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ELECTROPHYSIOLOGICAL INVESTIGATIONS ON RENSHAW CELLS

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When Renshaw cells are synaptically activated by antidromic volleys entering the spinal cord through ventral roots, the initial frequency of discharge is often over 1500/sec; and, after a few impulses at above 1000/sec, the frequency progressively declines until the terminal responses are 20 or more milliseconds apart (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956; Frank & Fuortes, 1956; Curtis & Eccles, 1958*a*, *b*). The initial high frequency certainly indicates that a very intense synaptic stimulation is evoking discharges immediately the cell has recovered from the absolute refractoriness following the previous discharge. It has been generally assumed that the later progressive decline in frequency of discharge results from a smooth continuous decline in the intensity of synaptic stimulation; nevertheless, there are experimental observations that suggest a discontinuity between an intense initial phase of synaptic stimulation and a subsequent low and prolonged activity. For example, the blocking drug, dihydro-β-erythroidine hydrobromide is very effective in suppressing all but the first two or three discharges, which persist with almost unchanged latency and frequency even after very large doses (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1956; Curtis & Eccles, 1958*b*; Brooks & Wilson, 1959). A further indication of an intense initial synaptic excitatory action is provided by the lengthened interval that often disturbs the rhythm between the second and third discharges (Eccles, Fatt & Koketsu, 1954, Fig. 6G, H; Frank & Fuortes, 1956, Fig. 12B).

In the present investigation synaptic stimulation of Renshaw cells has been examined both by studying the effect of variation in intensity of the stimulus on the time course of the repetitive response, and by employing intracellular recording to give the time course of the synaptically induced

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depolarization. A further variant of the intensity of synaptic stimulation has been obtained by making use of the depression that follows a conditioning volley in the same presynaptic pathway to the Renshaw cell. A systematic examination has also been made of the synaptic activation of Renshaw cells by antidromic volleys from a large variety of muscle nerves.

METHODS

The general experimental procedures with micro-electrode recording from the lumbosacral cord of anaesthetized spinal cats were similar to those already described in publications from this laboratory (Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Eccles & Fatt, 1955). Renshaw cells were recorded from extracellularly, and also, with varying degrees of success, intracellularly. They were synaptically excited by antidromic volleys in motor axons, which were set up by stimulation either of ventral roots or of various muscle nerves. In the latter case the appropriate dorsal roots had been severed so as to eliminate synaptic activation either through interneuronal pathways or via axon collaterals of reflexly discharging motoneurones. Many muscle nerves were mounted on stimulating electrodes, and could be rapidly tested in turn by a rotary switch device (see Eccles, Eccles & Lundberg, 1957). The nomenclature of the various muscle nerves is given in the description of Table 1.

RESULTS

Time course of synaptic activation of Renshaw cells

Variations in the intensity of the synaptic stimulus. When the antidromic volley in the alpha fibres of the ventral root was progressively decreased in size, the intensity of excitation of Renshaw cells decreased stepwise, as revealed both by the frequency and by the duration of their discharge (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954; Frank & Fuortes, 1956). Renshaw cells also displayed several intensities of response when excited by maximal antidromic volleys in the alpha fibres of different muscle nerves (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954). By graded submaximal stimulation of the muscle nerve evoking the most intense response of a Renshaw cell that cell could be made to respond in a manner closely resembling the responses evoked by maximal volleys in the less effective nerves. For example, Fig. 1A and G are virtually identical; as also Fig. 1B and H; and Fig. 1C and J, K, L. Thus it may be concluded that the responses evoked by antidromic volleys from different muscle nerves vary solely on account of the effectiveness of the respective synaptic excitatory actions, i.e. the variations have a purely quantitative basis. In parenthesis it may be noted that a similar conclusion has been reached for the monosynaptic activation of motoneurones for different afferent pathways, both homonymous and heteronymous (Eccles *et al.* 1957). It is therefore justifiable to employ both submaximal and maximal volleys from different muscle nerves (cf. Fig. 2A–E) when investigating the effects of variation in the amount of synaptic excitatory action on Renshaw cells.

When there was a smooth decline in the frequency of the repetitive response evoked by a maximal antidromic volley, as in Fig. 2E, progressive weakening of the synaptic excitation (E–A) caused, first, a shortening of the response and a more rapid decline of the frequency; but the first two or three discharges were much less affected until the stimulus was so weak that all later discharges had been eliminated (Fig. 2A, B). When the reciprocal of the response interval, i.e. the frequency, was

