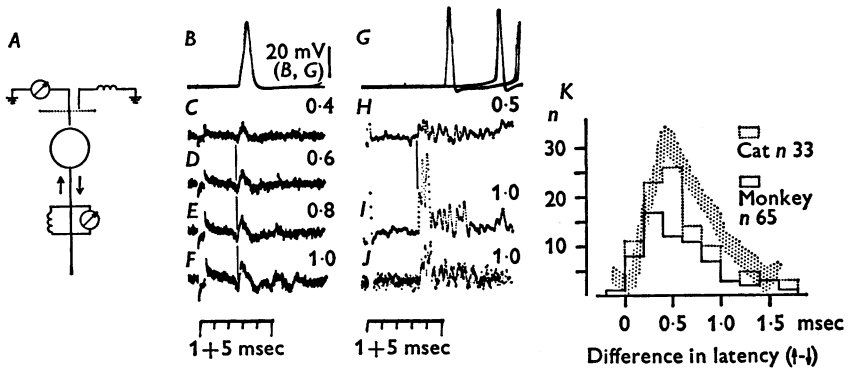

Fig. 3. A comparison of the latencies of the antidromic activation of a sample of pyramidal tract cells and of descending volleys evoked by stimulation of the surface of the motor cortex. *A*, diagram of experimental arrangement. In this and the following figures the stimulating and recording sites for evoking nerve impulses propagating ortho- or antidromically (arrows) are indicated on two sides respectively. Other indications as in Fig. 2. *B* and first spike in *G* are records of antidromic action potentials of pyramidal tract cells, followed by potentials evoked synaptically. Intracellularly recorded spikes from occasionally impaled cells are shown as their antidromic character leaves no doubt; note notches between IS and SD components and the all-or-none character of the first spike in *G*, even despite a relatively long latency of this potential; *C–F* and *H–J*, descending volleys recorded at L 1–L 2 from the surface of the lateral funiculus in monkey and at Th 11–12 from a dissected fascicle of the dorsolateral part of the lateral funiculus in cat respectively. Same sites from which potentials in *B* and *G* were evoked. Averaged records in *H* and *I*. *K*, a histogram of the differences between the latencies of antidromic and descending responses. Continuous and dashed lines, data for monkeys (sixty-five pyramidal tract cells) and cats (thirty-three pyramidal tract cells), respectively. Inserted record of the surface descending volley is from one of the monkeys.

The general correspondence between the time course of the descending volleys and the distribution of the latencies of antidromic cortical responses is well in keeping with the results of Patton & Amassian (1954) showing that

the descending volleys evoked by weak surface stimulation of the motor cortex are due to the direct excitation of the pyramidal tract cells. In view of this good correspondence, the latencies of the descending volleys in individual preparations will in the following be taken to indicate the shortest conduction time of the fastest conducting corticospinal tract axons (cf. also Landgren, Phillips & Porter, 1962).

2. Latencies of antidromic activation of pyramidal tract cells and of descending volleys evoked by intracortical stimulation

The records of descending volleys evoked by intracortical stimulation were taken with as high amplification as the noise level allowed in order to

