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DISTRIBUTION OF RECURRENT INHIBITION AMONG MOTONEURONES

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In recurrent inhibition the discharge of impulses from motoneurones exerts an inhibitory influence not only on motoneurones of the same species (homonymous motoneurones), but also on other motoneurones of diverse function (Renshaw, 1941; Lloyd, 1946, 1951; Eccles, Fatt & Koketsu, 1954; Holmgren & Merton, 1954; Granit, Pascoe & Steg, 1957; Henatsch & Schulte, 1958; Kuno, 1959; Wilson, 1959; Brooks & Wilson, 1959; Wilson, Talbot & Diecke, 1960). It is now generally agreed that the recurrent inhibitory pathway runs via motor axon collaterals to special interneurones in the ventro-medial part of the ventral horn (Renshaw, 1946), which in turn discharge impulses that directly inhibit motoneurones (Eccles, Fatt & Koketsu, 1954; Frank & Fuortes, 1956; Eccles, Eccles, Iggo & Lundberg, 1961). Undoubtedly recurrent inhibition provides a negative feed-back whereby motoneuronal discharge causes inhibition that is extensively distributed to neighbouring motoneurones of diverse function. There is no evidence that recurrent inhibition extends more than one segment along the cord, and it is strictly ipsilateral.

In the earlier investigations the distribution of recurrent inhibition appeared to have no functional significance in reflex co-ordination and a general suppressor function on motoneuronal excitability was postulated (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954; Holmgren & Merton, 1954; Hammond, Merton & Sutton, 1956). Subsequent investigators have attempted to discern additional functional meanings. Granit *et al.* (1957) found that recurrent inhibition was particularly exerted on tonic alpha motoneurones, which was confirmed by Kuno (1959), and proposed that recurrent inhibition served to stabilize the discharge of tonic motoneurones at low frequencies even during strong gamma excitation of muscle

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spindles (Granit & Rutledge, 1960). Brooks & Wilson (1959) also proposed a function related to the gamma-loop activation of motoneurones, but with a more general distribution to motoneurones of all types. When gamma motoneurones activate muscle spindles of a muscle, they evoke the discharge of Group Ia afferent impulses, which monosynaptically excite not only the motoneurones of that muscle, an homonymous action, but also the motoneurones of synergic muscles, and even of apparently unrelated muscles (Eccles, Eccles & Lundberg, 1957*a*; Eccles & Lundberg, 1958). These latter types of activation are usually weaker, and hence are more readily suppressed by the recurrent inhibition driven by the discharging motoneurones. Thus recurrent inhibition would tend to confine the operation of the gamma-loop mechanism to the alpha motoneurones belonging to the muscle containing the discharging annulo-spiral endings (cf. Granit & Rutledge, 1960), and hence subserve finesse of movement. Finally, Wilson, Talbot & Diecke (1960) investigated the distribution not only of recurrent inhibition, but also of the recurrent facilitation that was originally reported by Renshaw (1941) and later overlooked because it was depressed in anaesthetized preparations (Wilson, 1959; Wilson & Talbot, 1960). On the basis of this additional information they proposed that, besides the inhibitory stabilization of level of excitation of motoneurones and the sharpening of effectiveness of monosynaptic excitation, the recurrent facilitation serves to enhance the level of excitation of flexor motoneurones, which otherwise would be dominated by the more powerfully activated extensors.

The present investigation has been undertaken in relation to these various postulated functions of recurrent inhibition, which are tested by a more extensive survey of the distribution of recurrent inhibition than has hitherto been attempted. Altogether over 400 motoneurones belonging to fourteen different muscles of the cat hind limb have been studied intracellularly in order to discover the incidence of recurrent inhibition in response to antidromic volleys in fourteen different muscle nerves. In the original survey there were only thirty-six motoneurones and eight types of antidromic volley (Eccles, Fatt & Koketsu, 1954). The only other intracellular survey was restricted to twenty-eight gastrocnemius-soleus motoneurones and three types of antidromic volley (Kuno, 1959). A preliminary report has been published (Eccles, Iggo & Ito, 1960).

