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CORRELATION OF THE INHIBITORY POST-SYNAPTIC POTENTIAL OF MOTONEURONES WITH THE LATENCY AND TIME COURSE OF INHIBITION OF MONOSYNAPTIC REFLEXES

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Afferent volleys in the second or third sacral dorsal roots inhibit the contralateral motoneurones with a central latency which is so brief that Lloyd & Wilson (1959) have been led to draw several conclusions, which they have extended to the so-called direct or Ia inhibition as investigated with volleys in limb nerves (cf. Lloyd, 1960): that the central inhibitory path is monosynaptic; that the inhibitory post-synaptic potential of motoneurones is not the primary agent of this inhibition, but may be a secondary and later manifestation of the inhibition; that the spike potential recorded intracellularly in the soma cannot be used as an index of the reflex discharge of an impulse along the axon.

These conclusions stand as a challenge to an extensive conceptual structure that has been built upon a precise study of Ia inhibition both by intracellular recording from motoneurones, and by a careful correlation of these results with inhibition of monosynaptic reflexes discharged by the motoneurones (Eccles, 1953, 1957, 1958; Coombs, Eccles & Fatt, 1955*b*; Curtis, Krnjević & Miledi, 1958; Frank & Sprague, 1959; Curtis & Eccles, 1959). Thus it has been concluded that the inhibitory action of a Ia afferent volley is fully explained by the motoneuronal hyperpolarization produced by that volley (the inhibitory post-synaptic potential, IPSP), or more strictly by the IPSP together with the ionic currents that generate it. It has further been concluded, from an extensive series of investigations, that the central pathway for Ia or 'direct' inhibition is disynaptic, and the inhibitory interneurones on this path have been located and shown to have the requisite properties (Eccles, Fatt & Landgren, 1954, 1956; Jack, 1957; Curtis *et al.* 1958; R. M. Eccles & Lundberg, 1958). Finally, it has

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been shown that the IS component of the spike potential, as recorded intracellularly, is a direct antecedent of the impulse discharged along the axon in the ventral root (Araki & Otani, 1955; Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957*a*, *b*).

Evidently the contradiction extends to the very nature of synaptic action on neurones (cf. Lloyd, 1960), and its resolution is hence of urgency and importance. Crucial experiments have been designed for this purpose. Lloyd & Wilson (1959) make the point that it is essential to investigate the inhibition of impulses discharged along axons in the ventral root, rather than impulses recorded in the somas of motoneurones. The present investigation has conformed to this criterion, the latency of Ia inhibition being measured directly on the monosynaptic reflex spike, which has never previously been attempted except for one record published by Frank & Sprague (1959). These latencies and a series of other investigations establish that the IPSP accounts precisely for Ia inhibition of monosynaptic reflexes. In the discussion it is argued that the conclusions of Lloyd & Wilson (1959) are refuted, and that the contradiction is thereby resolved.

METHODS

The animals (ten cats) were under light Nembutal (pentobarbitone; Abbott Laboratories) anaesthesia with the spinal cord divided at the second lumbar segment. For the investigation of Ia inhibition of motoneurones supplying hind-limb muscles, nerves to quadriceps (Q) and to posterior biceps-semitendinosus (PBST) muscles were mounted on stimulating electrodes, and the ventral roots from L 5 to S 1 were severed. The S 1 or the lower half of the L 7 ventral root was mounted for monophasic recording of reflex spikes produced by Group I PBST volleys. In order to define accurately the onset of inhibition as illustrated in Figs. 1–3, it was essential to have a large and relatively stable reflex spike potential. In most experiments facilitation or potentiation of the monosynaptic reflex was required for this purpose. This was achieved either by facilitation by a reflexly subliminal PBST volley about 1·5 msec earlier, or by potentiation by a brief tetanus (450/sec for 200 msec) of the PBST nerve ending about 150 msec earlier. This latter procedure was preferable and was adopted in our later experiments, the interval between successive tests being lengthened to 7 sec instead of the 3·5 sec which was employed when single or double stimuli were applied to the PBST nerve. If the latency of inhibition is to be demonstrated by the reflex spike responses, the interval between the inhibitory (Q) volley and the excitatory (PBST) volley must be chosen so that the onset of the inhibition occurs during the rising phase of the reflex spike potential (cf. Figs. 1–3). Furthermore, the sweep speed must be so fast that the superimposed traces of the inhibited spikes and the control spikes are clearly distinguishable. This was accomplished by expanding an appropriate part of the sweep so that it covered the whole face of the tube. When the spike was potentiated as above, the sweep was accelerated about 200 times so as to give records such as those of Figs. 1 A–F, 3. Similar procedures were employed when investigating the inhibitory action of the contralateral S 3 afferent volley on the monosynaptic reflex spikes which an ipsilateral S 3 afferent volley evokes in the equivalent ventral roots. The afferent volleys were always recorded by an electrode on the appropriate dorsal root at its entry into the spinal cord, such electrodes being at the upper L 6 and S 1 levels for Q and PBST volleys respectively. The arrival time of the volley at the cord was taken to be the peak of the first (positive) deflexion, as indicated in Figs. 1–3.

When recording potentials electrotonically transmitted along the ventral roots, the spinal cord was rotated by traction on the denticulate ligament and slightly raised from its bed by small balls of cotton wool. In order to discover and utilize the ventral-root filaments containing the highest proportion of axons of PBST motoneurones, the S1 or lower L7 ventral roots were carefully split into filaments which were mounted one at a time on fine platinum hooks employed as recording electrodes. The proximal electrode was within 1 mm of the exit of the root, while the distal was at least 15 mm away. Root splitting of large ventral roots had the additional advantage that it made it easier to record from the ventral-root fibres as soon as they emerged from the spinal cord. It was not as a rule necessary for the much smaller S3 ventral root.

The procedure for intracellular recording from motoneurones has already been described (Brock, Coombs & Eccles, 1952; Coombs *et al.* 1957*a*). Since IPSPs were being recorded, the electrodes were filled with 0·6 M-K_2SO_4 or 2 M-K citrate solution. The excitability of motoneurones was tested during IPSPs by means of square pulses applied through the intracellular micro-electrode in the depolarizing direction (cf. Fuortes *et al.* 1957; Coombs, Curtis & Eccles, 1959).

RESULTS

The central latency of Ia inhibitory action on motoneurones

It has been generally accepted that a comparison between the central latencies of Ia inhibitory action and of Ia monosynaptic excitatory action cannot simply be derived from an investigation of the latest time at which an inhibitory volley can be placed relative to an excitatory and yet exert an inhibitory action on the height of the reflex spike potential as recorded in a ventral root. Even under the most favourable conditions the monosynaptic reflex discharge is composed of individual discharges dispersed over a considerable fraction of a millisecond, as is indicated diagrammatically in Fig. 1I, J. With such a critical disposition of the volleys the inhibition would be exerted on the latest reflex discharges that contributed to the summit height of the spike potential, as indicated diagrammatically in Fig. 1K, L (cf. Eccles *et al.* 1954, 1956; Frank & Sprague, 1959; Lloyd & Wilson, 1959); yet this presumed effect has not been investigated, except in one illustration by Frank & Sprague (1959), who, however, did not utilize it in measuring the latency of central inhibitory action.