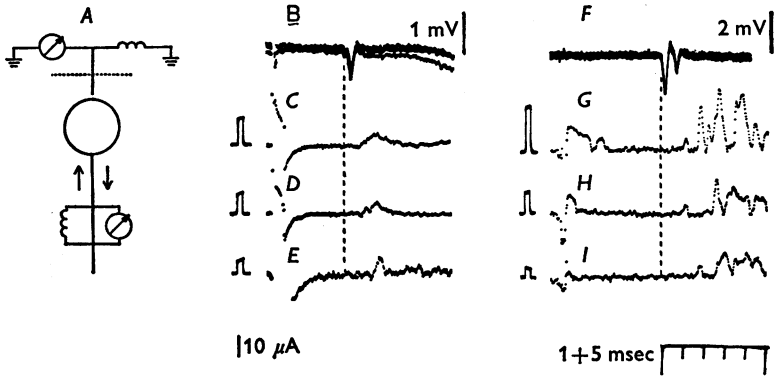

Fig. 4. A comparison of the latencies of the antidromic activation of pyramidal tract cells and of the earliest components of the descending volleys evoked by intracortical stimulation. *A*, diagram of experimental arrangement: indications as in Fig. 2 and 3. *B* and *F*, extracellular records of antidromic potentials in pyramidal cells. *C–E* and *G–I*, descending volleys recorded at L 1–2 from the surface of the lateral funiculus in monkey and at Th 11–12 from a dissected fascicle of the dorsolateral part of the lateral funiculus in cat, respectively. (Note higher amplification in *E* than in *C*, *D*.) Stimulation of the same sites from which records in *B* and *F* were evoked. Averaged records of descending volleys. Amplitudes of the stimulating intracortical pulses are given to the left of corresponding records, with the calibration under *E*. Records in the right column (*F–I*) are from the same preparation as the records in Fig. 3*G–J*.

increase the probability of detecting the smallest responses. The amplification and the general recording conditions were the same as in the control experiments described under Methods, in which they proved to be sufficient for distinguishing spike potentials in single fast conducting axons running at different depths from the surface of the spinal cord. Accordingly we shall present the results of this study under the assumption that the recording conditions in the main series of experiments allowed discernment of

responses in single corticospinal tract fibres as well (see Discussion for evaluation of this assumption). In Fig. 4 are examples of descending volleys evoked by intracortical stimulation that was applied close to pyramidal tract cells in monkey (*C–E*) and in cat (*G–I*). The volleys were evoked by stimuli of increasing (from bottom to top) strength which activated an increasing number of pyramidal tract cells, as is indicated by an increase in number and in amplitude to individual components. In the illustrated cases the earliest components of the descending volleys had clearly longer latencies than the latencies of the recorded antidromic spikes (*B* and *F*). A similar comparison has been made for responses evoked from the vicinity of twenty-one different pyramidal tract cells in monkey and twenty-three such cells in cat; its results are summarized in Fig. 5. In Fig. 5*B* are plotted the differences between the latencies of the earliest components of the descending volleys, indicated by (↓) in *A* and *B*, and the latencies of the antidromic responses (↑) that were previously recorded with the intracortical electrode. The length of each line corresponds to the difference which was found at one electrode position at the indicated strength of intracortical stimulation. To simplify the interpretation of these differences we plotted them only for the cases in which the stimulating electrodes were close to the cells with the highest conduction velocity; the latency of the antidromic spikes of the selected cells did not exceed the minimal one, indicated by the latency of the surface descending volley (cf. the preceding paragraph) by more than the rising time of the latter. This limitation was introduced mainly because of the fact that even weak intracortical stimuli usually activated a number of pyramidal tract cells (as judged from a number of components of the descending volleys) in addition to the ones recorded from and the earliest components of the descending volleys might be set up in the axons of any of these cells. If the pyramidal tract cells originally recorded from belonged to the slowly conducting fraction, while some of the other cells activated by cortical stimulation had much faster conducting axons, the revealed differences would be considerably underestimated. Not knowing if the earliest components of the descending volleys were set up in the axons of the cells, the antidromic spikes of which were recorded at the site of stimulation we decided to compare the latencies of these earliest components also with the conduction time of the fastest conducting corticospinal tract fibres. The latter is given by the latency of the descending volley evoked from the surface of the cortex. In Fig. 5*D* are therefore shown the differences between the latencies of the descending volleys evoked by the intracortical ($\downarrow i$) and by the surface ($\downarrow s$) stimulation. As for Diagram *B*, the length of each line corresponds to the difference at one electrode position and at a given stimulus strength. Both diagrams show that the majority of the descending volleys reached the recording

