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POST-SYNAPTIC EFFECTS OF CORTICAL STIMULATION ON FORELIMB MOTONEURONES IN THE BABOON

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SUMMARY

1. The arm area of the baboon's precentral motor cortex was stimulated by brief surface-anodal pulses, and the post-synaptic potentials elicited in contralateral forelimb motoneurones were studied by intracellular recording.

2. Strong cortical stimuli elicited a rapid series of excitatory and, in some cells, inhibitory post-synaptic potentials (EPSPs and IPSPs respectively). Comparisons with the simultaneously recorded response of the pyramidal tract indicated that these post-synaptic potentials were due to a repetitive discharge of fast pyramidal fibres. Thus, the later synaptic events were mostly due to a repetition of the early monosynaptic EPSP and early IPSP respectively.

3. Inhibition was seen more often in cells whose monosynaptic EPSP had a small maximal size than in those whose monosynaptic EPSP was larger. The net depolarization produced by a strong cortical stimulus was related to the maximal size of the early monosynaptic EPSP.

4. In the Discussion, an interpretation is suggested for previous findings concerning the spinal distribution of late synaptic effects elicited by cortical stimulation.

INTRODUCTION

In the monkey, there is a monosynaptic excitatory connexion between fast conducting cortico spinal fibres and spinal alpha motoneurones (Bernhard, Bohm & Petersén, 1953; Preston & Whitlock, 1960, 1961; Landgren, Phillips & Porter, 1962). Thus, in the spinal motoneurones of the monkey, a weak and brief surface-anodal cortical stimulus will usually elicit a brief EPSP of monosynaptic latency, sometimes alone and sometimes succeeded by a brief IPSP (Hern, Landgren, Phillips & Porter, 1962;

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Landgren *et al.* 1962). The latter is presumably caused by fast pyramidal fibres via a spinal interneurone (Preston & Whitlock, 1961; Landgren *et al.* 1962; Phillips & Porter, 1964). A stronger cortical stimulus will also give rise to later excitatory and, at least sometimes, inhibitory events in the motoneurones. Such delayed cortical effects (referred to below as 'late' excitation, 'late' inhibition or 'late' synaptic effects respectively) are often very powerful (Bernhard *et al.* 1953; Phillips & Porter, 1964), and this has been found to be the case also in animals whose 'extrapyramidal' connexions between the cortex and the spinal cord have been destroyed (Preston & Whitlock, 1960, 1961). The late synaptic effects should therefore to a great extent be due to activity of fibres in the pyramidal tract. No further studies seem to have been made, however, concerning the origin of the late synaptic effects.

A strong and brief cortical stimulus will give rise to a prolonged discharge in the pyramidal tract (Adrian & Moruzzi, 1939; Patton & Amassian, 1954, 1960), and this obviously has to be taken into account when trying to interpret the late synaptic effects on motoneurones (cf. Landgren *et al.* 1962; Phillips & Porter, 1964). In the monkey, such a prolonged discharge of the pyramidal tract will be recorded by a gross electrode as a rapid series of distinct 'waves' (Patton & Amassian, 1954, 1960). The initial wave, the 'D wave', will be elicited alone by weak surface-anodal cortical stimuli (Hern *et al.* 1962; Phillips & Porter, 1964; Gorman, 1966), and it is due to the direct electrical stimulation of fast cortico-spinal cells (Patton & Amassian, 1954, 1960). The later waves, the 'I waves', are due to the indirect excitation of cortico-spinal cells via intracortical synapses (Patton & Amassian, 1954, 1960). Recent studies on baboons have shown that the I waves almost exclusively reflect a semi-synchronous repetitive discharge of fast cortico-spinal fibres belonging to the same group as those responsible for the D wave (Kernell & Wu, 1967). The fast cortico-spinal fibres have monosynaptic connexions with spinal motoneurones (see above), and the I wave discharge of these fibres would therefore, in the primate, be expected to be directly responsible for much of the late excitation elicited in motoneurones by strong cortical stimulation. That this is actually the case will be shown by the present experiments on baboons, in which the synaptic events elicited in motoneurones by cortical stimulation were studied in relation to the pyramidal tract waves. The results suggest that inferences concerning the spinal distribution of pyramidal monosynaptic excitation might be drawn from previous findings concerning the distribution of late synaptic effects.

METHODS

The experiments were performed on young baboons of either sex weighing between 4·7 and 6·4 kg. The methods of anaesthesia, cortical stimulation and pyramidal tract wave recording were the same as those of a preceding paper (Kernell & Wu, 1967). Thus, during the experiments the animals were breathing a mixture of 50–70 % nitrous oxide in oxygen, and anaesthesia was deepened as required by the intravenous injection of hexobarbitone (Hern *et al.* 1962). The arm area of the precentral motor cortex was stimulated unifocally at one fixed site with brief (0·2 msec) surface-anodal pulses, and pyramidal tract waves were recorded with an electrode resting lightly against the dorsolateral surface of the cervical spinal cord. Pyramidal tract waves were recorded about 12–18 mm cranial to the insertion of the micro-electrode used for intracellular recordings.

Intracellular records from motoneurones innervating muscles of the forearm and hand were obtained in segments C 7–8. The motoneurones were situated contralateral to the side of cortical stimulation. The single-barrelled micro-electrodes were filled with 3 M-KCl or 2 M potassium citrate, and their resistance was generally between 5 and 15 MΩ. The cathode follower output was connected to two amplifiers in parallel. One of the amplifiers was always d.c.-coupled, and the other one was either d.c.- or a.c.-coupled (time constant 0·1 sec). In order to control cord pulsation, a saddle-shaped celluloid plate pressed lightly against the dorsal surface of the cord. The micro-electrode was inserted through a hole in this plate (Hern *et al.* 1962). Ventral and dorsal roots were intact, and the arm was denervated with the exception of the branch innervating extensor digitorum communis. The motoneurones were identified by their antidromic response to stimulation of fore-limb nerves. The nerves prepared for stimulation were: ulnar (in upper arm), median (in upper arm), radial (at elbow) and the branch of the radial nerve innervating extensor digitorum communis. Rectal temperature was kept between 37 and 39° C.