In Fig. 1A–F the inhibitory Ia volley in quadriceps (Q) nerve preceded the excitatory PBST volley by intervals ranging from 0·5 to 1·0 msec, as measured from the traces recorded by an electrode on the dorsal root at the upper L6 segmental level. A second electrode at the entry of the upper S1 dorsal root revealed that the BST volley entered the spinal cord 0·17 msec earlier than its arrival at the L6 level, as indicated by the electrical potential it generated there. The records have been arranged so that the spike potentials of the Q volleys are in the same vertical line (the dotted line to the left). Each record was formed by the superposition of about eight traces of the inhibited reflex spike and eight traces of the uninhibited reflex spike. It will be noted that the traces of reflex spikes in

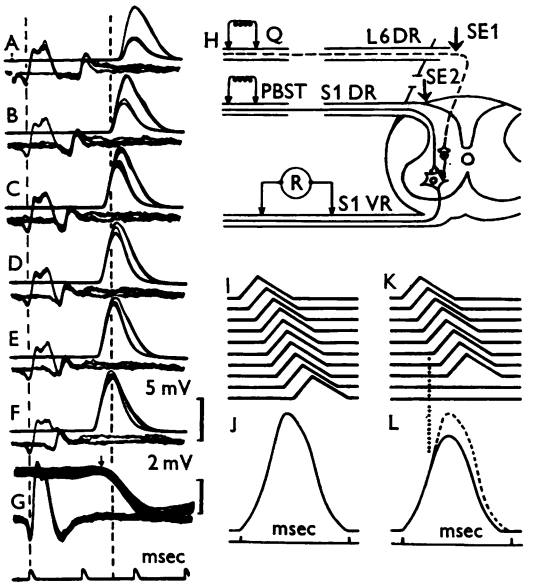

Fig. 1. The experimental arrangements are shown diagrammatically in H. In A–F a monosynaptic reflex spike response (monophasically recorded from S 1 ventral root (upper traces)) was generated by an afferent volley from PBST, which also produced a diphasic spike in the records from the dorsum of the cord (lead SE 1 in H) at the upper L 6 level (lower traces). This PBST volley reached the cord at various times after a maximum Group I afferent volley from quadriceps, which is at a fixed position in A–F, its arrival time at the upper L 6 level being given by the left perpendicular broken line. The superimposed traces for each of the testing intervals A–L were formed by first photographing eight traces for the BST volley alone at 7 sec interval to give the control reflex spike, and then a further eight traces with quadriceps volley in addition. It will be seen that at each testing interval the inhibited reflex spike deviates from the control. The second vertical broken line at 1·59 msec from the first passes through the onset of the inhibition so signalled by the reflex spikes for all but record A, where evidently the inhibition was operative before the beginning of the testing reflex response. In G there is an IPSP produced by the Q volley and intracellularly recorded from a PBST motoneurone at the S 1 segmental level a little later in this same experiment. The record of the Q volley at the upper L 6 segment was at higher amplification, but otherwise very similar, and it is exactly synchronized with the Q volleys in A–F, the sweep speeds being identical. Note different potential scales for the reflex spike and for the IPSP. The manner of production of a record such as C or D is illustrated in the constructions, I–L. In I there are schematic reflex spikes in ten fibres dispersed over 0·5 msec, as in a normal monosynaptic reflex, which is derived as in J by summation. Inhibition, beginning at the dotted line in K, delays the onset of the 6th to 8th spikes, as shown, and suppresses the 9th and 10th. As a consequence the summed reflex potential (the continuous line in L) deviates from the control (the broken line in L) at a point just later than the dotted line.

Fig. 1 C closely resemble the construction in Fig. 1 L. As would be expected in the several records at the different test intervals the onset of inhibitory depression of the reflex spike occurred at virtually the same time after the inhibitory volley, the respective points of divergence lying very close to the perpendicular broken line 1·59 msec later than the line giving the arrival time of the inhibitory volley at the upper L 6 segment. In Fig. 1 A the inhibition had already begun before the onset of the testing spike.

An alternative method for measuring central latency (Fig. 2) has the advantage of giving a reflex spike potential that is more expanded in

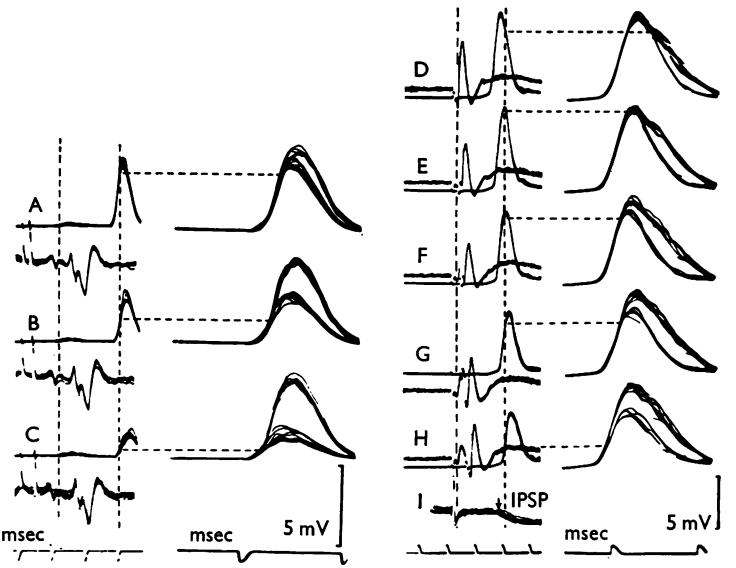

Fig. 2. A–C; PBST monosynaptic reflex spike response in S 1 ventral root and its inhibition by the quadriceps volley as in Fig. 1, but in a different experiment. D–H; ipsilateral monosynaptic spike response in S 3 ventral root and its inhibition by the contralateral S 3 dorsal-root volley. In each set of records the left traces show the inhibited reflex spike (upper beams) and the afferent volley recorded on the dorsal-root entry (lower beams). The inhibitory volleys are at a fixed position in the sweep and the excitatory volleys are shifted from the left to the right in the series of A to C and D to H. The right-hand traces in each series illustrate the monosynaptic reflex spike with a faster sweep velocity, the inhibited responses (8 traces) being superposed upon the control ones (8 traces). The horizontal broken lines are drawn from the diverging points of the inhibited and control responses in the faster sweep to the corresponding points on the companion slower records, through which the vertical interrupted lines are drawn so as to indicate the onset time of the inhibition, as in Fig. 1. The vertical lines to the left indicate the time of arrival of inhibitory volleys at the spinal cord, at upper L 6 level in A–C and at S 3 level in D–H. Since the afferent volley was recorded at upper S 1 level in A–C, the first vertical line was drawn delayed by 0·13 msec. In I, a specimen IPSP recorded intracellularly from a motoneurone in S 3 region is illustrated with the same time base, its onset being marked by a small arrow.

time, so that the first point of divergence of the control and inhibited spike potentials can be more accurately determined than in Fig. 1. From this point horizontal lines are drawn to the companion slower records on which the inhibitory latency is measured as in Fig. 1, and in Fig. 2A–C this latency is 1·82 msec. Since in this series the afferent volleys were recorded at S1 level, an allowance of 0·13 msec was made in drawing the first vertical line in order to allow for the measured propagation time of the Q volley to its entrance into the cord at upper L6 level.

Altogether by the one or other method the inhibitory latency of Q volleys on PBST monosynaptic reflexes has been measured in experimental series in six preparations. The values have ranged from 1·59 to 1·90 msec with a mean of 1·72 msec.

Inhibitory latencies determined as in Figs. 1 and 2A–C can be readily compared with the latencies of the IPSPs produced by Q volleys in PBST motoneurones at the same segmental level. For example, in Fig. 1G there is the IPSP recorded shortly afterwards in a PBST motoneurone. When arranged in this manner the onsets of the IPSPs have been found to precede the beginnings of the reflex spike inhibition by 0·06–0·3 msec (mean 0·21 msec), which corresponds approximately to the conduction time of the reflex spike from the cord to the proximal recording electrode. For example, in the experiment of Fig. 1 there was 0·28 msec between stimulation of the ventral root through the proximal recording electrode and the onset of the antidromic spike potential as recorded in the soma (Coombs *et al.* 1957*a*). Thus the central inhibitory latency as derived from Fig. 1 is 1·31 msec, which is to be compared to 1·37 msec for the latency of the IPSP in Fig. 1G. This excellent agreement between the latencies of the IPSPs of motoneurones and the reflex spike inhibitions has been seen in every experimental series.