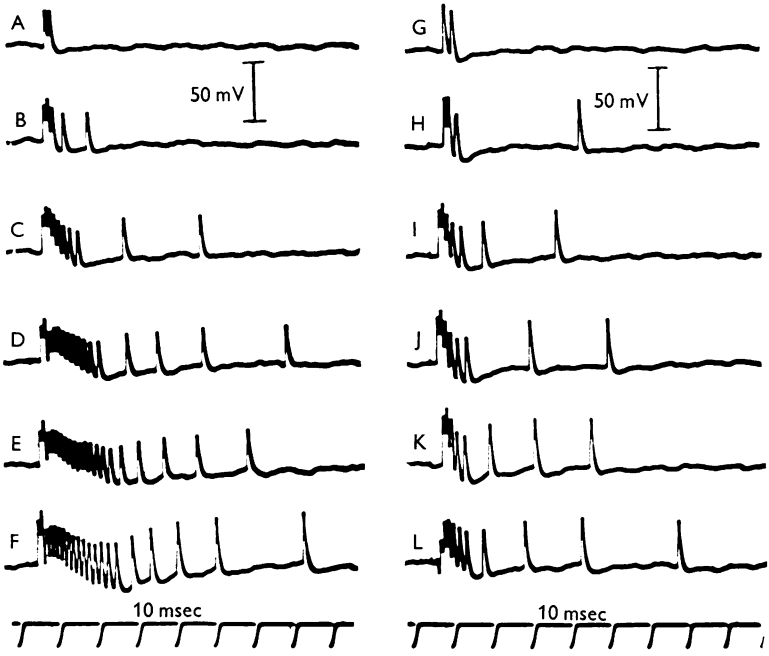


Fig. 1. Intracellular responses of a Renshaw cell evoked by submaximal alpha volleys from the medial gastrocnemius nerve, of increasing size from A–D and by maximal volleys in E, F. G–L are responses of same cell evoked by maximal alpha volleys from a series of other muscle nerves: plantaris, soleus, anterior biceps, inferior gluteal, lateral gastrocnemius and posterior biceps respectively. The series of A–E was taken a little later than F–L, the resting potential and spike potential having meanwhile declined. Same voltage and time scales throughout.

plotted for the successive discharges (Fig. 2G), it might be inferred for the range of responses of Fig. 2A–F that the synaptic stimulation diminished first in duration and only later in intensity. However, this inference is inadmissible because the initial very high frequencies of discharge (Fig. 2C–F) would be largely determined by the duration of Renshaw-cell refractoriness.

When a Renshaw cell was activated by various sizes of antidromic volley in the ventral root (Fig. 2H–L), the respective repetitive responses

may be plotted (Fig. 2M), as in Fig. 2G, to show the time course of the decline of frequency. Comparison of Fig. 2G and M shows that the two modes of grading synaptic excitation of Renshaw cells yielded closely parallel results, as has already been illustrated by the respective series of records, A–E and G–L in Fig. 1.

When attempting to derive the time course of the synaptic excitatory action from curves such as those of Fig. 2G and M, it may first be assumed

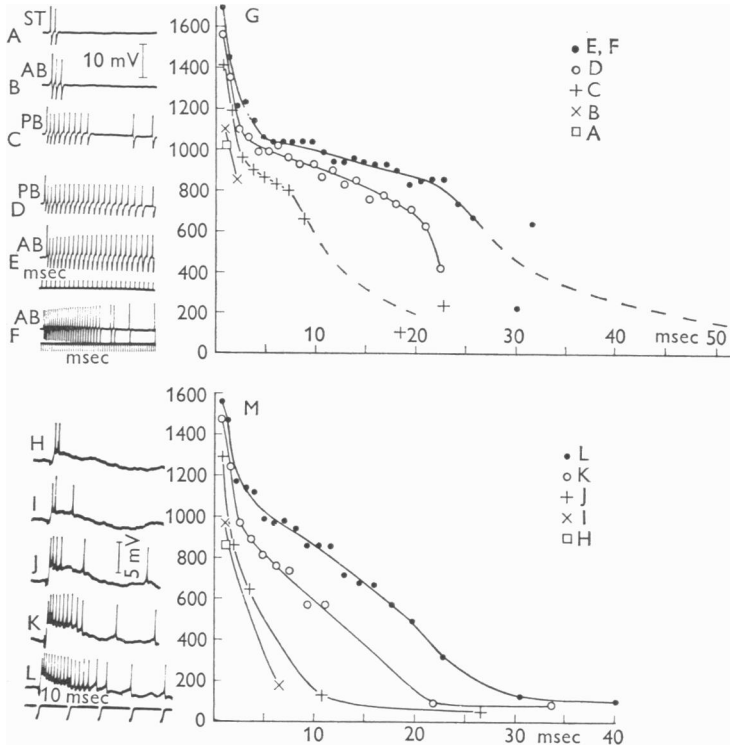
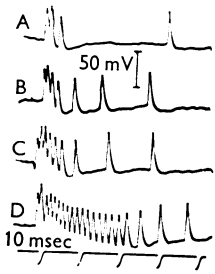


Fig. 2. A–F are responses of a Renshaw cell recorded extracellularly and evoked respectively by antidromic volleys from the three muscle nerves that were effective: maximal semitendinosus, submaximal anterior biceps, submaximal posterior biceps, maximal posterior biceps, and maximal anterior biceps at fast (E) and slower speed (F). The intervals between the successive responses are plotted as frequencies (ordinates) in G, much as has been done for interneuronal responses by Hunt & Kuno (1959), the abscissae being the time of each discharge after the initial discharge. Thus the curves of G (see symbol lettering) plot the time courses of the frequencies of discharge for each of the responses in A–F. The terminal portions of two curves are shown as interrupted lines because of the scatter of the points. H–L are another series for the repetitive responses of another Renshaw cell (with poor intracellular recording) evoked by an antidromic volley in L7 ventral root, which was maximal in L and with four grades of submaximal stimulation in H–K. The intervals between the successive spike discharges are plotted in M in the same way as in G, as again indicated by the symbol lettering.