The results refer to forty-seven motoneurones of spike size 50–85 mV. Records were also obtained from thirty-five other motoneurones which were less suitable for quantitative studies, because the membrane potential was too unstable or the penetration too short-lasting to allow a full investigation to be made of the response to different strengths of cortical stimulation. Data obtained from these latter thirty-five cells confirm on all relevant points, the main results.

RESULTS

Early and late synaptic events. In the fore-limb motoneurones of the present experiments, a weak surface-anodal cortical stimulus evoked a brief EPSP with a latency between 2·3 and 3·1 msec (Fig. 1*A*, 2·4 mA and *B*, 2·3 mA). Previous authors have shown that this EPSP is monosynaptically generated by fast cortico-spinal fibres (see Introduction), and in the present paper it will be referred to as the 'early EPSP' or the 'early excitation'. The only other post-synaptic event that could be elicited with a similar stimulus threshold was a brief IPSP with an onset 0·9–1·6 msec later than that of the early EPSP (Fig. 6*A*, 3·6 mA). Previous workers have concluded that this IPSP is probably generated by fast cortico-spinal fibres via a spinal interneurone (see Introduction), and it will here be referred to as the 'early IPSP' or the 'early inhibition'. An early IPSP was seen only in some of the motoneurones. In all the cells, the early EPSP and IPSP were succeeded by later synaptic events at stronger cortical stimuli (e.g. Fig. 1*A* and *B*). In many cells, the late effects appeared to be

purely excitatory, whereas in some neurones a mixture of excitation and inhibition was seen. Inhibition will be treated separately in a later paragraph.

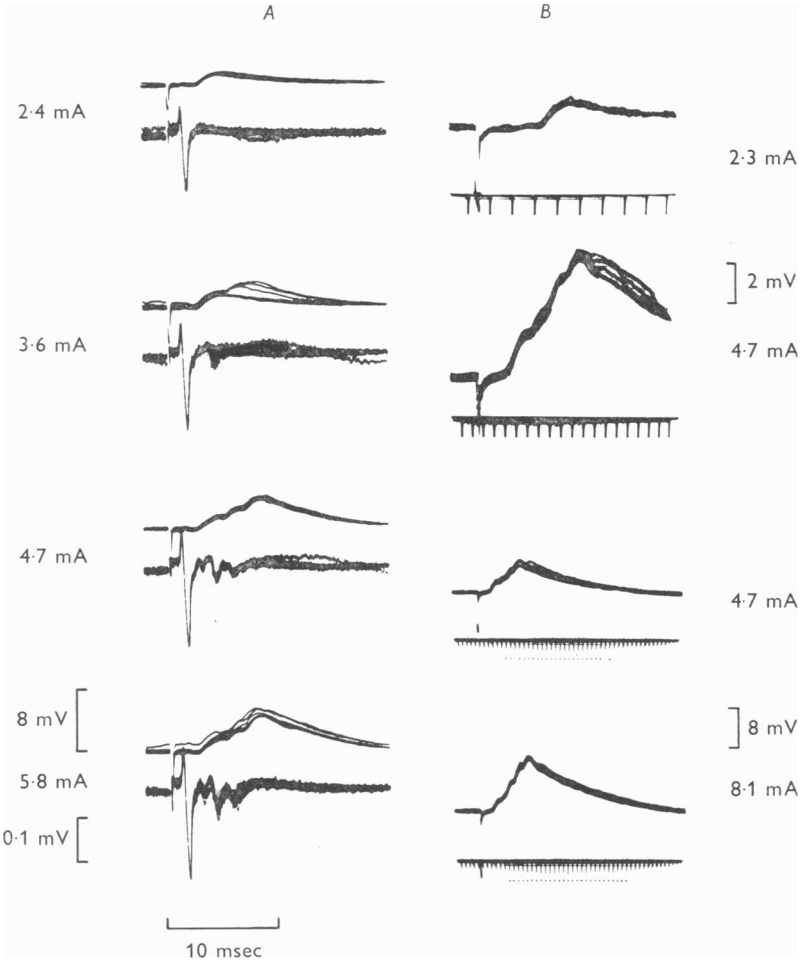

Fig. 1. *A*. Ulnar motoneurone, spike size 51 mV. Post-synaptic potentials (upper superimposed traces) elicited by brief surface-anodal cortical pulses at the indicated intensities (mA). The pyramidal tract waves (lower superimposed traces) were recorded simultaneously with an electrode resting on the dorsolateral cord surface. Single stimuli were repeated once a second. Negativity downwards. Micro-electrode filled with potassium citrate. Voltage calibrations: 8 mV for intracellular records, 0·1 mV for pyramidal tract waves. *B*. Radial motoneurone, spike size 66 mV. Post-synaptic potentials elicited and recorded as in *A*. KCl microelectrode. Voltage calibrations: 2 mV for the two upper records, 8 mV for the two lower ones. Time: msec. Intracellular records in *A* and *B* obtained with d.c.-coupled amplifier.