Similar investigations on the latencies of reflex spike inhibition and of IPSPs have been performed on the inhibition of motoneurones in the S3 segmental level by volleys in the lowest threshold afferent fibres of the contralateral S3 roots. As is illustrated in Fig. 2D–H and Fig. 3, the latency of inhibition of the reflex spike could be similarly determined and compared with the latency of the IPSP of the same motoneurones. The series of Fig. 2D–H resembles Fig. 2A–C in the method of deriving the inhibitory latency, which was 1·74 msec for the spike, as compared with 1·53 msec for the IPSP in I. In the series of Fig. 3A–D the monosynaptic reflex spikes were facilitated by a submaximal S3 volley 2 msec earlier, as described in Methods. The latency of the inhibitory action of the contralateral S3 volley on the reflex spikes as measured between the two vertical lines was 1·58 msec, while in E and J the latencies of the intracellularly recorded IPSPs, as measured to the arrows, were 1·38 and 1·20 msec,

respectively. In the series F–I on the same preparation the reflex spikes were potentiated by a brief conditioning tetanus, as described in Methods. The contralateral inhibitory action on the very large reflex spike was much reduced, but the great stability of the reflex allowed the latency to be accurately measured, the interval between the two vertical lines giving a value of 1·50 msec.

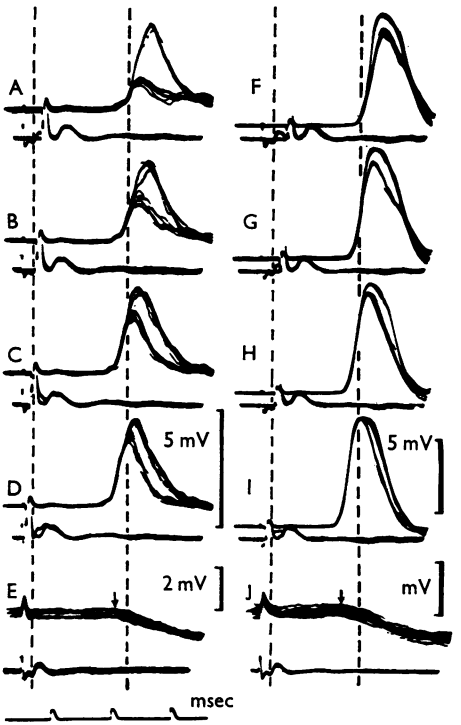

Fig. 3. The latency measurement of inhibition in the S3 ventral root, with two different methods of potentiation of the reflex. Arrangements of records are the same as in Fig. 1. The monosynaptic reflex by the ipsilateral dorsal root stimulation was potentiated in A–D by a subliminal conditioning stimulus and in F–I by tetanic stimuli (see Methods). E and J show IPSPs recorded intracellularly from two different motoneurones in the S3 segment of the same preparation.

The inhibitory latency of reflex spikes in the S3 ventral root was measured in many series in five separate experiments, the mean value being 1·57 msec (range, 1·31–1·78). When allowance was made for the conduction time to the proximal recording electrode on the S1 ventral root (0·2–0·3 msec), there was again excellent agreement between the latencies of the inhibitory action on the reflex spike and of the IPSP, the mean value for the latter being 1·32 msec (range 1·20–1·40).

In contradistinction, Lloyd & Wilson (1959) calculated that the latency

for reflex spike inhibition was as much as 1·0 msec briefer than the latency of the IPSP generated by Ia afferent volleys. It will be shown in the discussion that this discrepancy derives from errors in their assumptions and in the calculations derived therefrom.

Since there is now such excellent agreement between the central latencies for inhibition as tested by the IPSP and by the inhibition of reflex spikes, it is justifiable to use the former measurements when comparing the central latencies for Ia inhibitory and excitatory action on motoneurones, a procedure which has been routinely employed (Brock *et al.* 1952; Eccles *et al.* 1954, 1956; Curtis *et al.* 1958; Frank & Sprague, 1959). In the present experiments the mean central latency for the IPSP was 0·75 msec longer than for the EPSP of S 3 motoneurones and 0·91 msec longer for inhibition of PBST motoneurones. As previously noted (Eccles *et al.* 1956), the latter value is longer because of the longitudinal path of 10–15 mm down the spinal cord. With approximate equality of the inhibitory and excitatory pathways the latency differential is reduced to about 0·75 msec. The presynaptic impulses responsible for the inhibitory action on PBST motoneurones have been recorded in the immediate environment of the inhibited motoneurones only 0·3 msec before the onset of the IPSP (Eccles *et al.* 1956), hence the same time of 0·3 msec is occupied by excitatory and inhibitory presynaptic impulses in initiating the EPSP and the IPSP respectively. The latency differential of about 0·75 msec cannot therefore be attributed either to differences in the times involved in the actual synaptic mechanisms or in the lengths of the respective central pathways. Frank & Sprague (1959) reported that there was a briefer latency differential (range 0·3–0·7 msec) for the EPSPs and IPSPs at the S 2 and S 3 levels, which is briefer than values observed by Curtis *et al.* (1958) and in the present experiments. However, their EPSPs and IPSPs showed a shortening of latency by as much as 0·6 msec as the stimulus strength was increased. In the absence of records from the dorsal roots showing the timing of the volleys entering the spinal cord, the abnormally large latency shifts remain inexplicable, but suggest that the brief latency differentials of 0·3–0·7 msec cannot be regarded as in conflict with the mean differential of about 0·8 msec obtained in all other investigations.

The time course of the Ia inhibitory action

In the standard procedure the time course of the inhibitory action of a single Ia afferent volley has been determined by observing the depression of a testing monosynaptic reflex at various intervals after the inhibiting volley. Inhibitory curves so determined have always had a sharp rising phase, maximum inhibitory action being observed at stimulus intervals of 0·5–1·0 msec, but there have been very large differences in the decaying

phases reported by different investigators. In the first description there was an approximately exponential decay with a time constant of about 4 msec (Lloyd, 1946), but this curve was only rarely obtained by Laporte & Lloyd (1952), who attributed the much more rapid decay that was usually observed to a superimposed disynaptic excitatory action of the Ib afferent impulses which contaminated the conditioning volley. However, Bradley, Easton & Eccles (1953) regularly found this fast initial phase of decay even when the conditioning volley had been shown to have no appreciable admixture of Ib impulses. It was later postulated that the time course of this inhibitory curve was due to two superimposed factors. The hyperpolarization of the IPSP would be expected to give an inhibitory curve with an approximately exponential decay, as observed originally by Lloyd; but superimposed thereon is an initial brief depressant action produced by the intense ionic currents that flow for about 2 msec through the patches of post-synaptic membrane activated by the inhibitory transmitter (Coombs, Eccles & Fatt, 1955*c*; Curtis & Eccles, 1959). Finally, Jack, McIntyre & Somjen (1959 and personal communication) have suggested that, when the spinal cord is in particularly good condition, the membrane potential of the motoneurone is so high that no hyperpolarization is produced by inhibitory synaptic action. The Ia inhibition would thus be due entirely to the ionic currents, and consequently would have a duration of about 2 msec. This extreme position was based on two experimental findings. Occasionally very brief inhibitory curves were observed, there being no detectable inhibitory action after the initial intense phase; and, in contrast to excitatory post-synaptic potentials, no inhibitory post-synaptic potentials could be recorded as a result of electrotonic transmission to the ventral root as it emerged from the spinal cord. The intracellularly recorded IPSP was thus postulated to be a consequence of the lowering of membrane potential due to the impalement by the micro-electrode (cf. Lloyd & Wilson, 1959).