that the various motor-axon collaterals converging on a Renshaw cell have similar time courses for their synaptic excitatory action. At least there has been no significant difference between the motor-axon collaterals for the motoneurones supplying different muscles, and no difference would be expected between axon collaterals from the lower-threshold and the higher-threshold alpha motor fibres. When there was a sufficiency of convergent impulses on a Renshaw cell, the initial intensity of activation was so high that the frequency (over 1500/sec) would be close to the limit set by the refractoriness of the cell; and even with considerably lower frequencies (around 1000/sec) refractoriness must be a significant factor in setting the frequency. The curves for the more powerful synaptic stimulations thus give a misleading impression of the time course for the intensity of the synaptic stimulus, because the refractoriness of the discharging cell prevents the initial intense phase from evoking a commensurately high frequency of discharge. The curves for the less powerful synaptic stimulations also give an erroneous time course, because a synaptic stimulus below threshold is given a zero value, as occurs after the first two or three responses with the weakest stimuli in Fig. 2G, M. However, some indication of the time course of the synaptic stimulus may be derived by noting the times at which the same response frequencies were produced by stimuli of different strengths. For example, the peak frequency in Fig. 2A was comparable with the frequency at a point 10 msec later in Fig. 2E, so it is probable that the respective intensities of synaptic stimulation were then similar. A complicating factor would be introduced by the possible action of accommodation in slowing the frequency in the latter part of Fig. 2E. Likewise, in the series of Fig. 2H–M, the peak frequency of the weakest response (Fig. 2H) was comparable with the frequency of the strongest response (L) at a point 10 msec later. An intermediate stimulus strength (J) showed a decline from an initial frequency of 1290/sec to 135/sec at 10 msec later; yet, with the response evoked by the maximum stimulus (L), the initial frequency of 1560/sec had declined only to 860/sec at 10 msec later. Evidently the synaptic stimulus was much greater in this latter case, but it could cause only a limited increase in the initial frequency. In general, therefore, the curves of Fig. 2G, M may be interpreted as indicating that the synaptic excitatory action produced by a single impulse had a very intense phase lasting 2 or 3 msec followed by a slowly declining tail of much lower intensity.

As already mentioned, an abnormally long interval may occur between the second and third responses to a very powerful synaptic stimulation. For example, in Fig. 3 this was regularly present in responses evoked by a maximum antidromic volley in the medial gastrocnemius nerve (D), but did not occur with the responses to the weaker stimulations provided by

other muscle nerves (A–C). The break in rhythm is further illustrated by the filled circles in the plotted curves (E). A regular observation has been that the lengthened interval is a little too brief (by about 0·2 msec in Fig. 3E) to be explicable merely as due to the dropping out of one response.



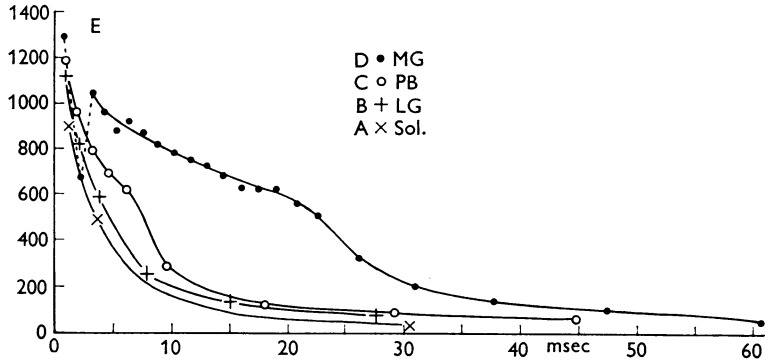


Fig. 3. A–D, repetitive Renshaw cell responses evoked by maximal antidromic volleys in the various muscle nerves as indicated. Same cell as in Fig. 1, with a faster sweep speed, in order to show the break in rhythm between the second and third responses in the largest response (D). In E the measurements from the series partly shown in A–D are plotted as in Fig. 2G, M. The plotted points for the medial and lateral gastrocnemius volleys are the means of two closely similar records. The curve joining the first three points for medial gastrocnemius (●) is drawn as a dotted line.

Similar findings occur with antidromic volleys set up by graded stimulation of the ventral root (Fig. 4A–D). The break in the rhythm was not present with the two weakest stimuli (A, B), but was very evident with the strongest stimulus (C), particularly when observed at high speed (D). Again, the lengthened interval was about 0·2 msec too brief to be attributable to the dropping of one discharge. Presumably, when extremely large the synaptic depolarization caused some disorganization of the spike-generating mechanism, just as occurs with cathodal depression of nerve

fibres or motoneurones (Coombs *et al.* 1955). Sometimes a small abortive spike was detectable at the time of the missing discharge (cf. Eccles, Fatt & Koketsu, 1954, Fig. 6G, H).