METHODS

The ten cats used were lightly anaesthetized with pentobarbital sodium. The spinal cord was cut at the upper lumbar region (lumbar 1 or 2) and all the ipsilateral dorsal roots from sacral 3 to lumbar 5 were cut. Up to fifteen nerves supplying the muscles of the hind limb were dissected free in the leg and set up for electrical stimulation. All the motor nuclei innervating knee and ankle muscles lie in the lumbar 6 and 7 and sacral 1 segments and

almost all the limb-muscle nerves, including the inferior and superior gluteal nerves, arising from this part of the cord were tested. A full investigation of the Renshaw inhibition for lumbar 5 and 6 segments has not been attempted because of the technical difficulty of working with many of the muscle nerves originating from these segments.

The techniques for intracellular recording from motoneurones have already been published in detail (Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Eccles & Fatt, 1955*a*). Single micro-electrodes filled with either 0·6 M-K_2SO_4-agar, or occasionally 3 M-KCl, were used. With KCl-filled electrodes the inhibitory potentials were tested at different membrane potential levels to see whether the inhibitory potential was masked by a change in the inhibitory equilibrium potential due to the leakage of Cl^- ions from the electrode (Coombs, Eccles & Fatt, 1955*b*; Eccles, Eccles & Lundberg, 1957*b*). The time constant of the amplifier (500 msec) was sufficiently long for recording the recurrent or Renshaw inhibitory post-synaptic potentials (RIPSPs) without appreciable distortion. Invasion of a motoneurone by an antidromic impulse in its own axon was prevented by passing between the micro-electrode and earth a brief pulse (0·1–1·2 msec) of hyperpolarizing current (see Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957). In this way it was possible to employ a maximum antidromic volley in the homonymous nerve without the complication of an after-hyperpolarization in the motoneurone under observation. While thus avoiding depression of the Renshaw IPSP on account of superposition on the after-hyperpolarization, there was, nevertheless, still some depression because the IPSP was superposed on, and hence occluded by, the hyperpolarization that decayed for many milliseconds after the blocking pulse. By comparing the IPSPs of Fig. 1 *R* and *T*, the occlusion is seen to diminish the Renshaw IPSP to about 60 %. Throughout this paper there has been no correction for this effect, hence all homonymous IPSPs will be underestimated.

Nomenclature. The following abbreviations have been used, especially in figures: AB = anterior biceps; SM = semimembranosus; ST = semitendinosus; PB = posterior biceps; MG = medial gastrocnemius; LG = lateral gastrocnemius; Sol = soleus; Pop = popliteus; Pl = plantaris; FDL = flexor digitorum longus + flexor hallucis longus; Per = peroneal, supplying all three peroneal muscles as well as tibialis anticus, extensor digitorum longus and brevis; SG = superior gluteal; IG = inferior gluteal; T = tibial, supplying flexor digitorum brevis and the small foot muscles; Q = quadriceps; Grac = gracilis.

RESULTS

Post-synaptic potentials generated by recurrent inhibition (RIPSP)

As illustrated in Figs. 1, 2 and 3, maximum antidromic volleys in the motor fibres of many muscle nerves produce RIPSPs in a motoneurone. These diverse RIPSPs vary greatly in size, but have similar time courses: a central latency of rather more than 1 msec; a maximum at about 5 msec later; and a total duration of about 40 msec (cf. Eccles, Fatt & Koketsu, 1954). Since the equilibrium potential for the RIPSP (about -80 mV) is only about -10 mV from the resting potential (Coombs *et al.* 1955*b*), a linear increase in the inhibitory synaptic action will give a progressively diminishing increase in the size of the RIPSP, i.e. there is an interaction, or occlusion, between superimposed RIPSPs, which increases with the sizes of the RIPSPs (Fig. 3). Usually, the RIPSPs generated by single muscle volleys have not been larger than -2 or -3 mV, so there is no serious error in employing the amplitudes of the RIPSPs in Figs. 1 and 2

as measures of the respective inhibitory synaptic actions without making any correction for occlusion.

A total of 474 motoneurones were collected in the present experiments. As judged by the magnitude and instability of the resting and action potentials, many of the motoneurones were damaged by the penetration of the micro-electrode, and consequently were discarded as unreliable when assembling the statistical analyses in Tables 1 and 2. Because the sulphate-filled electrodes tended to be noisy and to block frequently, the resting potential measurements were not considered to be sufficiently reliable to provide an index of the condition of the cell. Instead, the size of the antidromic spike potential has been used and all cells with a spike potential of less than 50 mV were rejected.