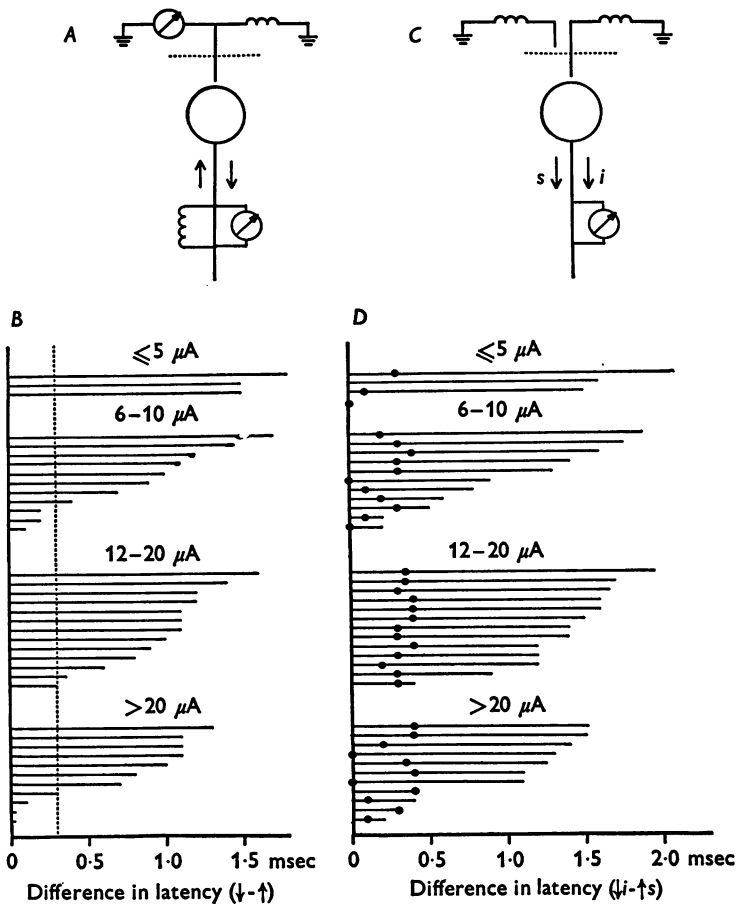

Fig. 5. Differences between the latencies of descending volleys evoked by intracortical stimulation and the latencies of antidromic activation of pyramidal tract cells. *A*, experimental arrangement for the data in *B*. *B*, differences between the latencies of antidromic spike potentials recorded in pyramidal tract cells and the latencies of descending volleys evoked by stimuli applied in the vicinity of these cells. Differences for stimuli of different strengths as indicated; those between the zero and the dashed vertical line may be insignificant. *C*, experimental arrangement for data in *D*. *D*, differences between the conduction time in the fastest conducting corticospinal tract fibres, as indicated by the latencies of the descending volleys evoked by stimulation of the surface of the cortex and of the descending volleys evoked by intracortical stimulation. Filled circles indicate the delay with which the pyramidal tract cells recorded at the site of intracortical stimulation were activated antidromically (as in Fig. 3*K*). Further explanations in the text.

electrode that was in contact with the lateral funiculus with latencies much longer than the corresponding conduction times of a fast component of the corticospinal tract fibres. Even if differences equal to or smaller than 0·6 msec in *B* (0·3 msec possibly due to current spread and shortening of the latencies of antidromic responses, as discussed above, plus 0·3 msec required for one synaptic delay, cf. Jankowska & Roberts, 1972) and 0·3 msec in *D* (required for one synaptic delay) were considered insignificant the occurrence of about three-quarters of the earliest components of the descending volleys would be incompatible with the direct activation of the pyramidal tract cells by intracortical stimuli.