Another common type of disorganization of the spike mechanism is illustrated in Fig. 4E–H. Here the spike had a double composition resembling the compound IS–SD spike of motoneurones, and in the initial

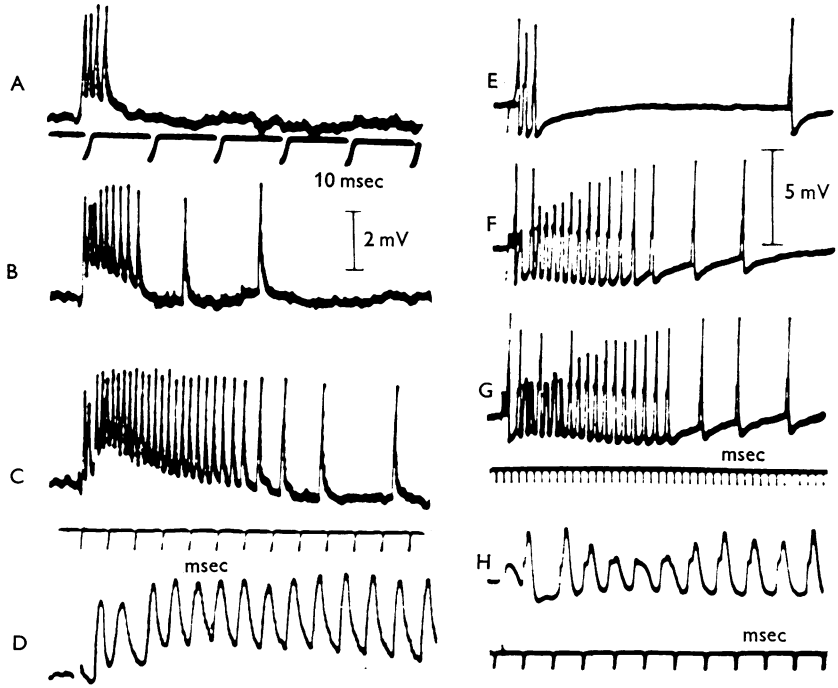


Fig. 4. A–D, series of Renshaw cell responses recorded intracellularly as in Fig. 3A–D, but for a different cell and evoked by antidromic volleys set up by stimulation of L7 ventral root, submaximal in A, B and maximal in C. D is same response as C, but at a faster sweep speed as shown above it. Same potential scale throughout. (D, H are at lower amplification.) E–H, responses evoked in another Renshaw cell as in A–D, G and H being evoked by maximal antidromic volleys in L7 ventral root. H at the sweep speed indicated below it.

high-frequency phase most of the spike potentials were largely composed of the initial small spike. In the fast record (H) it is seen that this initial small spike was fairly uniform throughout the response, while there was great variability in the later spike. Presumably this fragmentation of the spike is attributable to the high frequency of spike generation, rather than to the intensity of the background synaptic depolarization. It did not occur with the lowest frequency of discharge evoked by the weakest stimulation (Fig. 4E). On analogy with motoneurones it is probable that

the initial small spike was associated with the discharge of an impulse along the axon (Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957*a*, *b*).

Excitatory post-synaptic potentials of Renshaw cells. On rare occasions the intracellularly recorded Renshaw cell responded by a depolarization

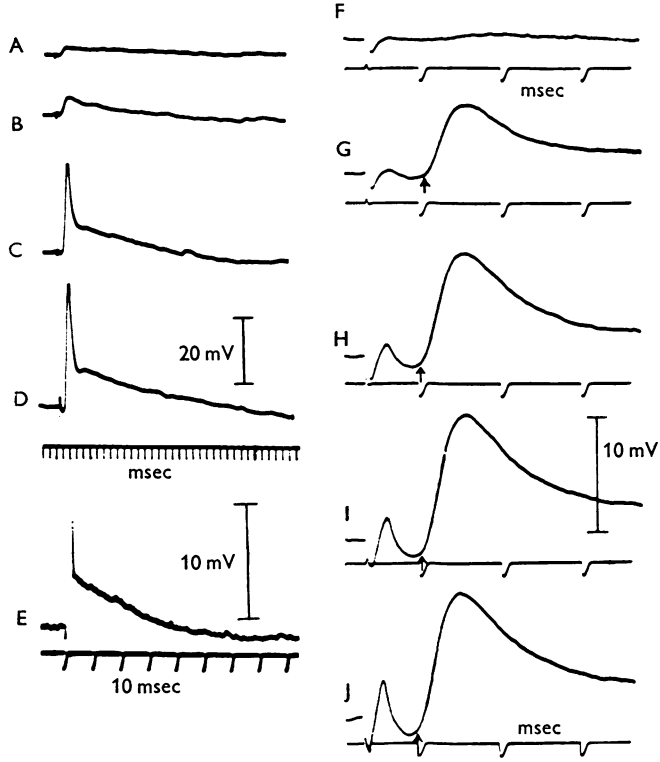


Fig. 5. Excitatory post-synaptic potentials (EPSPs) recorded intracellularly from a Renshaw cell with a recorded membrane potential initially of −60 mV. A–D show graded sizes of EPSPs evoked by submaximal (A–C) and maximal (D) antidromic volleys in L7 ventral root. E shows maximal response recorded at slower sweep speed and with a long time constant (1 sec) of the amplifier. F–J are fast records of EPSP evoked by progressively increasing antidromic volleys, as shown by the initial diphasic wave with first deflexion upwards. The arrows indicate times of onset of the EPSPs. Series E–J at higher amplification than A–D, as indicated. Upward deflexions signal positivity relative to the indifferent earth lead i.e. membrane depolarization.

(the excitatory post-synaptic potential, EPSP) uncomplicated by spike potentials. A response of this type has already been illustrated (Eccles, Fatt & Koketsu, 1954, Fig. 6I), but it was at that time suggested that the initial brief peak of depolarization was a spike potential. A much larger

potential of the same type is illustrated in Fig. 5. The responses of this Renshaw cell were so unusual that its identification could be regarded as established only after the full investigation described below.