In order to compare the total amount of recurrent inhibition which

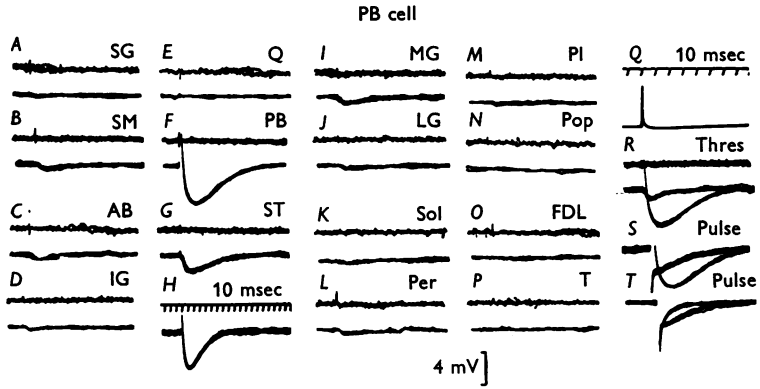

Fig. 1. Intracellular records from a posterior biceps motoneurone to illustrate the recurrent or Renshaw post-synaptic inhibitory potentials (RIPSPs) generated by antidromic volleys from different muscle nerves. Lower traces of *A–P* give the responses evoked by maximum antidromic volleys from the various muscle nerves as indicated by the symbols, which may be identified by reference to the paragraph, under Methods, labelled 'nomenclature'. Downward deflexion signals increasing negativity of the micro-electrode tip, i.e. hyperpolarization, *H* shows PB response at a slower sweep speed, all other records being at speed given in time scale of *Q*. Upper traces were taken from the dorsal surface of the cord. In *R* the RIPSP from PB was sometimes recorded since the stimulus was at the threshold of the axon itself. When the axon was activated the RIPSP was obscured by the larger after-hyperpolarization. In *S* a rectangular pulse was applied in about 50 % of the records, so preventing the invasion of the cell. In *T* the effect of such a pulse alone (upper record) is compared to the pulse plus the posterior biceps volley. The difference between them indicates the size of the autogenous RIPSP. Spike evoked by the PB volley was 89 mV (*Q*); conduction velocity of the axon was 70 m/sec; duration of after-hyperpolarization (AHP) was 100 msec (*H*); resting potential (RP) was −67 to −62 mV. Micro-electrode was filled with 0·6 M-K_2SO_4 plus 1 % agar. Same potential scale for records except *Q*. Records in this and subsequent figures consist of superposed sweeps.

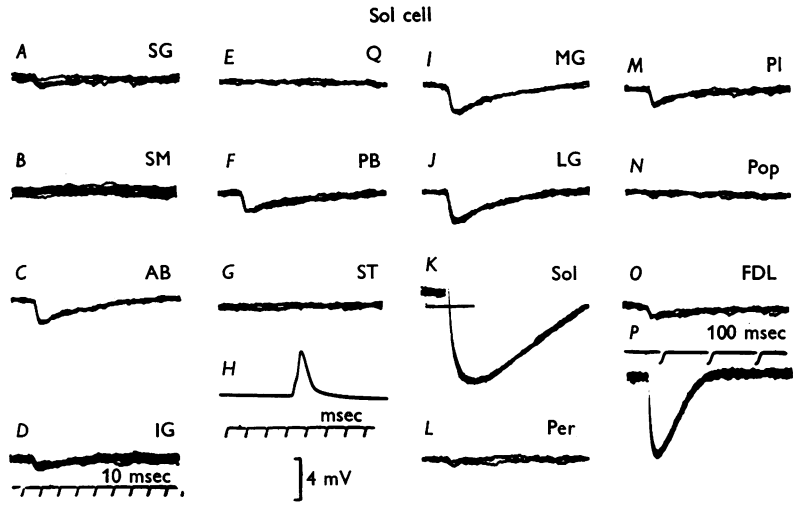

Fig. 2. Intracellular records as in Fig. 1, but from a soleus motoneurone in order to show the wide field of neurones from which it receives Renshaw inhibition. The size of the autogenous RIPSP is indicated by a line on the soleus record (*K*), where the large after-hyperpolarization follows the antidromic spike potential. This cell had a resting potential of −60 to −63 mV; the spike potential was 80 mV (*H*); the duration of the after-hyperpolarization was 140 msec (*P*); and the conduction velocity of its axon was 67 m/sec. Same potential scale for records except *H*, and time scale of *D* for all except *H* and *P*.