Excitation. In Fig. 1*A* at 3·6 mA, the early EPSP is, in one of the superimposed traces, succeeded by a later EPSP looking almost identical to the first one (see also Fig. 2*A*, 2·2 mA and *B*, 2·4 mA). In other traces, two such late EPSPs are apparently evoked in rapid succession (Fig. 1*A*, 3·6 mA). At 4·7 and 5·8 mA the two late EPSPs are larger and less variable. Following the last of these EPSPs, the membrane potential returns smoothly towards the base line (Fig. 1*A*). These results are typical. In all

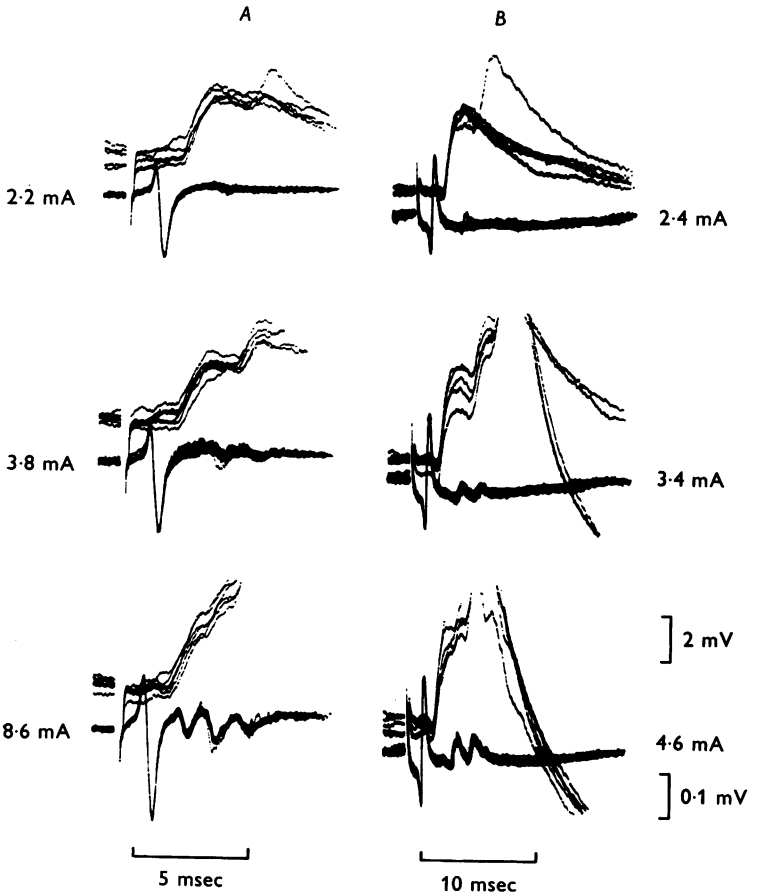

Fig. 2. *A*. Extensor digitorum communis motoneurone, spike size 76 mV. Postsynaptic potentials and pyramidal tract waves elicited and recorded as in Fig. 1*A*. Potassium citrate micro-electrode. *B*. Ulnar motoneurone, spike size 85 mV. A spike was elicited in about half the number of trials at 3·4 mA and in all trials at 4·6 mA. Pyramidal tract waves recorded with negativity upwards. Potassium citrate micro-electrode. Same voltage calibrations for *A* and *B*: 2 mV for intracellular records, 0·1 mV for pyramidal tract waves. Intracellular records in *A* and *B* obtained with a.c.-coupled amplifier (time constant 0·1 sec).

the cells, the depolarizing phase of the late excitation occurred in such distinct and rapidly recurring steps. Other examples of such steps are shown in Figs. 1*B* and 2, and similar steps are seen also in several of the illustrations of previous workers (Preston & Whitlock, 1961; Phillips & Porter, 1964). New steps could appear successively with an increase of stimulus intensity. The latency of a particular late EPSP-step was, however, remarkably constant once it had appeared. Even with a very large increase of stimulus intensity, its latency would shorten only by some tenths of a msec or less (Figs. 1 and 2). Just at threshold, the latency would sometimes vary by some tenth of a msec. Often successive steps occurred at very brief intervals (1–2 msec), but they could generally be clearly separated at a high gain and a rapid sweep. Only in a few cells, some of the latest steps would become less distinct at the very highest stimulus intensities.

These findings indicate that most of the late excitation produced by cortical stimulation is actually made up of a rapid series of distinct EPSPs similar to the early monosynaptic one. The early EPSP is monosynaptically generated by the D wave discharge of fast pyramidal fibres, and late EPSPs would therefore be expected to be monosynaptically generated by the I wave discharge of the same group of fibres (see Introduction). The I waves and the late EPSPs should then often tend to have about the same cortical stimulus threshold. Furthermore, the delay between the early EPSP and a particular late EPSP should be the same as the delay from the D wave to the I wave responsible for the late EPSP in question. In other words, late EPSPs should be elicited by the I wave discharge with the same monosynaptic latency as that between the D wave and the early EPSP.

In Fig. 2*A*, the post-synaptic potentials are shown, together with simultaneously recorded pyramidal tract waves (lower traces). At 2·2 mA, the cortical stimulus is just at threshold for the I_2 wave (Kernell & Wu, 1967) and it is then also just at threshold for one late EPSP. A late EPSP-step with the same latency is obtained also at 3·8 and 8·6 mA (Fig. 2*A*). The interval between this late EPSP and the early EPSP is almost exactly the same as the interval between the onset of the negative-going phases of the I_2 wave and the D wave respectively. At 8·6 mA (Fig. 2*A*) a new EPSP-step has appeared, which has an even shorter latency, and the I_2 wave is now also preceded by an earlier I wave, the I_1 wave (Kernell & Wu, 1967). This new EPSP is delayed with respect to the early EPSP by almost exactly the same amount of time as that between the I_1 wave and the D wave (Fig. 2*A*). Findings similar to those of Fig. 2*A* are shown for another motoneurone in Fig. 2*B* (for this latter cell and for the one of Fig. 6*A*, the pyramidal tract waves are atypically recorded with negativity

upwards). In Fig. 3, from another motoneurone, all the single-sweep records were obtained with the same stimulus strength. This case further demonstrates the close correspondence that was often found between one late EPSP and one particular I wave. It is seen (Fig. 3), that the late EPSP is large when the I_2 wave is large, it is small when the I_2 wave is small, and it is absent when the I_2 wave is absent.