The membrane potential was about −60 mV for the first records of Fig. 5; it gradually declined thereafter. A maximum alpha volley in the L7 ventral root evoked the large (36 mV) brief depolarization (D) that after about 2 msec merged into a much slower decaying depolarization. The full duration of this depolarization is shown in E to be about 60 msec. Progressive diminution of the alpha volley from maximum size (D) was associated with a diminution of both the fast and slow components of the depolarization (C). With the responses to smaller volleys the fast component was relatively lower, being but little above the slow component in B, and at about the same level in A. This is also well shown in the very fast records of Fig. 5, F–J. The fast component was barely identifiable in the weakest response (F), but showed progressive increment in G, H and I, there being no further increase when the alpha volley finally reached maximum (J).

The onset of the fast response was obscured in F–J, as it was superimposed on the downward (negative) deflexion of the extracellularly recorded soma spike potential of the alpha motoneurones. However, it can be detected as a sharp bend in the curve at the points marked by the arrows, which give it a latent period of about 0·4 msec from the phase of maximum positivity in the field potential produced by the approaching antidromic impulse. If a small allowance be made for the time of propagation of the antidromic impulse up the motor-axon collaterals, the actual synaptic delay for the onset of the depolarization of the Renshaw cell would correspond well with the value of about 0·3 msec calculated for other synaptic delays in the central nervous system (Eccles, 1957).

The smooth contour and relatively long duration (2 msec) of the brief depolarization establishes that it was not due to the superposition of a brief spike-like process on a more prolonged EPSP. This conclusion receives further support from the various gradations in its size (Fig. 5A–D, F–I). It may, therefore, be concluded that the potentials illustrated in Fig. 5 are EPSPs set up in a Renshaw cell by the summed action of several excitatory impulses converging on this cell. At least several of these impulses generated EPSPs having fast and slow components.

In Fig. 6A–H, from the same cell, a second maximum alpha volley was set up in the L7 ventral root at various intervals after the first. Even at relatively long intervals there was a considerable diminution both of the fast and slow components of the second EPSP. As shown by the plotted points (Fig. 6J) derived from the series partly illustrated in A–H, full recovery took about 150 msec, and at the shortest intervals (0·7–5 msec)

the depression was to about 40% of the control. As measured by the addition to the slow EPSP produced by the testing volley, the slow component of the EPSP was even more depressed at the shortest intervals. The time course of recovery was approximately the same for both the fast

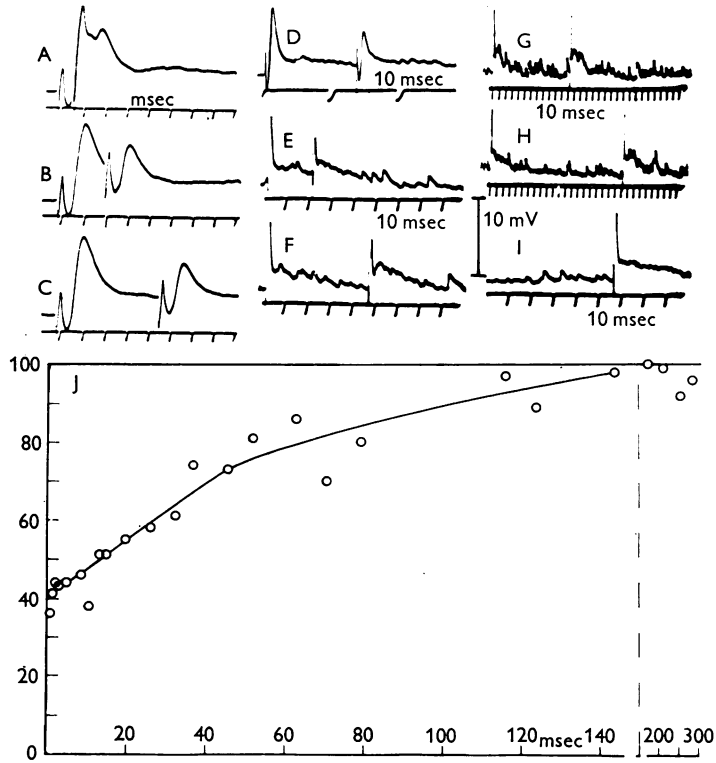


Fig. 6. A–I, same Renshaw cell as in Fig. 5, but responses evoked by two maximal antidromic volleys at various intervals as indicated by the stimulus artifacts. Note changes in sweep speed as indicated by the time bases for each record. Same potential scale throughout. J. Sizes of initial brief EPSP evoked by the second volley (calculated as percentages of the mean control size) are plotted against stimulus intervals for the series partly shown in A–I. Note the great compression of the time scale beyond the interrupted line at 150 msec.

and slow components. Renshaw (1946, Fig. 5) illustrated the depression of discharge that occurred with conditioning by a preceding antidromic volley in the same nerve fibres. There was considerable depression with a volley interval in excess of 50 msec, and more recently (Eccles, Fatt & Koketsu, 1954) it has been reported that the depression persisted for as long as 100 msec. Presumably this depression of Renshaw cell discharges is sufficiently explained by the depression of the second EPSPs illustrated in Fig. 6.

Patterns of activation of Renshaw cells

The input pattern from individual muscle nerves on to Renshaw cells has now been examined in conditions where nearly all the muscle nerves arising from the lower lumbar and upper sacral cord could be stimulated. The only nerves to hind-limb muscles which were not stimulated were the obturator nerve and sartorius nerve. The convergence on to Renshaw cells could thus be examined in almost full detail. Nine of the cells were dominated by one muscle nerve, which was at least twice as effective as any other muscle nerve in causing a discharge of impulses from the cell (Figs. 1F–L, 3A–D, 7A–D). All the remaining cells were excited really effectively by only 2 or 3 muscle nerves.