It has therefore been important to redetermine the Ia inhibitory curve under conditions in which complicating excitatory Ib actions could be excluded. Invariably it has been found that the initial intense phase has declined smoothly into a much more slowly decaying component, exactly as described by Bradley *et al.* (1953) and as usually found by Jack *et al.* (1959). For example, in Fig. 4 very weak stimulation of the quadriceps nerve was employed to inhibit the monosynaptic reflex spike evoked by a maximal Group I BST volley in S 1 ventral root. The stimuli for the two inhibitory curves plotted in Fig. 4B were 1·2 and 1·4 times threshold, and well below the threshold for Ib afferent fibres, which by the double stimulation technique (Bradley & Eccles, 1953; Eccles, Eccles & Lundberg, 1957*a*) was determined at 1·7 times threshold. The broken lines indicate

for these two inhibitory curves the approximate course of the inhibition that could directly arise on account of the hyperpolarization recorded in the IPSP of Fig. 4A. The inhibitory curve that had the least slow component is shown in Fig. 5G, together with specimen records of the inhibited reflex spike at several testing intervals (Fig. 5A–E), and in Fig. 5F tracings of the quadriceps afferent volley and a specimen IPSP. An unusual procedure was employed in assessing the abscissae in Fig. 5G: instead of the conventional plotting of intervals between the entry of the inhibitory and

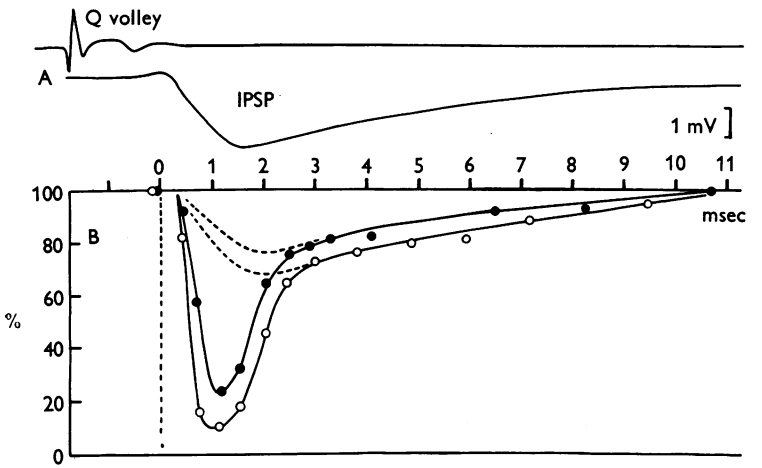

Fig. 4. Inhibitory curve in which the inhibitory action of a quadriceps Ia afferent volley is tested by the size of a monosynaptic reflex spike discharged into the S1 ventral root from BST motoneurones. In B the ordinates show the percentage size of the reflex spike, the abscissae the testing volley intervals. ●, inhibitory stimulus 1·2 threshold, ○ 1·4 threshold for Ia fibres. The dotted lines indicate the approximate time courses of the components of inhibition attributable directly to the hyperpolarization of the IPSP, which is seen in the traced record in A. The afferent quadriceps volley (upper trace of A) is recorded at the upper S1 level.

excitatory volleys into the cord, the abscissae in Fig. 5G are measured from the entry of the inhibitory volley to the summit of the reflex spike potential. As a consequence the latency of origin of the curve (1·8 msec) is the result of approximately the same method of measurement of the latency of inhibition as is given by the interval between the vertical lines in Fig. 1. It is seen likewise to be a little longer than the latency of the IPSP in Fig. 5F.

The manner in which a Ia inhibitory curve is built up from the responses of individual motoneurones is illustrated by testing for the range of intervals over which a motoneurone is inhibited by a Ia inhibitory volley of constant size. If the testing monosynaptic excitation is diminished in size, the range of the inhibited intervals increases as illustrated in Fig. 6. In

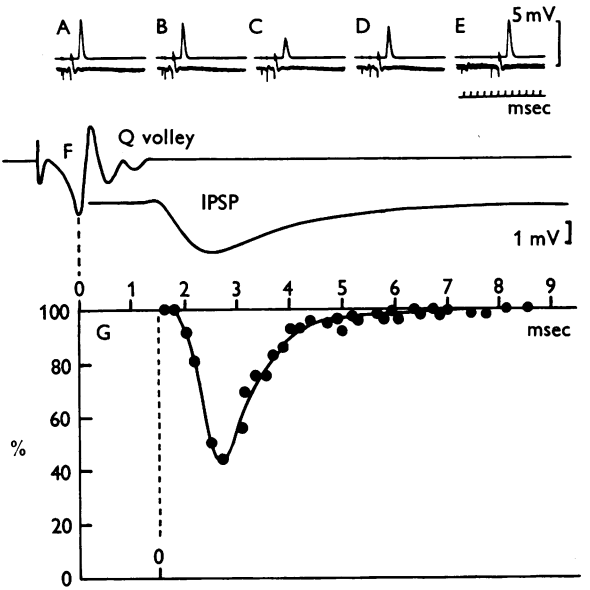

Fig. 5. An example of the inhibitory curve in which the quadriceps volley showed a relatively small later effect upon the PBST monosynaptic reflex (compare with Fig. 4). A–E specimen records of inhibited spikes (upper beams) at five different intervals. The lower beams are potentials recorded on the dorsal surface of the cord at upper S 1 level. A specimen IPSP recorded intracellularly from a motoneurone in the same preparation is traced in F together with the record of the quadriceps afferent volley. In G the ordinates give the percentage sizes of the reflex spikes and the abscissae are measured from the arrival of the quadriceps volley to the summit of the reflex spike. The zero point in the time scale is at the arrival of the quadriceps volley at the upper S 1 level, its entrance to the upper L 6 level being at 0·17 msec later. Zero volley interval, as in Fig. 4B, is shown below.

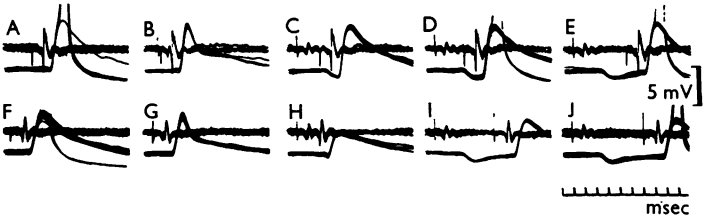

Fig. 6. The difference of ranges of intervals over which a motoneurone, activated by Ia EPSP of different sizes, is inhibited by Ia inhibitory volley of a constant size. The lower traces are potentials intracellularly recorded from a PBST motoneurone and upper ones the afferent volleys recorded at upper S 1 level. A–E, test volley maximal for Group Ia; F–J, test volley about 2/3 maximal for Ia.

the series A–E the testing reflex spike was evoked by a maximal Group Ia volley, and as shown in the control (A) it evoked a spike in all but one of about eight superimposed recordings. There was complete inhibition only at the relative short testing intervals of 0·36 and 2·14 msec (B, C), i.e. at the maximum of the inhibitory curve. In the series, F–J, the testing reflex spike was evoked by a Group Ia volley that was only about two thirds maximal, which evoked a reflex discharge in only two of the eight superimposed tracings. There was now complete inhibition with testing intervals ranging from 0·18 msec (G) to 5·3 msec (I).