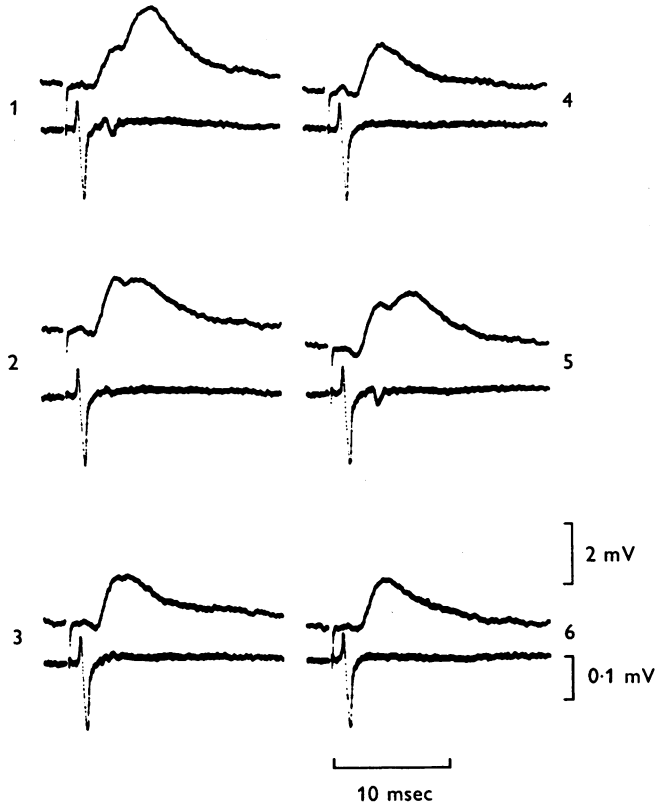

Fig. 3. Ulnar motoneurone, spike size 51 mV. Post-synaptic potentials (upper traces) and pyramidal tract waves (lower traces) elicited by cortical stimulation at 3·6 mA. Consecutive single records, stimulus being repeated once a second. Negativity downwards. Potassium citrate micro-electrode. Voltage calibrations: 2 mV for intracellular records, 0·1 mV for pyramidal tract waves. Intracellular records obtained with a.c.-coupled amplifier (time constant 0·1 sec).

A correspondence between the stimulus threshold for one particular late EPSP and one particular I wave, such as that demonstrated by Figs. 2 and 3, was often found during the present experiments. In Fig. 4, relative latencies are compared for twenty-five such cases (filled circles). The latency of late EPSPs, measured from the onset of the early EPSP, has

been plotted against the latency of the corresponding I waves, the latter being measured from the onset of the negative-going phase of the D wave. The EPSP latency could be equal to or up to 0·4 msec longer than the I wave latency, and the mean difference between the two latencies was only 0·2 msec (Fig. 4). These late EPSPs must almost certainly have been monosynaptically generated by fast pyramidal fibres discharging during the respective I waves.

In some cases, two late-EPSP-steps would tend to appear at a stimulus intensity for which only one I wave was clearly visible (Fig. 1*A*, 3·6 mA).

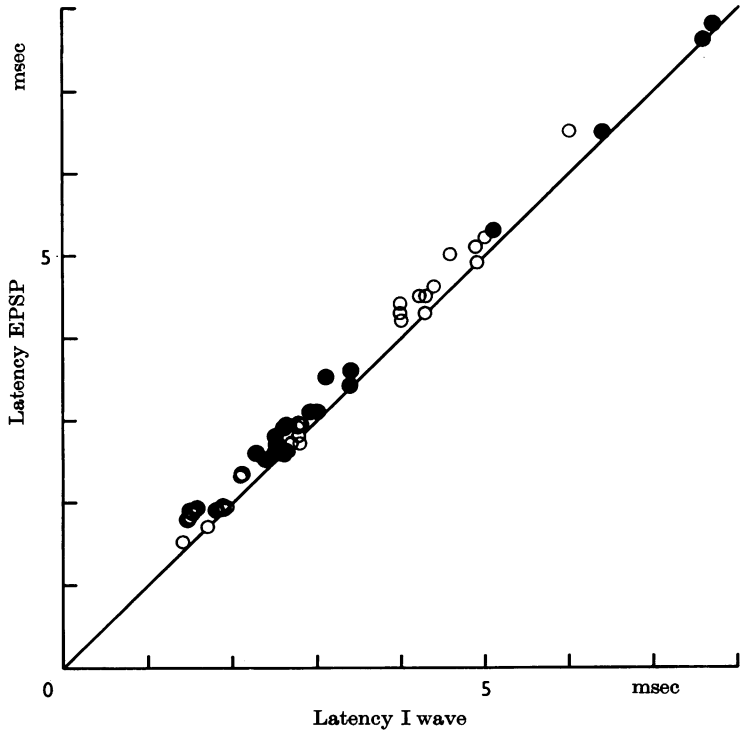

Fig. 4. Diagram showing relation between latency of late EPSP-steps (ordinate) and latency of simultaneously recorded I waves (abscissa). EPSP latency measured from onset of early EPSP to onset of late EPSP. I wave latency measured from onset of negative-going phase of D wave to onset of negative-going phase of I wave. Diagonal line is the unity line. *Filled circles*: values from cases in which the cortical stimulus threshold for one particular EPSP corresponded to the stimulus threshold for one particular I wave. These values represent twenty-five late EPSPs from seventeen different motoneurones. *Open circles*: values obtained by plotting latency of other late EPSPs against the simultaneously recorded I wave latency which was most similar. Open circles represent twenty-four late EPSPs from thirteen different motoneurones. For many of the motoneurones, some of the late EPSPs are represented by filled circles, whereas others from the same cell are represented by open circles.