TABLE 1

The convergence of antidromic volleys in muscle nerves on to 18 Renshaw cells. Each muscle nerve was tested separately and in the columns are the numbers of impulses discharged from the respective Renshaw cell in response to a maximal antidromic volley in the alpha motor fibres. Numbers in bold type show the contribution from the dominant muscle nerve. The symbols labelling the columns correspond to the following muscle nerves, and the same convention is employed in the text: Q, quadriceps; SM, semimembranosus; SG, superior gluteal; FDL, flexor digitorum longus+flexor hallucis longus; Per., peroneal; Pl., plantaris; Pop., popliteus; LG, lateral gastrocnemius; AB, anterior biceps; ST, semitendinosus; IG, inferior gluteal; PB, posterior biceps; MG, medial gastrocnemius; Sol., soleus; T, posterior tibial nerve (flexor digitorum brevis plus medial and lateral plantar). Cells 10, 11 and 18 are illustrated in Figures 7, 2 and 1, respectively

Renshaw cell number	L6 and upper L7					Mid L7					Lower L7 and S1			
	Q	SM	SG	FDL	Per.	Pl.	Pop.	LG	AB	ST	IG	PB	MG	Sol.
1	6	5	**13**	.	.	.	.	.	.	.	.	.	.	.
2	.	.	**13**	.	2	.	.	.	.	.	.	.	.	.
3	.	.	**10**	.	5	.	.	.	.	.	.	.	.	.
4	.	.	**7**	2	.	.	.	.	.	.	7	.	.	.
5	.	3	**7**	3	**8**	.	.	.	.	.	.	.	.	.
6	.	.	**10**	3	3	.	.	3	**8**	3	3	.	.	.
7	.	**17**	4	.	8	.	5	.	.	.	.	.	.	.
8	.	**16**	.	.	8	.	.	.	.	.	3	.	.	.
9	.	.	.	.	.	**14**	9	9	.	.	.	1	.	.
10	.	.	.	.	.	6	.	**18**	.	.	.	.	8	6
11	.	.	.	.	.	.	.	.	**32**	2	.	**28**	.	.
12	.	.	.	.	.	.	.	.	**20**	9	.	**13**	.	.
13	.	.	.	.	.	.	.	.	.	**10**	.	**12**	1	.
14	.	.	.	.	.	.	.	.	.	.	5	**22**	.	.
15	.	.	.	.	.	3	.	6	.	5	7	**12**	9	.
16	.	.	.	.	.	.	.	**26**	.	.	.	.	**26**	**17**
17	.	.	.	.	.	.	.	5	2	.	5	4	**13**	.
18	.	.	.	.	.	2	.	8	7	.	7	10	**21**	4

Table 1 has been prepared so that the muscle nerves are arranged in the approximate sequence of their motoneuronal nuclei along the long axis of the ventral horn. When they are arranged in this way, an important feature of the input pattern to these 18 Renshaw cells becomes con-

spicuous; namely, that Renshaw cells tend to be excited by collaterals from the axons of adjacent motoneuronal nuclei, as for example in Fig. 7. When 2 or 3 muscle nerves were dominant, they were invariably from adjacent nuclei in the cord. An apparent exception to this rule (cell 4),

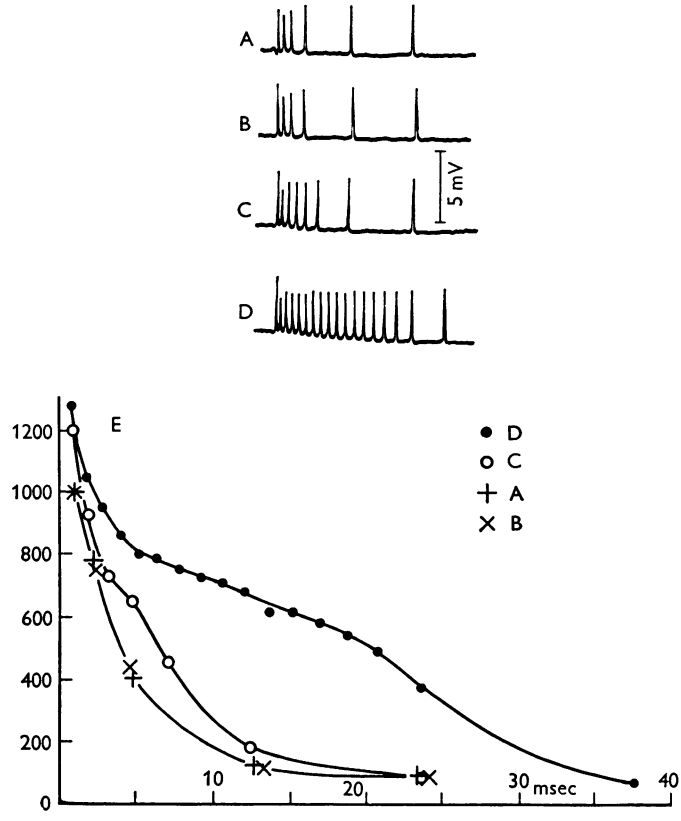


Fig. 7. A–D, Renshaw cell responses recorded intracellularly as in Fig. 3 and evoked by maximal antidromic volleys in the muscle nerves. A, plantaris; B, soleus; C, medial gastrocnemius; D, lateral gastrocnemius. All other antidromic volleys were ineffective. E, Responses illustrated in A–D are plotted as in Fig. 2G, H, the lettering of the symbol giving the reference to the records A–D. Note that the curves for A and B are virtually identical.