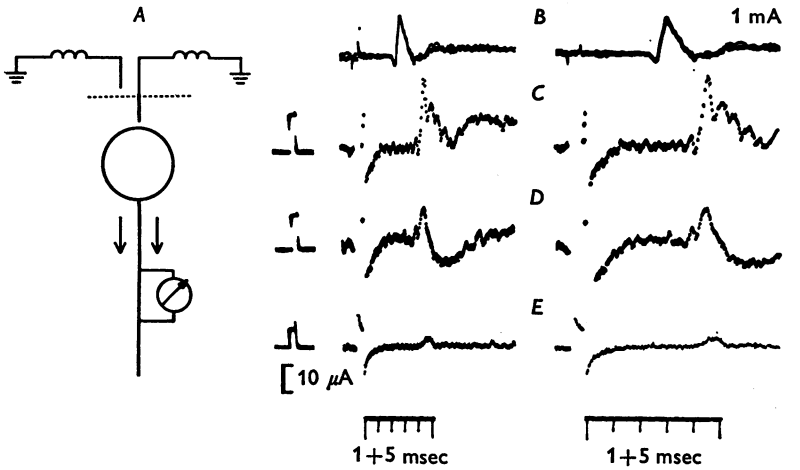

Fig. 6. Latencies of the descending volleys evoked by intracortical stimulation in relation to the total duration of the descending volleys evoked by surface stimulation in a monkey. *A*, diagram of the experimental arrangement for intracortical (right) and surface (left) stimulation. *B*, surface descending volley. *C–D*, descending volleys evoked by intracortical stimulation with decreasing strengths, the corresponding stimulus pulses shown to the left. Left and right-hand records were taken simultaneously with different sweep speeds.

The numbers of tests with stimuli of different strengths were too small to allow a correlation between the proportion of cells which were probably directly activated (i.e. those which showed the differences between the latencies of antidromic and descending responses $\leqslant$ 0·3 msec) and the parameters of stimulation. The data of Fig. 5 give the impression nevertheless that the probability of a direct excitation would be much higher with stronger stimuli.

Taking into account not only the first but also the later components of the descending volleys evoked by intracortical stimulation reveals that the

proportion of pyramidal tract cells excited indirectly must exceed the proportion of cells excited directly to an extent even greater than indicated by Fig. 5. For instance, the records of Fig. 6 show that practically all but the earliest components of a descending volley evoked by intracortical stimulation (*C–E*) may have latencies longer than the latest components of the descending volley evoked by surface stimulation (*B*). In view of the close relation between the duration of the surface-induced volley and the distribution of the conduction velocities in the corticospinal tract fibres this would indicate that the intracortical stimulation had mainly indirect effects on the pyramidal tract cells.

The results presented up to now were obtained when the tip of the intracortical stimulating electrode was in close proximity to the pyramidal tract cells and when the strength of stimulation did not exceed 25–30 μA. In the case of some cells the effects of stimulation in more superficial and/or deeper layers were also checked. For a few of the cells in which direct excitation was observed it was most readily evoked when the electrodes passed the cell bodies and were inserted 200–400 μm deeper (cf. Fig. 7*I* and *J*, arrows). At these deeper electrode positions the antidromic spikes of the cells had either a much smaller amplitude or could not be differentiated from the antidromic field potential and potentials of other cells. It is thus likely that the direct excitation of the cells was then due to the depolarization of their axon. If so, the higher probability of a direct excitation of the pyramidal tract cells by stronger stimuli, as suggested by Fig. 5, might be explained by the spread of current to the axons of the cells.

It should be noted in this context that the amplitudes of the extracellular spikes recorded in the present study were usually much larger than in the experiments of Stoney *et al.* (1968). Whether this was due to different types of electrode or to their different positions in relation to the pyramidal tract cells is difficult to say. However, if in the experiments of Stoney *et al.* (1968) the electrodes passed relatively close the initial segments of the axons (where the extracellularly recorded spikes may be smaller but the excitability higher) it would explain the larger proportion of cells directly excited in their cat material).