In other instances, one late EPSP-step would have about the same stimulus threshold as two different I waves. Thus, in such cases the appearance of one particular late EPSP could not be connected with the appearance of one particular I wave. Even in these cases, however, there existed for each late EPSP-step an I wave with a similar relative latency, providing that the stimulus intensity was well above the threshold for the respective late EPSPs. This is demonstrated by the values marked as open circles in Fig. 4. These values were obtained by measuring, in cases other than those shown by filled circles, the relative latency of all the late EPSP-steps and of all I waves. The latency of each EPSP-step was plotted against the simultaneously recorded I wave latency which was most similar. In the diagram (Fig. 4) the open circles are seen to be distributed along the same line as the filled circles. The difference between the EPSP latency and the I wave latency is equally small for both groups of values (open and filled circles respectively). Thus, all the late EPSP-steps are almost certainly monosynaptically generated by fast pyramidal fibres discharging during the I waves. I waves and late EPSP-steps could start as late as 7·5 msec or more after the onset of the D wave and the early EPSP respectively (Fig. 4).

The threshold for a late EPSP-step was never much lower than that for the I wave with the same relative latency. It is not surprising that the threshold for an EPSP-step could be somewhat lower than that for the corresponding I wave, because a substantial number of pyramidal fibres must presumably take part in an I wave discharge before it can be seen in recordings from the surface of the spinal cord (Kernell & Wu, 1967). The threshold for a late EPSP-step could sometimes be much higher that that for the corresponding I wave. In Fig. 1*A* at 4·7 and 5·8 mA, for instance, three I waves are elicited but only two late EPSPs are seen (corresponding in time to the I_2 and I_3 wave respectively). For each particular I wave, new pyramidal fibres are recruited over a wide range of stimulus intensities, and some of the fibres connected to a given cell might not have been recruited even at the strongest stimuli employed (around 12 mA; Kernell & Wu, 1967). Late EPSPs would start to appear at a stimulus strength between around 1·3–5·5 mA, and for about half the number of neurones its threshold was between 2 and 3 mA. When considering the present values of stimulus intensity it should be remembered that the stimulating electrode was kept at one fixed site, i.e. no search was made for the 'best cortical point' for each particular motoneurone.

The early EPSP would reach its maximal amplitude at a stimulus intensity which was often a little lower and sometimes a little higher than that at which late EPSPs started to appear. Once the maximal size of the early EPSP had been reached, the stimulus intensity could be doubled

without any further change in the size of the early EPSP (cf. Phillips & Porter, 1964). As is apparent in Figs. 1–3, the size of the early EPSP could be quite accurately measured in the presence of late EPSPs (Phillips & Porter, 1964). The latter did not start earlier than 1·5 msec after the onset of the early EPSP (Fig. 4), and the rise time of the latter averaged $1{\cdot}4 \pm 0{\cdot}05$ (S.E., range 0·9–2·1 msec, 46 cells) msec. There was never any substantial increase in the size of the early EPSP at times later than 1·5 msec after its onset. For forty-seven cells the maximal size of the early EPSP varied from less than 0·2 mV up to 9·5 mV (Fig. 5). The size of the early EPSP was not found to be related to the spike size of these cells.

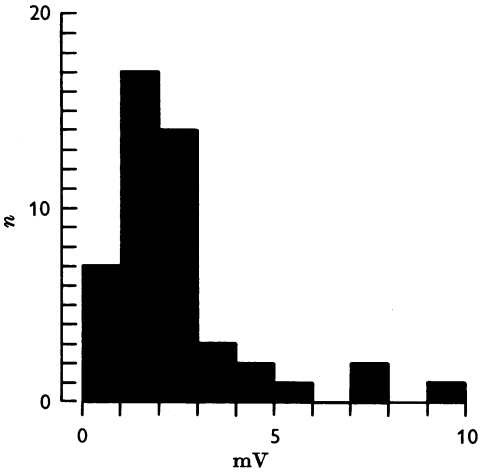

Fig. 5. Histogram showing maximal size of early monosynaptic EPSP elicited by cortical stimulation in forty-seven fore-limb motoneurones. In all cases it was ensured that the EPSP had attained its maximal size, i.e. that its amplitude did not show any further increase with an increasing intensity of cortical stimulation.

At strong cortical stimuli, some late EPSP-steps would often acquire about the same amplitude as the maximal early EPSP of the same cells (Fig. 1*A*, 5·8 mA), and in several cases some late EPSPs could even be a little larger than the early one (Fig. 1*B*, 8·1 mA). Previous workers have shown that the size of the early EPSP may be more than doubled by a fast tetanic stimulation (around 200/sec) of fast pyramidal fibres (Landgren *et al.* 1962; Phillips & Porter, 1964). The I wave discharge is a correspondingly rapid repetitive discharge of fast pyramidal fibres (Kernell & Wu, 1967), and late EPSPs could thus well be larger than the maximal early EPSP; actually this could presumably be so even if a smaller number of synapses were responsible for the late EPSP than for the early one.

As would be expected, late EPSPs tended on the whole to be larger in cells having a large early EPSP than in those having a small one. Thus, as

will be shown below (Fig. 7), the 'maximal' late synaptic depolarization which a cortical stimulus may elicit in a cell was related to the maximal size of its early EPSP. However, before analysing such results concerning total net depolarization, inhibition has to be considered.