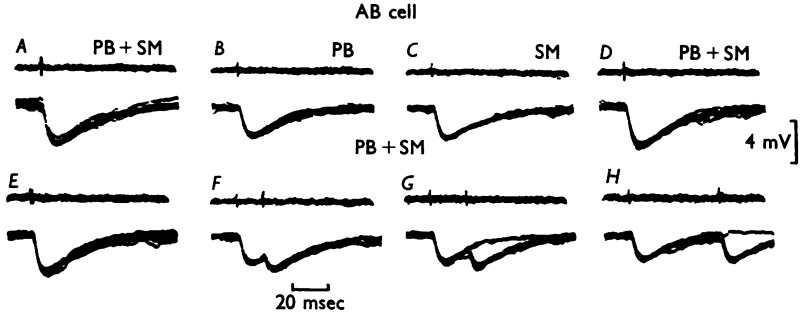

Fig. 3. These records from an anterior biceps motoneurone illustrate the RIPSPs produced by interaction of antidromic volleys from two different muscle nerves. In each record the upper trace is from a lead on the surface of the cord and the lower is the intracellular record. The RIPSPs were generated by maximal volleys in the alpha motor axons in posterior biceps (PB) and semimembranosus (SM) alone and together as indicated by the symbols; with synchronized volleys in *A*, *D* and *E* and with SM following PB at various intervals in *F*–*H*. At 50 msec there was almost no sign of the occlusion which was so evident in *E*–*G*. Antidromic spike (not shown) was 90 mV; autogenous RIPSP by pulse method was 3·2 mV; resting potential, −62 mV.

could be generated in different motoneurones, the peak amplitudes of the RIPSPs produced in an individual motoneurone by maximum antidromic volleys from each muscle nerve alone have been added together to give a cumulative figure (the sum of the autogenous and heterogeneous in Table 1), which was as large as 22 mV in one Sol motoneurone. These aggregate values give a useful basis for comparison between different species of motoneurones, but it is evident that synchronous volleys in all the muscle nerves could not generate such a large RIPSP which would be

TABLE 1. The mean values for the action potentials, conduction velocities, duration of after-hyperpolarizations and Renshaw IPSPs for the motoneurones of the hind limb. The nuclei are arranged in descending order of duration of after-hyperpolarization. The RIPSP is entered under autogenous RIPSP, i.e. that generated by volleys in the motoneurone's own muscle nerves and heterogeneous RIPSP, i.e. the sum of the individual RIPSPs generated by volleys in all the other muscle nerves. The standard error of each mean is given, with the number of cells tested in parentheses

Motoneuronal nucleus	Action potential (mV)	Conduction velocity (m/sec)	After-hyperpolarization: Duration (msec)	After-hyperpolarization: % of cells longer than 110 msec	Renshaw IPSP (autogenous) (mV)	Sum of all Renshaw IPSPs (heterogeneous) (mV)
Soleus	67 ± 3·2 (11)	64 ± 1·8 (12)	186 ± 3·5 (9)	100	1·46 ± 0·22 (7)	12·8 ± 1·6 (11)
FLD + FHL	66 ± 3 (15)	75 ± 2·1 (14)	118 ± 5·6 (13)	54	0·5 ± 0·25 (13)	3·4 ± 0·95 (16)
Plantaris	67 ± 2·3 (23)	75 ± 2·2 (15)	110 ± 4·9 (22)	45	1·16 ± 0·36 (9)	3·5 ± 0·65 (23)
Semimembranosus	72 ± 2·1 (10)	75 ± 5 (6)	110 ± 4 (10)	40	2·47 ± 0·12 (6)	6·6 ± 1·55 (6)
Anterior biceps	64 ± 1·8 (29)	76 ± 1·7 (31)	109 ± 3·9 (27)	37	2·06 ± 0·28 (17)	5·3 ± 0·36 (29)
Lateral gastrocnemius	66 ± 2·3 (24)	79 ± 2·1 (16)	105 ± 4·3 (19)	31	1·32 ± 0·11 (16)	4·1 ± 0·56 (24)
Medial gastrocnemius	62 ± 2·3 (32)	73 ± 1·1 (21)	103 ± 4·1 (25)	40	0·93 ± 0·2 (19)	2·9 ± 0·5 (30)
Gluteal	68 ± 2·3 (19)	—	103 ± 2·9 (18)	21	1·52 ± 0·2 (12)	2·9 ± 0·45 (19)
Posterior biceps	64 ± 1·6 (43)	79 ± 0·4 (46)	99 ± 2·5 (39)	10	1·52 ± 0·34 (29)	3·1 ± 0·49 (44)
Popliteus	69 ± 3·4 (7)	80 ± 2·9 (9)	98 ± 5 (6)	16	1·37 ± 0·25 (4)	3·8 ± 0·32 (7)
Peroneal	65 ± 2·1 (18)	85 ± 7·3 (4)	95 ± 2·5 (11)	18	0·45 ± 0·23 (8)	1·4 ± 0·42 (18)
Semitendinosus	66 ± 2·9 (13)	78 ± 0·5 (15)	89 ± 4·0 (9)	0	1·5 ± 0·25 (12)	1·9 ± 0·49 (13)