The disadvantage of testing for inhibition by its diminution of a testing reflex discharge is that the ordinates give the relative population of the discharging motoneurones and do not give a direct measure of the intensity of inhibitory action. Such a measure is provided if the test stimulus is a brief rectangular current pulse that is applied in a depolarizing direction through the intracellular electrode during the IPSP (Frank, 1959; Eccles, 1958). At each testing interval during the inhibition, the pulse was varied in intensity until it evoked a spike potential in approximately one half of the trials (Fig. 7A–H). The spike potential was recorded either by the intracellular electrode, or from the appropriate ventral root filament. Figure 7K shows inhibitory curves obtained by this technique, the ordinates being measured as relative excitabilities, i.e. as reciprocals of the threshold current intensities. When the current pulse was very brief, as in A–D (0·2 msec), the time course of the inhibitory curve (Fig. 7K, short rectangles) corresponded closely with the time course of the IPSP (Fig. 7J). However, when, as in Fig. 7E–H, longer current pulses (0·8 msec) were employed, the same IPSP produced a much larger depression of excitability at the optimum intervals (Fig. 7K, long rectangles), but this brief intense phase was followed by a longer low-intensity phase where the depression was little more than that with the briefer test pulses. There is some uncertainty how such long pulses can be related to a curve that expresses the time course of the inhibitory depression. The whole duration of the pulse is evidently concerned in generating the discharge of an impulse. The best compromise is probably to regard the inhibitory curve as being defined by the mid points of the pulses, as in Fig. 7K. The time course of depression, as revealed by the long pulses, corresponds closely to the inhibitory curve determined by depression of monosynaptic reflexes (Figs. 4, 5). The relationship of these various types of inhibitory curves to the IPSP and the post-synaptic currents that generate it will be considered in the Discussion.

The electrical potential changes produced by Ia inhibitory action

Lloyd & Wilson (1959) and Jack *et al.* (1959 and personal communications) have reported that synaptic inhibitory action on motoneurones was not associated with any electrotonic spread of a hyperpolarization down their axons and so to the ventral root, though excitatory synaptic action on those same motoneurones caused the spread of a large depolarization. However, if the motoneurones were depolarized, the inhibitory action was observed to depress this depolarization, i.e. to produce a relative hyper-

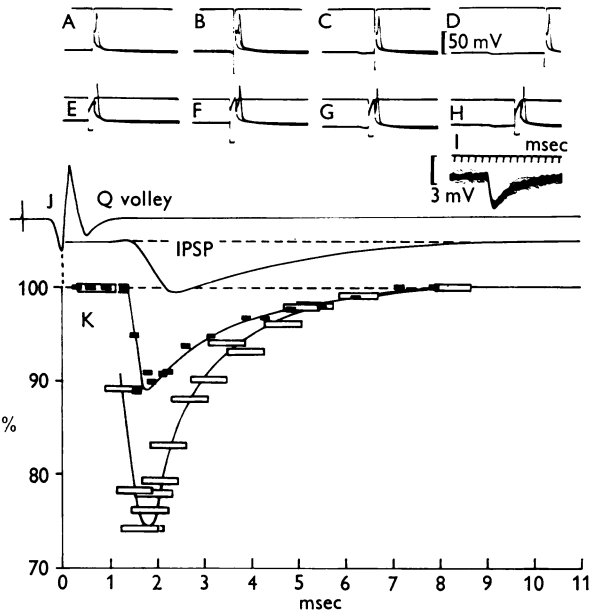

Fig. 7. In A–H depolarizing rectangular current pulses (the upper records with the pulses recorded downwards) were applied through an intracellular electrode (filled with 2M potassium citrate) in a biceps-semitendinosus motoneurone and adjusted in strength so that a spike potential was evoked in about half of the trials, as shown in the lower records, there being about 10 superimposed traces in each record. With A–D and E–H the pulse durations were 0·2 and 0·8 msec respectively and the control threshold current strength was 3·3 times larger in A than in E. In B–D and F–H the pulses tested excitability at various times relative to an IPSP, set up by a Ia quadriceps volley, which is shown at higher amplification and at the same sweep speed in I, and also plotted in J together with a tracing of the quadriceps volley (Q), as it entered the cord at the upper L6 level. In K the reciprocals of the threshold current strengths (relative to the control strength), for the series partly shown in A–H, are plotted on the same time scale as for J, zero time being measured relative to the entry of the Q volley into the cord. The 0·2 and the 0·8 msec pulses are shown respectively as solid and hollow rectangles of appropriate length. In drawing the two inhibitory curves it is assumed that the pulses test the excitability at the mid point of their duration.

polarization by electrotonic spread. These observations have given further support to the suggestion discussed above: that under conditions of normal resting potential, inhibitory synaptic action produces virtually no IPSP, and that the IPSP recorded by an intracellular electrode owes its existence to membrane depolarization consequent on the electrode penetration.

When attempting to record inhibitory hyperpolarizations that have propagated electrotonically from motoneurones down the motor axons and so to the ventral root, it is important to realize that these potentials would be very small. On the average the IPSPs intracellularly recorded

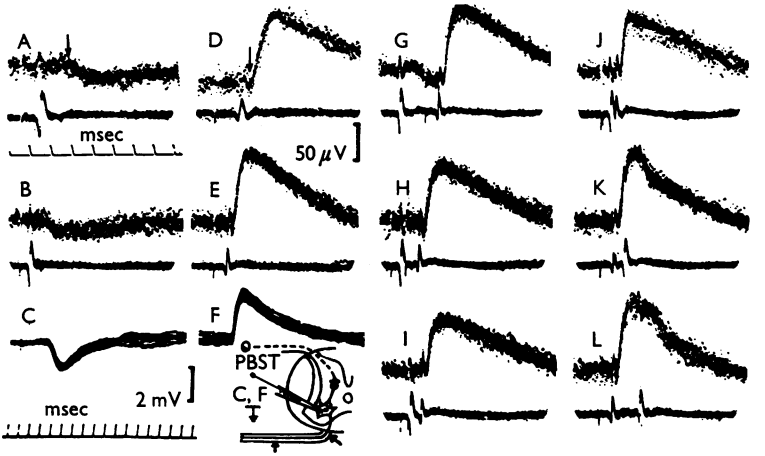

Fig. 8. C and F are intracellular records (see C, F in the inset diagram) of IPSPs and EPSPs evoked in a BST motoneurone at S 1 segmental level by a Ia quadriceps and a BST volley respectively, as shown in the inset diagram. The upper traces of the remainder are the potentials electrotonically conducted from the motoneurones along their motor axons and recorded from an isolated filament of the S 1 ventral root, one electrode being on the filament about 1 mm from its exit from the cord, the other at least 20 mm distally on the isolated filament, as shown by the two arrows in the inset diagram. A and B show potentials produced by a Ia quadriceps volley, D and E by a Ia BST volley. A and D are recorded at a faster time base, as shown, all other records being at same time base. G–L illustrate the interaction of EPSP and IPSP recorded in the ventral root when they are evoked at various volley intervals, as shown by the dorsal root records (from upper L 6) in the lower traces.

from PBST motoneurones in response to Q Ia volleys are several times smaller than the monosynaptic EPSPs generated by maximum Ia volleys in the PBST nerve. Furthermore, only a relatively small fraction of the fibres in any ventral root filament would be PBST motor axons, i.e. there would be a very effective shunting of any IPSP potential. Thus very high amplification must be employed and the potential change must be lifted out of the background noise by superposition of a great many superimposed traces. Figure 8 A and B shows that with such precautions a quadriceps

Ia afferent volley produced a well defined IPSP in a S1 ventral root filament about 1 mm after it emerged from the spinal cord. Monosynaptic EPSPs produced by a PBST volley were several times larger (D, E). Below B and E are shown on the same time scale the intracellularly recorded IPSP (C) and EPSP (F). The faster records, A and D, enable the latencies (note arrows) and rising phases of the ventral root IPSPs and EPSPs to be measured and compared. Comparison of B with C and E with