Inhibition. Clear signs of an early inhibition (Fig. 6*A* 3·6 mA) were seen in fifteen out of the thirty-three cells studied with electrodes filled with potassium citrate. As would be expected, early inhibition was seldom

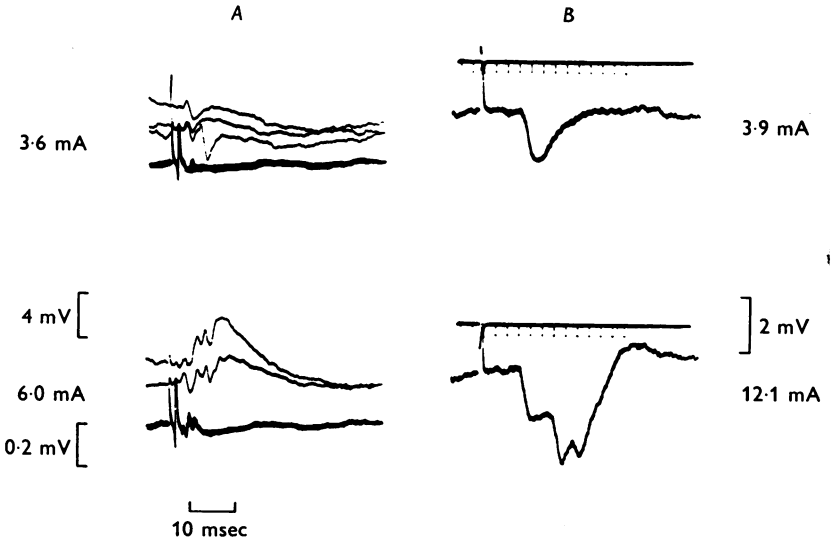

Fig. 6. *A*. Radial motoneurone, spike size 75 mV. Post-synpatic potentials elicited and recorded as in Fig. 1*A*. Owing to a somewhat noisy base line, the intracellular traces are not well superimposed. Pyramidal tract waves recorded with negativity upwards. Potassium citrate micro-electrode. Voltage calibrations: 4 mV for intracellular records, 0·2 mV for pyramidal tract waves. *B*. Median motoneurone, spike size 58 mV. One representative trace is shown for each stimulus strength. Potassium citrate micro-electrode. Time: msec. Intracellular records in *A* and *B* obtained with a.c.-coupled amplifier (time constant 0·1 sec).

seen in cells penetrated by KCl filled electrodes (seen only in one out of fourteen such cells) (Landgren *et al.* 1962; Phillips & Porter, 1964; cf. Eccles, 1964).

Figure 6*A* demonstrates that several of the properties of late inhibition were similar to those of late excitation. At 3·6 mA (Fig. 6*A*), a prominent early IPSP is seen to succeed the small early EPSP in all the three traces. The stimulus strength is just at threshold for the I_2 wave, and in one of the intracellular traces a distinct late IPSP succeeds the early one (Fig. 6*A* 3·6 mA). At 6·0 mA the D wave is succeeded by three prominent I waves. The late synaptic effect is now apparently a mixture of excitation

and inhibition. The early IPSP is succeeded by two sharp hyperpolarizing steps, i.e. presumably two late IPSPs. The intervals between the three inhibitory steps are similar to the intervals between the D, I_2 and I_3 waves respectively.

The neurone of Fig. 6*B* was the only one among the present cells in which practically no early excitation was obtained (0·2 mV or less), and the cell would therefore, on the present view, be expected to receive unusually little late excitation. In Fig. 6*B* it is seen that a stimulation of 3·9 mA elicits a prominent early IPSP (latency 3·5 msec), and at 12·1 mA two distinct late IPSPs are obtained. The depolarizing overshoot at the end was inconstant and probably not significant. In all neurones except this one (Fig. 6*B*) the late synaptic events had at least some net depolarizing effect, although this depolarization could be interrupted by inhibitory impacts (Fig. 6*A* 6·0 mA). It should be noted, however, that in some cells the effect on a spike discharge could have been largely inhibitory although a net depolarization was obtained at a higher membrane potential than that of the firing level. In several cells with a marked late inhibition and little excitation, the late synaptic events gave rise to a net hyperpolarization as the membrane potential decreased towards the end of a penetration (cf. Phillips & Porter, 1964).

Clear signs of late inhibition were seen only in eight of the motoneurones. To judge from this small material, however, the results of Fig. 6 were typical. Thus, late inhibition usually appeared to occur in distinct steps, and this is evident also in several of the illustrations published by Phillips & Porter (1964). The cortical stimulus threshold for late IPSPs was usually about the same or somewhat higher than that for simultaneously recorded I waves, and the intervals between the inhibitory steps often seemed to be related to the intervals between the various pyramidal tract waves (Fig. 6*A*). The results suggest that, as would be expected, much of the late inhibition is caused by the I wave discharge of fast pyramidal fibres via the same group of interneurones as that responsible for the early inhibition.

If late inhibition is, as it were, a repetition of early inhibition, then a late inhibition would be expected to be visible mostly in cells which also show clear signs of an early inhibition. This was actually the case. Signs of late inhibition were seen in seven out of fifteen cells with an early inhibition, but only in one out of eighteen cells lacking signs of early inhibition. All these cells were studied with electrodes filled with potassium citrate. The preferential occurrence of late inhibition in cells possessing an early one was statistically significant (chi-square test, $0{\cdot}001 < P < 0{\cdot}01$). When studied at a high gain and a rapid sweep, a mixture of late excitation and inhibition often looked like a fast repetition of the early EPSP-IPSP sequence. It should be noted, however, that in several cells possessing an

early inhibition no clear signs of late inhibition were seen, and that even if late IPSPs were visible, they were often seen to succeed some, but not all, of the excitatory steps. Such irregularities in the behaviour of late inhibition are not surprising when considering that the pathway most probably includes an interneurone. Furthermore, a small amount of late inhibition might well have been invisible in many of the cells because it was concealed by strong excitatory impacts, or because the membrane potential was too high (cf. Phillips & Porter, 1964).