far beyond the potential required to reach the equilibrium potential for the IPSP. Not only would there be occlusion between the RIPSPs generated by the different muscle volleys, but a further occlusive influence would occur at the level of the Renshaw cells themselves, because there is a very effective convergence of the axon collaterals from the motor fibres to many different muscles on to the same Renshaw cell (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1961).

With a few very sigificant exceptions the largest RIPSP was generated by an antidromic volley in the muscle nerve to which the impaled motoneurone belonged (Table 2, Fig. 9). The measurement of this autogenous RIPSP required that antidromic invasion of the impaled cell should be blocked by means of a short hyperpolarizing pulse through the recording

electrode as described in Methods. This autogenous RIPSP was largest with semimembranosus and AB motoneurones (Table 1). Exceptions to the rule were soleus and flexor longus digitorum motoneurones (Figs. 2, 9, 10; Table 2), where the autogenous RIPSP was smaller. The small number of alpha motor fibres in the soleus nerve presumably accounts for the poverty of its autogenous RIPSP.

Factors governing the intensity and pattern of the RIPSP received by a motoneurone

Type of motoneurone. Measurements of axonal conduction velocity and duration of after-hyperpolarization have been made on 146 alpha motoneurones. After pooling these motoneurones into eight categories according to conduction velocity, it is found that the mean durations of the after-hyperpolarization for each velocity category lie along the curve shown in Fig. 4. Evidently, those axons with conduction velocities of less than 70 m/sec come from motoneurones with relatively long after-hyperpolarizations. This confirmation of earlier investigations (Eccles, Eccles & Lundberg, 1958; Kuno, 1959) provides further support for the assumption that cells with long after-hyperpolarizations are tonic alpha motoneurones. If an arbitrary figure of 110 msec is taken to be the lower limit for the after-hyperpolarizations that are classed as belonging to tonic motoneurones, then the Sol sample in Table 1 exclusively comprised tonic motoneurones, whereas at the lower end of the scale ST was exclusively phasic. However, such a rigid criterion for tonic and phasic classification must be regarded as of doubtful validity.

Motoneurones with long after-hyperpolarizations always received a larger total RIPSP than motoneurones with brief after-hyperpolarizations, though the receptive field may be just as extensive. For example, a comparison of Figs. 1 and 2 shows typically that a soleus motoneurone received a larger aggregate RIPSP from much the same receptive field as for the PB motoneurone. Both the aggregate RIPSP and the duration of the after-hyperpolarization have been determined for 210 motoneurones. After pooling these motoneurones into eleven categories according to the duration of the after-hyperpolarization, the mean aggregate RIPSP was determined for each category and plotted as in Fig. 5. It is seen that there is an approximately linear relationship, the motoneurones with the longest after-hyperpolarizations having the largest aggregate RIPSPs. If an arbitrary figure of -9 mV is taken to indicate a very large aggregate RIPSP, then 75 % of the Sol motoneurones are in the group having very large RIPSPs. The SM comes next with 50 % of its cells in that same group followed by AB with 17 %.