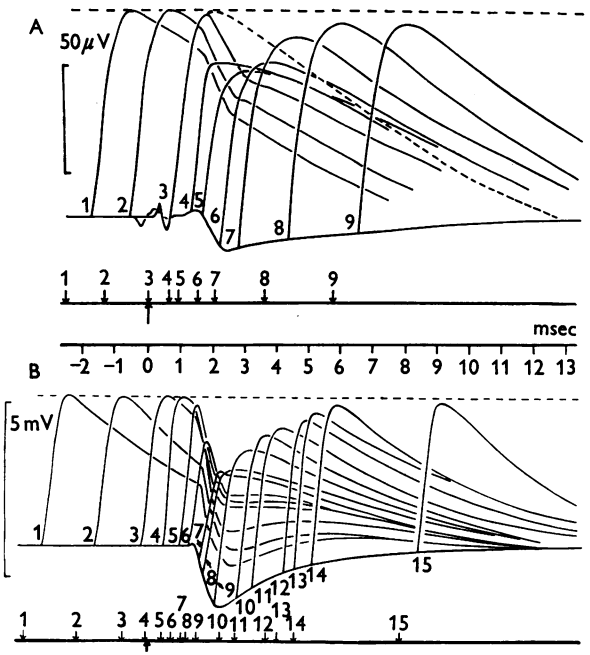

Fig. 9. Traces showing the interaction between IPSP and EPSP recorded electrotonically in S1 ventral root filament (A) and intracellularly from a motoneurone (B). Ia afferent fibres in PBST and quadriceps nerves are stimulated to give the excitatory and inhibitory volleys, respectively. Original records of A are in Fig. 8 G–L. Numbers at the foot of EPSPs are corresponding to those on the downward arrows which indicate the arrival time of PBST Ia afferent at upper S1 level. Upward arrows indicate the arrival of quadriceps volley at the same level. Same time scale for A and B.

F shows that latencies were virtually the same for the intracellular and for the ventral root IPSP and EPSP respectively; but, as would be expected, the summit was later and the decay much slower than with the respective intracellular potentials.

In G to L of Fig. 8 IPSP and EPSP were interacting at various volley intervals, B and E being the control records. Traces of these records in Fig. 9A show that the interaction closely paralleled the interaction ob-

served with intracellular recording (Coombs *et al.* 1955*b*; Curtis & Eccles, 1959). For example, in Fig. 9B there are plotted the intracellular potentials recorded from a motoneurone whose axon contributed to the potentials of Fig. 9A. In both types of recording it is seen that the IPSP was potentiated when the currents generating it were occurring during the depolarization of the EPSP. This potentiation occurred because the equilibrium potential for the IPSP was only at about 10 mV more hyperpolarization than the resting potential; hence a relatively small depolarization added considerably to the voltage driving the inhibitory ionic currents.

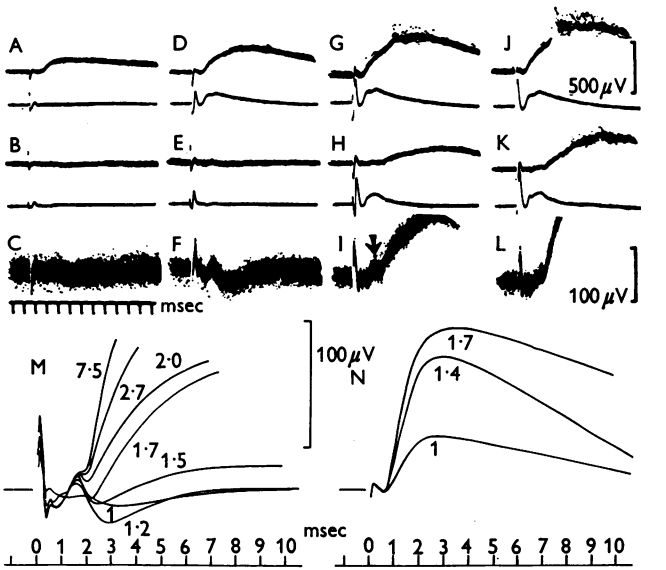

Fig. 10. Potentials recorded electrotonically on S3 ventral root. A, D, G and J; ipsilateral dorsal root stimulation. B, E, H and K; contralateral dorsal root stimulation. The stimulus is strengthened from the left to the right columns. In C, F, I and L the same potentials as in B, E, H and K, respectively, are shown with a higher amplification. Note the different voltage calibrations. Two sets of traces (M, N) below the photographs show the superposed potentials with different stimulus strengths, obtained by the contralateral dorsal root stimulation (M) and by the ipsilateral stimulation (N). Numbers in these figures indicate the strength of the dorsal root stimulation, relative to the weakest stimulus, which was just above threshold.

A hyperpolarizing potential was also recorded in the S3 ventral root in response to volleys set up by very weak stimulation of the contralateral S3 dorsal root. Such a potential was barely detectable with the low amplification of Fig. 10E, which seems comparable with that used by Lloyd & Wilson (1959), but was readily detectable at the higher amplification of Fig. 10F. The much smaller volley in Fig. 10B and C produced a very small hyperpolarization. These hyperpolarizations were much less

than the depolarizations produced by afferent volleys in the ipsilateral S 3 dorsal root (Fig. 10 A, D). When stronger stimulation was employed, the contralateral volley produced only a depolarization, in which, however, a small notch could be seen at the arrow in I, which presumably was produced by the onset of the now submerged inhibitory hyperpolarization. Not only was there a polysynaptic EPSP depolarization in H and I, but there was also an earlier small EPSP with a latency only a little longer than the ipsilateral monosynaptic EPSP in G, which presumably was due to the contralateral monosynaptic EPSP occasionally observed by Curtis *et al.* (1958) and by Frank & Sprague (1959). The superimposed traced records for contralateral S 3 volleys (Fig. 10 M) show the way in which the IPSP obtained with weakest volleys was submerged first by the contralateral monosynaptic EPSP and with stronger stimulation by the polysynaptic EPSPs. By contrast stronger ipsilateral stimulation merely added a later depolarization to the monosynaptic EPSP (Fig. 10 N). Possibly the contralateral monosynaptic EPSP contributed to the failure of Lloyd & Wilson to detect the hyperpolarizing IPSP. It may be mentioned in this context that the monosynaptic EPSP produced by Q volleys in soleus motoneurones (Eccles, Eccles & Lundberg, 1957 *b*) may submerge the Q IPSP propagated electrotonically along PBST motor axons, there apparently being a trace of this EPSP in Fig. 8 A, B, before the start of the IPSP.

The largest IPSP recorded from filaments of the S 1 or L 7 ventral root was 17 μV, and the mean value was 10 μV in seven experiments with the Ia IPSP generated by Q volleys and 28 μV in five experiments with the contralateral S 3 IPSP. The mean value for the Q IPSP was 11 % of the monosynaptic EPSPs similarly recorded and evoked by PBST volleys, but it seems possible to reconcile this relatively small size with the mean values of the IPSPs and EPSPs determined by intracellular recording from a random sample of the motoneurones concerned. Thus in the 28 BST motoneurones investigated in the same experiments, the mean IPSP was 29 % of the mean EPSP. The discrepancy (29 % as against 11 %) can at least be partly accounted for by the fact that PBST volleys generate an EPSP in many motoneurones, such as anterior biceps, which have no Q IPSP and which were rejected without being photographed. In addition, ventral root IPSPs in S 1 and lower L 7 root filaments are likely to be diminished by being superimposed on monosynaptic EPSPs which quadriceps Ia volleys generate in soleus motoneurones (Eccles *et al.* 1957 *b*).

DISCUSSION

For the first time the central latency of Ia inhibitory action on monosynaptic reflexes has been directly measured, and has been found to be in excellent agreement with the latencies of the inhibitory post-synaptic

potentials, IPSP, recorded intracellularly from several motoneurones randomly sampled out of the population giving the reflex spike. The discrepancy between the latencies of the reflex inhibition on the one hand and of the IPSPs on the other was always less than 0·1 msec. In contradistinction, Lloyd & Wilson (1959) asserted that the central latency for inhibition of reflex discharge was as much as 1·0 msec shorter than the central latency for inhibition of the soma spike response of the motoneurone and that the IPSP would just be early enough to account for this soma inhibition. Thus there is a discrepancy of about 1·0 msec between our direct experimental measurements and the value calculated by Lloyd & Wilson from various indirect measurements.