B. *Effects of repetitive intracortical stimulation*

Repetitive stimuli would not be likely to increase the effectiveness of the direct activation of the pyramidal tract cells, unless by providing an excitatory background secondary to the excitation of cortical circuitry and by lowering the thresholds for the submaximal direct effects. The indirect activation should be, on the other hand, greatly potentiated due to a temporal summation of synaptic effects of the stimulated fibres on pyramidal tract cells or on cells projecting to them. An increase of the descending volleys evoked by successive intracortical stimuli might then be a good

measure of the indirect activation of pyramidal tract cells. In the records of Fig. 7 the descending volleys were evoked by trains of three shocks of increasing strength (from top to bottom). As judged from the latencies of these volleys, the direct components (indicated by arrows) appeared only at the highest stimulus intensity and were evoked more readily at a deeper electrode postion. The components attributable to an indirect activation of pyramidal tract cells appeared following the second and

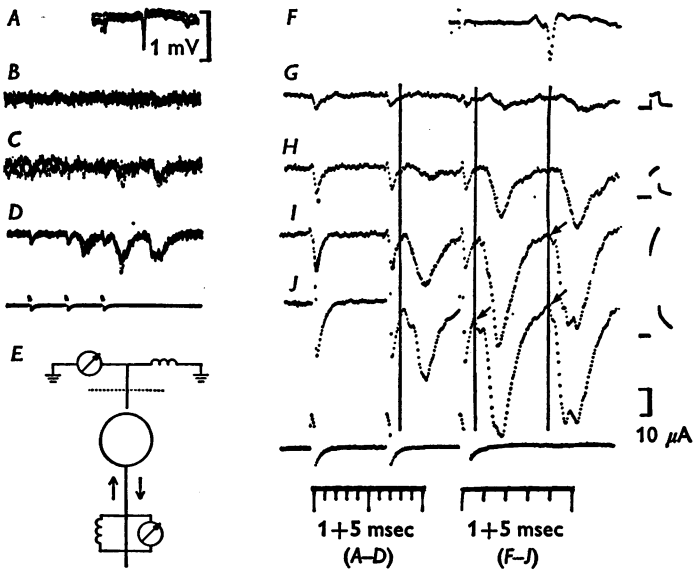

Fig. 7. Descending volleys evoked by repetitive intracortical stimulation. *A* and *F*, antidromic potential from a pyramidal tract cell in a monkey. *B–D* and *G–J*, descending volleys evoked by increasing strengths of intracortical stimulation at the same micro-electrode position as in *A* and *F*, recorded from a fascicle of the lateral funiculus dissected at L 1–2. *J*, intracortical stimulation with the same strength as for *D* and *I* but 300 μm deeper. Superimposed traces to the left and the corresponding averaged records to the right. The amplitudes of the stimuli are shown to the right and their timing on the lower traces in *D* and *J*. Arrows indicate descending volleys with the same latencies as the latencies of the antidromic potentials. In all the records, negativity is downwards.

third stimulus even when the first was too weak to give any response (*G*). When a response was evoked by the first stimulus (*H–J*) it grew with the second and third stimulus. Similar effects of repetitive stimulation were observed in all cases in which they were tested, both in monkey and in cat, under chloralose anaesthesia as well as under Nembutal. The frequency of stimulation was between 200 and 400/sec. With trains of longer duration and

moderate (4–20 μA) stimulus intensity the maximal responses were obtained after the third shock, as shown in Fig. 8*B*. Records of Fig. 8*C* and *D* show that the temporal facilitation of responses evoked by surface stimulation was much weaker, especially at higher stimulus intensities (cf. Patton & Amassian, 1954).

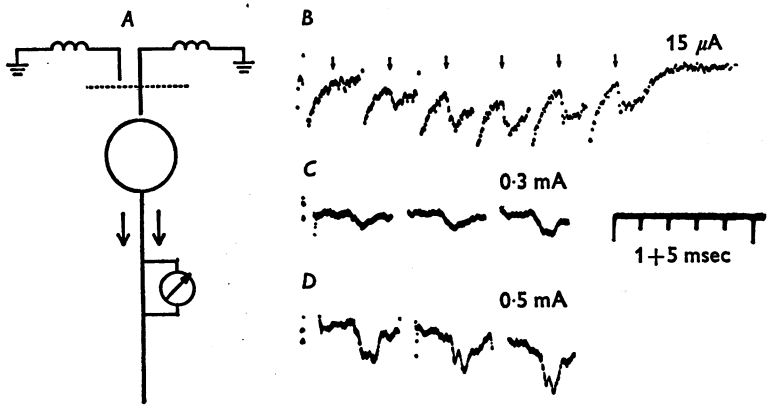

Fig. 8. Effects of repetitive surface and intracortical stimulation. *A*, diagram of experimental arrangement. *B–D*, records from a fascicle of the lateral funiculus in a cat. *B*, descending volleys evoked by intracortical stimulation in a cat. *C–D*, descending volleys evoked by two strengths of surface stimulation in another cat. Negativity downwards.