If the assumption that all alpha motoneurones with long after-hyperpolarizations are tonic is correct, the present results indicate that the tonic alpha motoneurones are more strongly inhibited by the recurrent collaterals than are the phasic motoneurones. This conclusion is in agreement with the results of the reflex experiments of Granit *et al.* (1957). There do not, however, appear to be two distinct populations of motoneurones;

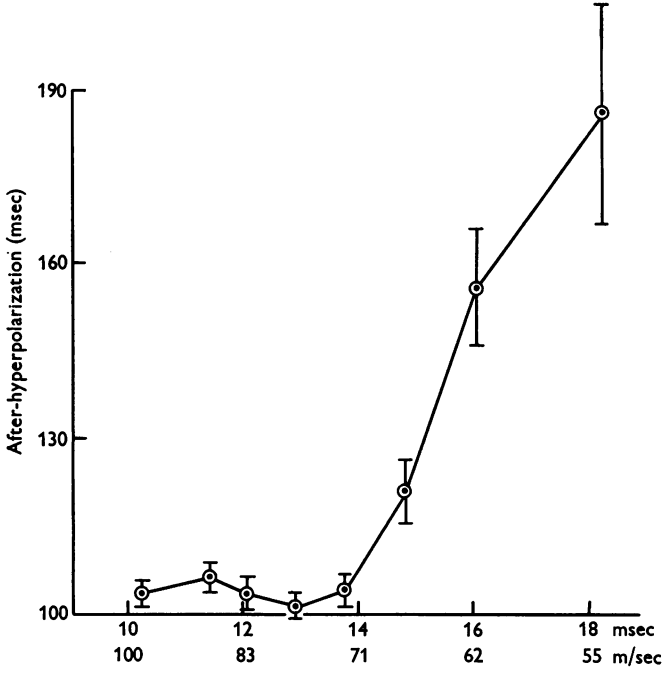

Fig. 4. Relationship between the conduction velocities of the axons and the durations of the after-hyperpolarization from 227 motoneurones. The times to travel 1 m are plotted as abcissae in linear scaling and the equivalent conduction velocities are also shown. On correction (assuming a $Q_{10} = 1{\cdot}7$) for the relatively low temperatures of the preparations (usually 34–35° C) the inflexion on the curve occurs at approximately 80 m/sec. The vertical lines indicate the sizes of the standard errors of the means at each point. Further description in text. The population was grouped into classes at intervals of 10 msec on the conduction velocity scale, except at either end where, because of the small sample available, the classes were larger.

all transitions exist both with the conduction velocities (Fig. 4) and with the after-hyperpolarizations (Fig. 5). The gamma motoneurones, on the other hand, do not develop a detectable RIPSP even though their axons are much more slowly conducting than those of the tonic alpha motoneurones (Granit *et al.* 1957; Eccles, Eccles, Iggo & Lundberg, 1960).

Location of the motoneurone. It was reported by Renshaw (1941) and by Eccles, Fatt & Koketsu (1954) that the effectiveness of recurrent inhibi-

tory action from muscle nerves on to a motoneurone was related to the proximity of this motoneurone to the motor nuclei of the various nerves. On the other hand, while recognizing the possible influence of proximity, Wilson *et al.* (1960) stressed particularly that a meaningful functional pattern could be discerned. Before attempting to relate the present results to the concepts developed by Wilson *et al.* three different procedures will be adopted in order to see how far the new evidence conforms to the