It is not clear how Lloyd & Wilson arrived at this value of 1·0 msec. For example, in the construction of their Fig. 3 the spike *ghi* begins about 0·2 msec earlier than in the actual records from which it was derived and the spike summit, *h*, is drawn about 0·35 msec earlier than in their Fig. 1E. Possibly the time scale of Fig. 1 is incorrectly labelled. Be that as it may, superposition of their Fig. 1E and F gives a situation resembling the superimposed records of our Fig. 3 and reveals that the latency for the reflex spike inhibition was about 1·35 msec, which is in good agreement with the latencies observed in the present investigation when due allowance is made by subtracting from these values the conduction times in the motor axons.

The discussion of Renshaw's (1942) results on the central latency of reflex inhibition can also be criticized. For example, the latency was measured from a point at which there was no inhibition, rather than from a point derived from extrapolation of the inhibitory curve to the level of zero inhibition, which gives 0·95 msec for the latency of reflex inhibition as measured to the beginning of the reflex spike discharge from the motoneurones. The time to the summit of the reflex spike potential illustrated by Renshaw (0·35 msec) should be added to this figure, which gives 1·30 msec for the central latency of inhibitory action. When there has been a negligible longitudinal component in the inhibitory pathway, the IPSPs have often been observed with central latencies even briefer than 1·30 msec (Fig. 3J; Eccles *et al.* 1956; Curtis *et al.* 1958; Frank & Sprague, 1959), and consequently would just be in time to produce a reflex inhibition having the latency displayed by the reflex inhibition illustrated by Renshaw. On the contrary, Lloyd & Wilson (1959) derive a value of 1·15 msec for the inhibitory latency and then perform the irrelevant operation of subtracting therefrom the central latency for reflex discharge for motoneurones (0·95 msec), arguing that the remainder (0·2 msec) is inadequate for an interneuronal relay. The correct procedure would be to subtract from 1·15 msec the inhibitory synaptic delay of 0·3 msec and then enquire if the residual 0·85 msec is sufficiently long for conduction along a pathway to the motoneurone that traversed an interneuronal relay. Recently interneurones have been found in the intermediate nucleus that discharged an impulse in response to a Ia afferent volley which had entered the spinal cord only 0·5–0·8 msec earlier (Eccles, Eccles & Lundberg, 1960); hence the transmission time of 0·85 msec is possible with a disynaptic pathway, though our remeasured value from Renshaw's experiment would be 1·0 msec, and thus readily accommodated to a disynaptic pathway.

The diagram of Fig. 11 serves to illustrate the various factors that determine the central latency of Ia inhibitory action on motoneurones. The times of this diagram are all based on experimental measurements, and it will be seen that the shortest latency for inhibition of monosynaptic

reflex discharge could occur with an approximate simultaneity of the excitatory and inhibitory afferent volleys. The excitatory volley is shown producing an EPSP of the motoneurones after a latency of 0·5 msec, which is made up from 0·2 msec conduction time and 0·3 msec synaptic delay. (ESD). However, the EPSP takes a further 0·4 msec to rise to the threshold for the earliest spike discharge, and the time for reaching threshold

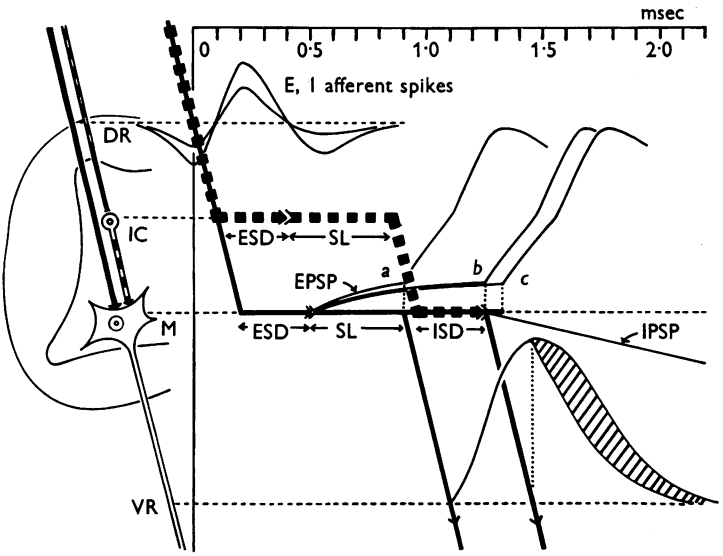

Fig. 11. Diagram showing time course of events during Ia inhibitory action on a monosynaptic reflex spike. The pathways are shown to the left with the monosynaptic excitatory line in black and the Ia inhibitory line in interrupted black. The remainder of the diagram is constructed both on the same time scale, as shown above, and on the spatial scale of the diagram to the left, except that the potential records are also shown in the conventional manner as rising from base lines at the respective recording sites: dorsal-root entry, DR; the motoneurone, M; and the ventral root, VR. The slopes of the lines on the spatial–temporal co-ordinates give the velocities, and delays at regions of junctional transmission are given by lengths of the horizontals there. Thus the monosynaptic path (the E afferent spike at DR) is shown delayed at the motoneurone by an excitatory synaptic delay ESD of 0·3 msec in initiating the EPSP, and a spike latency, SL, of 0·4 msec for the shortest latency (*a*) and 0·75 msec for the longest (*c*) involved in the EPSP initiating a spike discharge. The spikes discharges by *a* and *b* are shown propagating into the ventral root. An I afferent spike arriving synchronously with E, as shown by records at DR, and having an equivalent length of central pathway, is shown delayed for 0·75 msec at the intermediate cell (IC) relay (0·3 msec of excitatory synaptic delay plus 0·45 msec of spike latency); and thereafter propagating to the motoneurone, having a further delay of 0·3 msec (the inhibitory synaptic delay, ISD) before initiating the IPSP after a total central latency of 1·25 msec. It will be seen that this IPSP is just produced in time to delay or suppress (cf. Fig. 1K) all reflex discharges after *b* (for example *c*), and so is just able to diminish the height of the reflex spike as shown by the hatched area.

is as long as 0·8 msec with the latest discharges that contribute to the summit of the reflex spike potential in the ventral root, as is shown in the potential–time diagrams in this figure. Meanwhile the inhibitory volley will have entered the intermediate nucleus (IC), and, after a synaptic delay of 0·3 msec (ESD), will have produced an EPSP in the Ia intermediate cells, which rises to the threshold for generating the discharge of impulses after a further 0·45 msec, values which conform with experimental values for the quickest responses of intermediate neurones activated by Ia impulses (Eccles *et al.* 1956; Curtis *et al.* 1958; Eccles *et al.* 1960). Within about 0·4 msec (0·3 msec for the inhibitory synaptic delay, ISD) the impulses of the intermediate cells will be initiating an IPSP of the motoneurones that has a central latency of 1·25 msec and which is just in time to inhibit the discharge of the latest motoneuronal discharges contributing to the reflex spike potential. It will be evident from Fig. 11 that the interval between the inhibitory and excitatory volleys provides a most unsatisfactory basis from which to derive the central latency for inhibitory action, and this criterion has seriously misled Lloyd and Wilson.

There is satisfactory agreement between the time courses for Ia inhibition of monosynaptic reflexes (Figs. 4B, 5G) and the IPSPs that are recorded both from the ventral root and from motoneurones (Figs. 4A, 5F, 7I, J, 8A–F, 10C, F). The low residuum of inhibitory action persisting for more than 10 msec (Fig. 4B) would be expected if inhibitory synaptic action on motoneurones produced a hyperpolarizing potential that continued the depression of excitability beyond the brief phase of high ionic permeability (Curtis & Eccles, 1959). Thus it can be concluded that the IPSP recorded by an intracellular electrode is not simply a consequence of a depolarization produced by impalement with a micro-electrode, as suggested by Lloyd & Wilson (1959) and Jack *et al.* (1959).