with equally effective inputs from superior and inferior gluteal nerves, can be accounted for by the imperfection of the tabular presentation. These two nuclei overlap in the ventral horn, and furthermore they are in the same cell column (Romanes, 1951). The influence of location was also apparent with the less effective muscle nerves. Thus cell 18 (Fig. 1) was excited most effectively by MG, but was also excited by PB, IG, AB,

Sol., Pop., LG and Pl. These nuclei are adjacent to MG, whereas Q, SM, SG and Per. were without effect and are more cephalad in the spinal cord. Motoneuronal nuclei in the same cell column in the ventral horn but separated longitudinally are less effective in exciting a Renshaw cell than are nuclei at the same level of the cord, but in different cell columns. Thus cells 7 and 8, which were fired optimally by SM, were not excited by AB, PB or ST, even though the latter are in the same cell column; whereas SG, Per. and Pop. were effective, yet lie in different cell columns in the ventral horn.

DISCUSSION

Synaptic excitation of Renshaw cells

The intracellular records of the EPSPs in Fig. 5 show a time course that corresponds closely with the time course that was postulated for the synaptic excitatory action generating the repetitive discharges of Figs. 1–4, 7. There is even direct evidence that submerged beneath the spike potentials of Figs. 1, 2H–L, 2A–D, 4A–D, there was an EPSP having a brief intense phase and a prolonged tail. For example, in the weakest response of Fig. 4A the four spikes were superposed on a depolarization that had a total duration of about 6 msec. With the next stronger response (B) the depolarization was about 10 msec in duration, but the second and third spikes were reduced in size, possibly owing to cathodal depression exerted by the large depolarization on which they were superposed. Finally, with the largest response (C) the spikes were superposed on a long tail of depolarization over 30 msec in duration, and between the second and third spikes appeared the break in rhythm that probably is further evidence of the initial intense phase of depolarization. The depolarizations of Fig. 4 were much less than those of Fig. 5, but the recording conditions were much inferior in Fig. 4, where the membrane potential and the spike potentials were only about -10 mV and 4 mV respectively.

The series of Fig. 2H–L similarly shows the spikes superposed on a prolonged depolarization up to 30 msec in duration, which is better seen when there were few spikes in H–J, than in L, for the depolarization was there largely submerged by the after-hyperpolarization that followed each spike. This after-hyperpolarization was very prominent in Figs. 1 and 4E–H. Evidently the generation of impulses had occurred at a locus remote from the site of recording.

It is surprising that the very large EPSPs of Figs. 5 and 6 failed to generate spikes. Comparable observations have recently been made with the cells of origin of the dorsal and ventral spino-cerebellar tracts (Eccles, Oscarsson & Willis, 1961; Eccles, Hubbard & Oscarsson, 1961), and with some cells of the dorsal horn (Eccles & Krnjević, 1959). It was suggested

that the spike-generating mechanism was suppressed by the depolarization resulting from injury by the micro-electrode. With motoneurones also the depolarization resulting from severe injury eliminates the production of spikes by the EPSP, there being a transitional phase of local responses. In all these examples the elimination of the spikes does not greatly affect the EPSPs. With motoneurones the time course is shortened as a consequence of the diminution of the membrane time constant (Eccles, 1961), the time course of the EPSP approaching nearer to that of the currents generating the EPSP. It may be concluded that the EPSPs of Figs. 5 and 6 give reliable information on the time course of the synaptic excitatory action that evoked the high-frequency discharges in other Renshaw cells. The high intensity of the brief initial synaptic excitation furthermore provides a sufficient explanation of the failure of large doses of dihydro-β-erythroidine to suppress the first one or two discharges (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1956; Curtis & Eccles, 1958*b*; Brooks & Wilson, 1959).

Nevertheless, an important question still remains to be answered. It can be assumed that the EPSPs of Fig. 5 were generated by a virtually synchronous bombardment of the Renshaw cell by one impulse in each of several motor-axon collaterals. There is no evidence of any delayed bombardment via interneurones, which would be revealed by irregularities on the declining phase of the EPSP. Thus an explanation is required for the fast and slow components of the EPSPs produced by single impulses.

One suggestion would be that the initial rapid decline in the EPSPs of Fig. 5 is due to rapid destruction of acetylcholine by cholinesterase that is localized close to the site of its liberation. The subsequent low intensity of EPSP could then be attributed to the action of acetylcholine that had diffused beyond the zone of high cholinesterase activity. Unfortunately there are no observations of the effects produced by anticholinesterases on EPSPs such as those of Fig. 5. However, the action of anticholinesterases on the repetitive discharges of Renshaw cells indicates that the initial intense phase of action is not changed (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1956), the only significant action being the intensification and great prolongation of the later low frequency component of the response.