DISCUSSION

The results of the present study lead to two main conclusions: (1) that a weak intracortical stimulaton is relatively ineffective in a direct excitation of pyramidal tract cells and (2) that the effects of such stimulation are mainly indirect, especially when repetitive stimuli are used.

The first conclusion depends greatly on the sensitivity of our recording conditions, because it is based on the negative results, i.e. frequent failures of the intracortical stimulation to evoke descending volleys with a latency as short as would be required for the direct activation of pyramidal tract cells. Control experiments (described under Methods) showed that under the same conditions it was possible to record potentials in single fibres set up by the intra-axonal stimulation. The potentials propagating along these single fibres could be detected even if they ran at a depth up to 1·5 mm from the surface and if their conduction velocity was as low as 60 m/sec. The sensitivity of the recording should thus be sufficient to detect potentials in single corticospinal tract fibres as well, since both their location and the conduction velocity would be within the same ranges. However, the amplitude of the unitary descending volleys might

be somewhat lower than the amplitude of the responses of the intra-axonally stimulated fibres because of possible small variations in the latencies of the spikes induced by intracortical stimulation (cf. Rosenthal, Waller & Amassian, 1967), which would affect the averaged records. The detection of the unitary responses depended also to some extent on the noise level and the background activity of the fibres running in the lateral funiculus. To keep the latter low, the level of anaesthesia (especially barbiturate) was kept as deep as the general state of the preparation allowed. Nevertheless, in view of the small amplitude of the unitary responses it cannot be excluded that some of them might have been overlooked if the noise level was too high.

Besides the results now reported our first conclusion finds its support also in some earlier observations. Landau *et al.* (1965) observed only small *D*-waves, even with a relatively strong intracortical stimulation, until the electrode tip reached the border between the gray and white matter. Stoney *et al.* (1968) similarly failed to find a low threshold region for direct excitation in the majority (twenty-six of thirty-nine) cells, although a possible divergence between the recording and the stimulating electrodes was given as a reason for this failure.

In contrast to the first our second conclusion is based on the positive results and would hold true whether or not all the small direct unitary responses were detected. The considerable amplitude of the descending volleys with longer latencies leaves no doubt that the fibres contributing to the indirect effects greatly outnumber those activated directly.

The relatively long latencies of the descending volleys evoked by intracortical stimulation are taken to indicate the indirect origin of these volleys for the following reasons. (i) They were longer than the latencies of the antidromic spikes of the pyramidal tract cells recorded at the site of the stimulation, even with the margin of 0·3 msec allowed for current spread while stimulating corticospinal tract fibres in the lateral funiculus. (ii) They were longer than the conduction times of the majority of the fast conducting corticospinal tract fibres as indicated by the latency and the duration of the direct component (Patton & Amassian, 1954) of the descending volleys evoked by stimulation of the surface of the cortex. In addition, most of these differences exceeded the 0·2–0·3 msec which might be allowed for excitation of the pyramidal tract cell axons at a certain distance from their bodies by surface positive pulses (Landau *et al.* 1965). (iii) The explanation proposed by Phillips (1956*a*, *b*) for delayed effects of surface stimulation and by Landau *et al.* (1965) for longer latencies of some responses to intracortical stimuli, namely that the spikes may be initiated in the dendrites and slowly conducted to the soma and/or to the initial segment, could hardly apply in our case. The intracortical stimuli

were applied in the immediate vicinity of the pyramidal tract cell bodies, where clear antidromic potentials were recorded from them with the same electrodes, and where the cells were often impaled with a further movement of the electrode. (iv) A major contribution to the late descending volleys by directly excited pyramidal tract cells with lower conduction velocities, the antidromic responses of which were not recorded at a given electrode position, is rather unlikely. It would require that the thresholds for the direct excitation of such apparently remotely located, slowly conducting cells be very considerably lower than the thresholds of the cells in a closer vicinity of the electrode tip. (v) The late descending volleys were greatly facilitated by preceding intracortical stimuli in a way typical for a temporal facilitation of synaptic responses.