It was noted above that clear signs of an early inhibition were seen in only about half the number of neurones studied with electrodes filled with potassium citrate. The appearance or absence of early inhibition was not found to be related to the spike amplitude of the respective cells. It was interesting to note, however, that an early inhibition was most often seen in motoneurones which received relatively little early excitation (cf. Fig. 6). Of the fifteen cells with a clearly visible early inhibition, only three had an early EPSP with a maximal size of 2·0 mV or more, whereas of eighteen cells without any evident early inhibition, twelve cells had an early EPSP of 2·0 mV or more (chi-square test, $0{\cdot}001 < P < 0{\cdot}01$). The preferential occurrence of early inhibition in cells with little early excitation was as significant when comparing the neurones with respect to the maximal size of the early EPSP at 1 msec after its onset. Thus, the early EPSPs of cells with early inhibition were not small merely because they failed to reach their full size before the onset of the IPSP. A small IPSP would, of course, be more difficult to see on top of a large EPSP than on top of a small one. The findings indicate, however, that cells receiving much early excitation do not receive a corresponding large amount of early inhibition.

Thus, the relation between early excitation and inhibition would in general be more strongly in favour of excitation when the early EPSP is large, and it would be more strongly in favour of inhibition when the early EPSP is small. This should be true also for the relation between late excitation and inhibition, as the latter apparently are predominantly due to a repetition of the early excitation and inhibition. Therefore, one would in general expect the 'maximal' net depolarization, produced by a strong cortical stimulus, to be related to the maximal size of the early EPSP. This will be shown to be the case in the following paragraph.

The 'maximal' net depolarization produced by a strong cortical stimulus. The peak amplitude of the net post-synaptic depolarization produced by a strong cortical stimulus, could be very large, in several cases up to 10 mV or more (Fig. 1*B*). In many cells, this 'total peak amplitude' could not be measured at strong stimuli because spike firing would occur. In twenty-four motoneurones the total peak amplitude could be studied at a stimulus intensity of 7 mA or more. At these high intensities of

cortical stimulation, differences in total peak amplitude between the various cells were not in any obvious way related to differences in cortical stimulus strength. The increase of total peak amplitude was generally small at stimulus intensities above some 7 mA, and in several cases no further increase of the total peak amplitude could be seen as the stimulus strength was increased above 7–10 mA. In all of these cells, the cortical stimulus caused some depolarization in excess of the early EPSP (the exceptional case of Fig. 6*B* is not included here), and the peak of the post-synaptic depolarization occurred at $7{\cdot}0 \pm 0{\cdot}4$ (S.E., measured for twenty of the cells) msec after the onset of the early EPSP. In Fig. 7 it is shown that for these cells there was an obvious relation between the 'maximal' total peak amplitude and the maximal size of the early EPSP.

The cells of Fig. 7 have been collected in three groups with small, medium and large early EPSPs, respectively, and for each group the mean value of the total peak amplitude has been plotted against the mean size of the maximal early EPSP. Vertical and horizontal bars give the standard errors of the respective means. The difference between the total peak amplitude of the two extreme groups is highly significant (t test, $P < 0{\cdot}001$). In the diagram, the difference between the total peak amplitude and the size of the early EPSP is given by the distance between the plotted values and the interrupted line (the unity line). The additional depolarization caused by late synaptic effects is clearly related to the amount of early excitation received by the cell. Furthermore, it should be noted that this is apparently also true when considering cells with and without clear signs of early inhibition together. Ten of the cells in Fig. 7 had an evident early inhibition.

The relation plotted in Fig. 7 actually indicates that, on an average, there was roughly a direct proportionality between the 'maximal' total peak amplitude and the size of the early EPSP. As would be expected, the relation between the total peak amplitude and the size of the early EPSP tended to be somewhat lower for cells with signs of early inhibition. For such cells the relation was $3{\cdot}0 \pm 0{\cdot}4$ (S.E., ten cells), and for those without inhibition it averaged $4{\cdot}0 \pm 0{\cdot}3$ (S.E., fourteen cells). The average number of excitatory steps on the rising phase of the potential (including the early EPSP) was $4{\cdot}1 \pm 0{\cdot}2$ (S.E., twenty-four cells) and it was not significantly different for cells with and without inhibition, or for cells with a large and small early EPSP respectively. The relation plotted in Fig. 7 was equally significant when considering only the cells lacking signs of early inhibition.

These findings strongly support the view that most of the build-up of late excitation was due to monosynaptic EPSPs generated by fast pyramidal fibres firing during the I waves. The duration of an early EPSP was

of the order of 15 msec (Fig. 1*A*; Landgren *et al.* 1962). Thus, if no further excitation were received by the cell, one would expect the decay of late facilitation to last only some 15 msec following the final late EPSP-step. This was the case for several motoneurones, whereas for others a gradual decay could last for various times up to 30 msec or more (Fig. 1*B*). In the latter motoneurones the decay of late facilitation might have been prolonged because of some later arriving asynchronous excitation whose