Possibly, therefore, diffusion accounts for the initial rapid decline in intensity of action of the synaptic transmitter. However, on account of the electric time constant of the cell membrane, the rate of decline of the EPSPs of Fig. 5G–J will be much slower than that of the transmitter (cf. Curtis & Eccles, 1959). It may be assumed that diffusion out of the synaptic cleft would cause the transmitter concentration in the synaptic cleft to decline much faster than the EPSPs of Fig. 5G–J (cf. Eccles &

Jaeger, 1958, Fig. 3B). The prolonged residuum of action could then be due to the more diffuse action of acetylcholine on the membrane receptors adjacent to synapses. It has recently been shown (Miledi, 1960) that such receptors normally exist in an extensive zone around the region of the neuromuscular junction.

In the slower records of Fig. 6 there is clear evidence of brief depolarizing potentials occurring in random fashion. Since they have a time course rather like EPSPs, they may be assumed to be examples of the synaptic noise that has been reported on motoneurones and attributed to background internuncial bombardment (Brock, Coombs, & Eccles, 1952). In the anaesthetized preparation in which the activity of Fig. 6 was recorded, repetitive discharges of impulses from motoneurones and so up to motor-axon collaterals are unlikely to be the cause of all the activity seen in Fig. 6E–H. At least seven of these potentials occurred in Fig. 6I before the antidromic stimulus, hence the random potentials of Figs. 6E–H were not generated by the antidromic stimulation. However, there are synaptic connexions from interneurones to Renshaw cells (Curtis, Phillis & Watkins, 1961) and discharges from such interneurones could cause the spontaneous activity in Fig. 6. Alternatively, the small potentials could be comparable to the miniature end-plate potentials first described by Fatt & Katz (1952), and shown by them to be due to the liberation of quanta of transmitter. It should be remembered that the transmitter is the same as at the neuromuscular junction, and that the axon collaterals making synaptic connexions with Renshaw cells are indeed branches of the same motor nerve fibres that produce the miniature end-plate potentials of muscle. Possibly such miniature synaptic potentials are responsible for the late irregular discharges seen in such records as Fig. 2J, K, L and for the spontaneous discharges that are given by many Renshaw cells (Eccles *et al.* 1956; Curtis & Eccles, 1958*a*).

Convergence on to Renshaw cells from different muscle nerves

The convergence of muscle nerves on to a Renshaw cell was investigated by testing in turn the responses of the cell to single maximum alpha volleys in the motor fibres of the various muscle nerves. Conceivably this technique would demonstrate an arbitrarily restricted field of motoneurones from which a Renshaw cell could be excited during normal activity, for in such activity the repetitive firing of motoneurones might lead to the recruitment of Renshaw cells that were not excited by single volleys. This possibility was tested in one experiment by investigating the effect of repetitive synaptic activation of Renshaw cells. If a single volley failed to excite a Renshaw cell, a succession of volleys was also ineffective. The convergence revealed by the technique used in the present

work will thus also be present during the repetitive firing of motoneurones during normal reflex activity.

Individual Renshaw cells in Table 1 tend to be dominated by one or two muscle nerves. When there was only one dominant line, the Renshaw cell might be labelled as belonging to that particular muscle. When there was more than one dominant line, a convergence of synergists was sometimes evident, as in cells 13 and 16 in Table 1. Other Renshaw cells, however, were dominated by muscles of unrelated reflex functions, e.g. cells 11 and 12 were excited maximally by the hip extensor, AB, and the knee flexor, PB. The possibility of interaction between antagonists at the same joint is, however, limited by the topographical arrangement of the motoneuronal nuclei in the spinal cord. The knee extensors, Q, are more cephalad than the knee flexors, PB and ST, the centres of the respective nuclei being separated by more than one segment. This separation is sufficient to prevent any significant interaction between those antagonists. The ankle extensors, MG, LG and Sol., are more caudal than the ankle flexors (supplied by the deep peroneal nerve), but there is more overlap of the respective nuclei than with the knee antagonists. The location of motoneurones supplying the ankle antagonists thus again limits the possibility of convergence on to the same Renshaw cell, but there may also be other factors which limit the convergence.

SUMMARY

1. A detailed investigation has been made of the repetitive responses evoked in Renshaw cells by various sizes of antidromic volleys in alpha motor fibres. Comparable responses of a Renshaw cell were observed whether the size was varied by graded submaximal stimulation of a ventral root or by antidromic volleys in the different muscle nerves that converged on the same cell.

2. An analysis of the responses when there was a smooth decline in frequency from an initial maximum indicated that the synaptic stimulation had an initial very intense phase, about 2–3 msec in duration, followed by a much longer phase of low intensity. The very intense initial phase was also indicated by the break in rhythm sometimes observed between the second and third discharges, and by the high resistance of the initial 2 or 3 discharges to the depressant action of drugs such as dihydro-β-erythroidine hydrobromide.

3. An intense initial phase of synaptic depolarization was also revealed by intracellular recording from Renshaw cells. Usually the time course was obscured by superimposed spikes; but these spikes were occasionally absent and the full time course of the synaptically induced EPSP was revealed. There was an initial depolarization about 2 msec in duration

and up to 36 mV in height and a later slowly declining tail up to 60 msec in duration.

4. The EPSP evoked by a second antidromic volley was depressed for as long as 150 msec after a preceding volley, which correlates with previous reports of a prolonged depression of the discharges that a second antidromic volley evokes from Renshaw cells.

5. An investigation has been made of the pattern of activation of Renshaw cells from a large variety of muscle nerves, and its significance has been discussed.

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