The delays of intracortically evoked descending volleys, taken to indicate the indirect way of setting them, were up to 1·5–2·0 msec. By analogy with the segmental latencies of mono-, di-, and trisynaptic responses in spinal neurones, the delays of up to 0·5–1·0 msec and between 1·1 and 1·5 msec might suggest one and two synaptic delays respectively. Those exceeding 1·5 msec might correspond to two or to three synaptic delays (cf. Jankowska, Lundberg, Roberts & Stuart, 1975). However, the results of Rosenthal *et al.* (1967) indicate that the corresponding delays may be longer at the cortical level in view of much longer delays between the onset of the e.p.s.p.s and the generation of the spike potentials in the cells. The distribution of the latencies of the potentials evoked in both pyramidal tract and other cells by surface stimulation of the cortex showed distinct non-overlapping groupings with mean intervals of 1·05 msec. The first e.p.s.p. and the following spike appeared within 0·55–1·6 and 0·9–1·9 msec, respectively. The second e.p.s.p. appeared after 2·0–2·8 msec. Rosenthal *et al.* (1967) concluded, therefore, that most of the indirect responses with latencies up to 2·0 msec are monosynaptically mediated. Asanuma & Rosén (1973) took a delay of 1·5 msec as the upper limit for the monosynaptic e.p.s.p.s following weak intracortical stimulation. According to data of both Rosenthal *et al.* (1967) and of Asanuma & Rosén (1973), practically all the earliest indirect (Fig. 5) and a great part of the later responses evoked in our study by intracortical stimulation might have been induced monosynaptically.

Whether or not all those volleys may be attributed to the cortico-spinal tract fibres is another problem. When the pyramidal tract cells were not directly activated the earliest components, those appearing with delays corresponding to one as well as more synaptic delays, would almost certainly be propagating in the cortico-spinal fibres. Otherwise one had to assume a selective activation of pyramidal tract cells other than the recorded ones (with the axon in the lateral funiculus) that would project only to rostral levels of the neuraxis and relay there. The delays of the later components of the descending volleys, on the other hand, would be compatible with

the mono- or disynaptic excitation of pyramidal tract cells and further relaying of their effects through some cells on which the pyramidal tract axon collaterals are terminating, e.g. propriospinal tract cells (Lloyd, 1941; Vasilenko & Kostyuk, 1965; Vasilenko, Zadorozhnyj & Kostyuk, 1967; Illert, Lundberg & Tanaka, 1974) or reticulospinal tract cells (cf. Kuypers, 1958; Kuypers & Lawrence, 1967). The abolition of the effects of intracortical stimulation on motoneurones by the lesions of medullary pyramids (Asanuma & Sakata, 1967) would speak against the relaying of the corticospinal effects via the rubrospinal system (activated by collaterals of corticospinal fibres as well as by corticorubral fibres (Tsukahara, Fuller & Brooks, 1968) because the projections to the red nucleus should not be affected by medullary lesions. The same might hold true also for great part of cortico-reticular connexions.