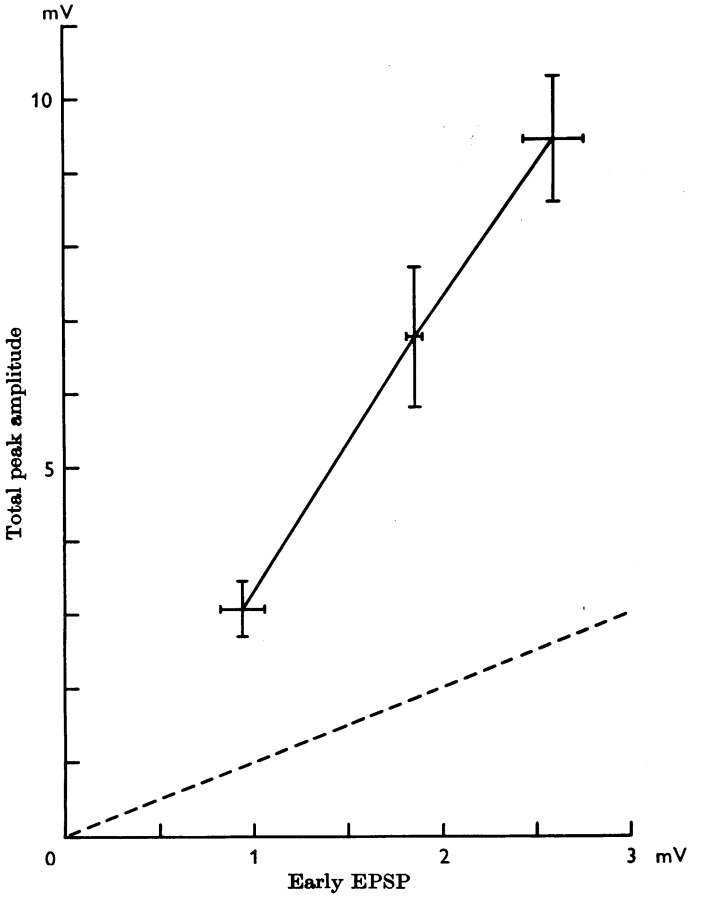

Fig. 7. Diagram showing, for twenty-four motoneurones, relation between total peak amplitude of post-synaptic depolarization elicited by a cortical pulse at 7 mA or more (ordinate) and maximal size of early monosynaptic EPSP (abscissa). The cells have been collected in three groups with small (0·6–1·5 mV, 9 cells), medium (1·7–2·0 mV, 9 cells), and large (2·1–3·3 mV, 6 cells) early EPSPs respectively. For each group, the mean value of total peak amplitude has been plotted against the mean size of early EPSP, and plotted mean values have been connected by straight lines. Horizontal and vertical bars give standard error of respective means. Interrupted line is the unity line.

origin cannot be determined from the present experiments. It should be noted that such a prolonged decay of late excitation was usually clearly seen only at the very strongest intensities of cortical stimulation.

DISCUSSION

The results have shown that most of the late facilitation which could be produced in forelimb motoneurones by a single cortical pulse consisted of monosynaptic EPSPs generated by repetitively discharging fast pyramidal fibres. The results also indicate that much of the late inhibition was caused by the discharge of fast pyramidal fibres via the same group of spinal interneurones as that which is presumably responsible for the early inhibition (Preston & Whitlock, 1961; Landgren *et al.* 1962; Phillips & Porter, 1964). The existence of some asynchronously arriving excitation and inhibition from other sources cannot, of course, be excluded. All the evidence tends to show, however, that such asynchronous effects must have been relatively unimportant, especially during the initial 5–10 msec following the onset of the early EPSP. The only evidence suggesting the existence of significant synaptic effects other than those due to a repetition of the early EPSP and IPSP was a prolonged decay of the synaptic depolarization, and this was obtained only in some cells, and only at the strongest intensities of cortical stimulation. Thus, even if the 'extra-pyramidal' connexions between the cortex and the spinal cord were destroyed, late synaptic effects elicited in motoneurones by cortical stimulation could to a large extent be due to the response of the cortex to the stimulus (the I wave discharge) rather than on pyramidal actions which were delayed because they were mediated by slow fibres or chains of spinal interneurones. A cortical stimulus is apparently not very useful for mapping out the latter type of functional connexions between the pyramidal tract and spinal motoneurones. The present results also suggest, however, that previous findings concerning the late synaptic effects in various motoneurone pools might give an indication of the relative amount of early excitation received by the respective cells. A prominent late facilitation would, on the present view, indicate that the cell in question receives a large amount of monosynaptic excitation from the cortex (cf. Fig. 7). Conversely, late actions whose net effect on spike discharge was inhibitory, would suggest that the cell receives only a small amount of monosynaptic excitation, but an appreciable amount of early inhibition. Previous studies with monosynaptic testing techniques have shown that, in the hind limb region of the monkey, the motoneurones innervating functional flexors tend to receive a marked late facilitation, whereas the net result is usually one of inhibition for motoneurones belonging to functional extensors (except gastrocnemius) (Uemura &

Preston, 1965). On the present view, these results would suggest that, in the hind limb, the pyramidal monosynaptic excitation is more marked in motoneurones of flexors than in those of extensors. It has also been shown that motoneurones of the fast head of triceps surae (gastrocnemius) receive a prominent late facilitation, whereas those of the slow head (soleus) receive a late inhibition (Preston & Whitlock, 1963). These results would suggest that, at least within triceps surae, the pyramidal monosynaptic excitation might be more marked in fast motoneurones than in the slow ones.

The present interpretation of the synaptic effects on motoneurones of a single brief but strong cortical stimulus is very similar to that previously advanced for the effects of a weak but more prolonged surface-anodal pulse. With the latter type of stimulus, a fast repetitive discharge of cortico-spinal neurones is caused directly by the steady current instead of by activating intracortical synaptic systems, and the excitatory effects elicited in motoneurones by a long surface-anodal pulse have also been considered to be due mainly to monosynaptic impacts generated by fast pyramidal fibres (Landgren *et al.* 1962). With either type of stimulus, a spike discharge would be expected to result predominantly in cells which, in comparison with other neurones, receive a large amount of monosynaptic excitation from the cortex (cf. Fig. 7).

During the present experiments, inhibition appeared to be relatively more powerful in cells receiving little than in those receiving much monosynaptic excitation. This might suggest that, between individual motoneurones, inhibition and excitation tend to be distributed in a reciprocal manner. It should be noted, however, that the inhibitory effect is likely to depend to some extent on the excitability of the postulated inhibitory interneurone, and this might well be influenced by other synaptic inputs.

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