In any case, it would not be expected on theoretical grounds that a prolonged steady membrane depolarization would provide conditions giving a hyperpolarizing inhibitory potential if there were no inhibitory potential at the resting membrane potential. There is independent evidence that the equilibrium potential for potassium, E_K, is at a more hyperpolarized level than the resting membrane potential, for the after-hyperpolarization that follows a spike response of the motoneuronal membrane is due to a raised K^+ permeability (Coombs, Eccles & Fatt, 1955*a*; Eccles, 1957); and it would be generally agreed that this after-hyperpolarization occurs in motoneurones independently of any lowering of membrane potential by injury, for it can readily be observed after electrotonic propagation to the ventral root (Eccles & Pritchard, 1937; Gasser, 1939; Lloyd, 1951). Thus the equilibrium potential for the IPSP would be identical with the resting membrane potential, E_R, only if the movement of K^+ ions made no contribution to the inhibitory post-synaptic action. Cl^- ions would therefore be almost exclusively concerned in the high ionic permeability occurring in the inhibitory response of the post-synaptic membrane, which would be in agreement with the general conclusion that with nerve and muscle the equilibrium potential for Cl^- ions (E_{Cl}) is identical with the normal resting membrane potential E_R (Conway, 1957; Hodgkin, 1958; Hodgkin & Horowicz, 1959; Keynes, 1954). If now E_R is lowered to

$E_{R'}$ by micro-electrode penetration, E_{Cl} would be at a more hyperpolarized level than $E_{R'}$, and inhibitory synaptic action would cause a hyperpolarizing response, even if none was present normally. But the difference between E_{Cl} and $E_{R'}$ would cause also a net influx of Cl ions until the redistribution caused the new equilibrium potential, $E_{Cl'}$, to be identical with $E_{R'}$ (cf. Boistel & Fatt, 1958). The time course of this distribution is quite rapid, having a half-time of about 15 sec (Coombs *et al.* 1955*b*; Eccles, 1957; J. C. Eccles, R. M. Eccles & M. Ito, unpublished observations), so a steady membrane depolarization resulting from electrode penetration would not account for IPSPs that persisted for more than 1–2 min; and hyperpolarizing IPSPs have been regularly observed during long periods of steady membrane potential.

A rectangular pulse of depolarizing current will generate the discharge of an impulse from a motoneurone when it has depolarized the initial segment of the motoneurone to a critical level (Araki & Otani, 1955; Frank & Fuortes, 1956; Coombs *et al.* 1957*a*, *b*). In doing this the applied current will pass partly through the membrane capacity, removing its charge so that the critical level is attained, and partly through the membrane conductance. When the stimulating current is as brief as 0·2 msec, it has to be very intense in order to depolarize the membrane to the critical level for impulse generation; and it would not be appreciably antagonized by the flow of inhibitory current. The inhibitory curve would be expected to be virtually identical with the time course of membrane hyperpolarization (the IPSP), as is actually seen in Fig. 7K (short rectangles). On the other hand, a stimulating current of 0·8 msec duration would be of much lower intensity (only 30% in Fig. 7), as may be seen by reference to the strength–latency curves for motoneurones (Frank & Fuortes, 1956; Coombs *et al.* 1959), and consequently it would be very effectively antagonized by the inhibitory current as well as by the hyperpolarization; hence the dual composition of such inhibitory curves (Fig. 7K, long rectangles). The excitatory synaptic currents generating the monosynaptic EPSP and spike discharge have an effective duration even in excess of 0·8 msec (Curtis & Eccles, 1959; C. Terzuolo & T. Araki, personal communication), so inhibitory current flow would tend to have the dominating influence in preventing the generation of spikes, the hyperpolarization being relatively unimportant, as is illustrated in the inhibitory curves of Figs. 4, 5.

The relative sizes of the two phases of inhibitory action could be quantitatively evaluated if the electric time constant of the membrane were known. Unfortunately there is a large discrepancy between the time constant calculated from strength–latency curves, which has a mean value of 1·1 msec (Frank & Fuortes, 1956; Coombs *et al.* 1959) and that derived from the time course of membrane potential change produced by a rectangular pulse (mean value 3·1 msec, Coombs *et al.* 1959). Presumably the membrane potential changes produced by depolarizing current pulses would be related to the former value of 1·1 msec rather than to the longer

value, and thus the dominance of the initial brief phase in Figs. 4, 5, 7K (long rectangles) is to be expected.

Wilson & Lloyd's statement that the 'latency for inhibition of soma spike is no measure of the latency of reflex inhibition' is a confusing half-truth. It is generally accepted that the spike recorded by a micro-electrode in the soma of a motoneurone is compounded of an initial IS (or A) spike and a later SD (or B) spike (Araki & Otani, 1955; Fatt, 1957; Fuortes *et al.* 1957; Coombs *et al.* 1957*a*, *b*) and that the former signals the generation of an impulse by the motoneurone and is responsible for the discharge of impulses along the motor axon, whereas the SD spike (often called soma spike) arises later by a secondary invasion. Thus, in the above statement, Wilson & Lloyd would be correct with this usage of 'soma spike' as the SD spike; their confusion arises because they use the same phrase 'soma spike' in two senses in the same argument, i.e. for all spikes recorded in the soma (IS plus SD) and for SD spike alone.

CONCLUSIONS

In contradistinction to Lloyd & Wilson (1959) it can be concluded:

(*a*) That the time course of IPSP gives a complete explanation of the observed inhibition of impulse discharge from motoneurones, both that arising reflexly and that in response to direct electrical stimulation.

(*b*) That there is a latency differential of about 0·8 msec between the Ia IPSP and the monosynaptic EPSP, which conforms with the requirements of a disynaptic inhibitory pathway.

(*c*) That inhibitory synaptic action on a motoneurone results from two processes: a brief phase of high ionic conductance of the inhibitory subsynaptic membrane; and a consequent hyperpolarization, the IPSP, which occurs even at the normal resting membrane potential.

(*d*) That the spike potential recorded in the soma of motoneurones provides a reliable indicator of the generation of impulse discharge from motoneurones, both under normal conditions and when under inhibitory action.

SUMMARY

1. The central latency of inhibition has been measured by observing the onset of its action upon the rising phase of a suitably timed monosynaptic reflex spike recorded in the ventral root.

2. With the inhibition of biceps-semitendinosus motoneurones by quadriceps Ia volleys and with the contralateral inhibition of motoneurones at sacral 3 level, the latency of inhibitory action on the reflex discharge of impulses from motoneurones into the ventral root has been found always to be within 0·1 msec of the intracellularly recorded inhibitory post-synaptic potentials (IPSPs).

3. In a reinvestigation it was confirmed that the time course of inhibitory action on monosynaptic reflexes by a Ia inhibitory volley had a brief intense phase and a longer low-intensity tail that persisted for about 10 msec.

4. A similar time course was also observed when the effect of the inhibitory volley was tested by the raised threshold to relatively long depolarizing current pulses (up to 1 msec) applied through the intracellular electrode. With testing by much briefer pulses the raised threshold had a time course resembling the IPSP.

5. IPSPs have been regularly recorded from ventral roots after electrotonic transmission from motoneurones.

6. These experimental results have been discussed in detail and it has been concluded that the time course of the IPSP gives a complete explanation of the observed inhibition of reflex discharge from motoneurones, the inhibitory post-synaptic currents being responsible for the initial brief intense phase that is superimposed upon an inhibitory effect attributable to the hyperpolarization of the IPSP.

7. It is shown that the central latency of inhibitory action exactly corresponds with the latency required for a pathway with an interpolated interneurone.

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