That the weak intracortical stimuli may activate pyramidal tract cells not only directly but also mono- or polysynaptically has been most elegantly and convincingly shown by Stoney *et al.* (1968) and by Asanuma & Rosén (1973) with direct recording from these cells (cf. also Rosenthal *et al.* 1967). In this respect our study does not reveal anything new. However, in the previous studies there had been no systematic analysis of the relative contributions by the two modes of activation of pyramidal tract cells to the effects produced by weak intracortical stimulation at either cortical or spinal level. Our results leave no doubt that the effects due to indirect activation of pyramidal tract cells are the dominant ones, although Asanuma and his collaborators took into account practically only the direct activation of pyramidal tract cells when calculating the numbers of cells activated by intracortical stimuli and distances between these cells and the electrode tip (Stoney *et al.* 1968; Asanuma, 1973). Their main argument against a major role of the indirect activation of pyramidal tract cells was based on the observations that the thresholds for electrical activation of fibres are relatively high, higher than the actually used strengths of intracortical stimuli that were sufficient to excite the pyramidal tract cells directly (Asanuma & Sakata, 1967; Stoney *et al.* 1968). Investigations performed after the original papers by Asanuma *et al.* (1968) were published showed, however, that the thresholds for excitation of fibres may be as low as 0·1–1·0 μA, depending on the position of the stimulating electrode in relation to the Ranvier's nodes (Jankowska & Roberts, 1972; Roberts & Smith, 1973) or preterminal segments of fibres (Baldissera, Lundberg & Udo, 1972). In the study on direct and indirect activation of neurones in the red nucleus (Baldissera *et al.* 1972) the thresholds for the indirect activation were in fact found to be considerably (about five times) lower than those for the direct activation. The latest published results by Asanuma & Rosén (1973) similarly reveal lower thresholds for synaptic effects in cortical cells than the lowest reported thresholds for a direct activation of pyramidal cells (Stoney *et al.* 1968).

In the case of red nucleus cells the direct activation was more easily seen with similar strengths of current (single pulses between 10 and 20 μA)

and with less sensitive recording conditions (without averaging the descending volley) than in studies on cortical effects, although for individual pyramidal tract cells the lowest reported thresholds were lower. The results of Baldissera *et al.* (1972) indicate that the direct effects of rubral stimulation were to a great extent due to the activation of the rubrospinal tract fibres on the medio-ventral side of the caudal part of the nucleus where they collect before crossing via decussatio tegmenti ventralis. If the low threshold direct activation of pyramidal tract cells was similarly due to stimulation of their axons, the smaller effectiveness of intracortical stimulation might perhaps be due to a less favourable trajectory of these axons or to too long distances between the tip of the stimulating electrode and their first Ranvier's nodes or initial segments. Asanuma & Sakata (1967) reported that the occasionally tested thresholds for evoking pyramidal effects from the white matter were higher than for effects evoked from the cortex. The results of Landau *et al.* (1965) indicate, however, that the thresholds for evoking the *D*-wave in the pyramidal tract are lower in the white than in the grey matter. A number of other observations would be also compatible with the direct stimulation of the pyramidal tract axons: a very narrow depth range (of the order of 50 μm) for the lowest thresholds direct activation of pyramidal cells (cf. Fig. 4 and 7 in Stoney *et al.* 1968), shorter latency tonic contractions and more stable responses to stimuli in the cortical layers ventral to the pyramidal cells layer (Asanuma & Ward, 1971) as well as more readily obtained direct activation of pyramidal tract cells with electrode positions ventral to them (Landau *et al.* (1965) and the results of this study). If these cells were only indirectly excited by stimuli applied further away from their axons one could even wonder if the weak electrical currents (of the order of 5–10 μA) would ever be sufficient for generating action potentials when applied near the soma and dendrites of cells as large as the pyramidal tract cells. To solve this problem additional experiments are necessary.

In view of the results of the present study the final functional interpretation of the effects of the intracortical stimulation will depend greatly on the network of the intracortical connexions. If the organization of cells and fibres within the cortex which make synaptic contacts with a given group of pyramidal tract cells is strictly columnar, the postulated focal character of a weak intracortical stimulation may be justified. However, if cells or fibres terminating on one group of pyramidal cells are making synaptic contacts not only with them but also with some other groups of pyramidal tract cells at shorter or longer distances, the focal effects would be only relatively focal. This problem will be discussed further in the following paper (Jankowska, Padel & Tanaka, 1975).

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