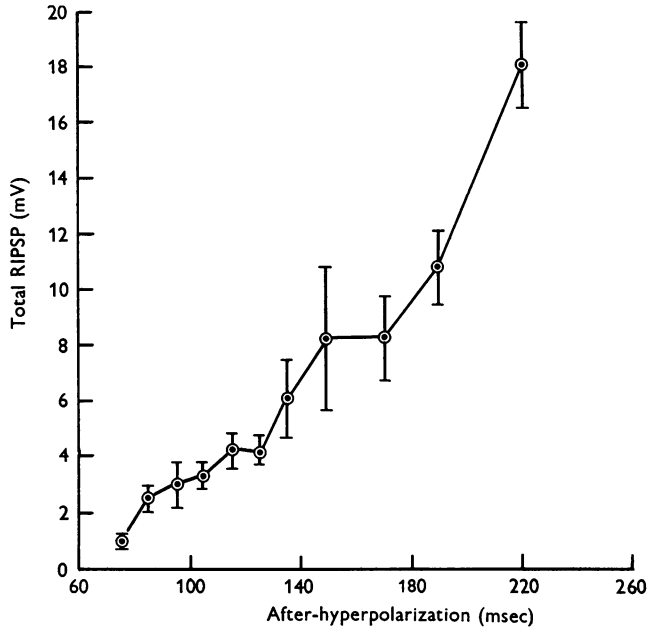

Fig. 5. Illustration of the relationship between after-hyperpolarizations of 210 motoneurones and the sums of all RIPSPs received by them from all the muscle nerves tested. Vertical lines indicate the sizes of the standard errors of the means. Further description in text. The population was arbitrarily grouped into classes at 10 msec intervals along the abscissa, except for the last four classes which were made larger to increase the size of the sample for statistical analysis.

proximity principle. In (i) and (ii) individual cells were tested whilst in (iii) populations of cells were examined.

(i) Figure 6 shows that when several cells in the same nucleus of one animal (AB in this example) were examined at the same segmental level, there was a fairly consistent pattern in the effectiveness of different muscle nerves in generating the RIPSP. The relative potency of these different nerves can be related in part to the proximity of the respective motor nuclei to the AB nucleus. For example, PB, SM and ST motoneurones lie in the same column of cells in the ventral horn and at overlapping segmental levels (Romanes, 1951), while the other potent nerves in Fig. 6

were MG and LG, which have their motoneurones just dorsal to the AB motoneurones, and IG with a just ventral location. These results for a single nucleus sampled at one level of the cord are illustrative of many series in the present investigation and are in general agreement with the proximity hypothesis.

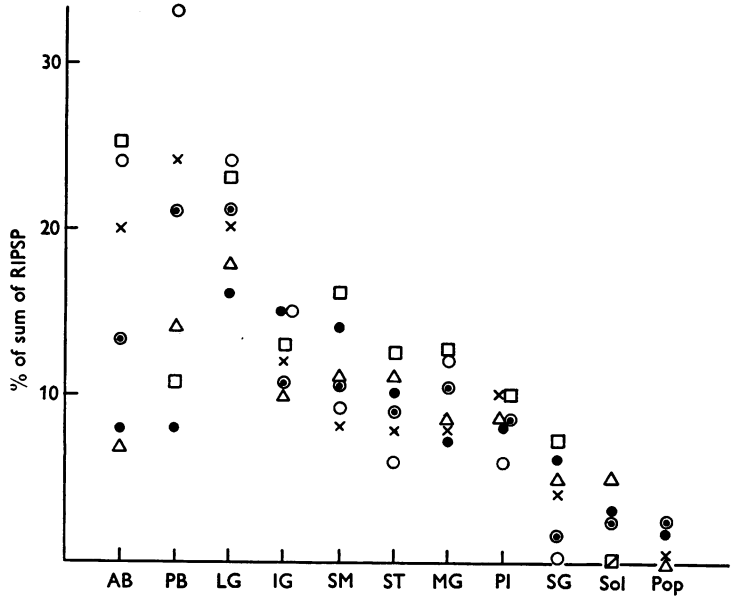

Fig. 6. In six anterior biceps motoneurones indicated by the six different symbols the RIPSPs received from all eleven nerves are plotted as percentages of the sums of all the RIPSPs. These neurones were all in the same region of the anterior biceps nucleus of one cat. The nerves are arranged approximately in descending order of effectiveness.

(ii) In an alternative procedure the RIPSP production has been determined for motoneurones in the same nucleus but at several different segmental levels. For this investigation the PB nucleus is particularly appropriate since it extends through nearly two segments. For example, the RIPSPs produced in PB cells by the most effective three muscle nerves were recorded at three segmental levels several millimetres apart, the mean values being plotted in Fig. 7*A*. The much greater effectiveness of the AB volley at the most rostral level (level I at lower L7) may be correlated with the more rostral location of its motor nucleus. This segmental gradient of effectiveness is even better illustrated in the larger sample of motoneurones plotted in Fig. 7*B*, where the mean RIPSPs recorded from all species of motoneurones were plotted as in Fig. 7*A*. The motor nuclei of PB, MG and IG correspond closely in their caudal extension, which is

several millimetres beyond the AB nucleus, as is indicated in the locations marked in Fig. 9. The segmental gradients of effectiveness of various muscle nerves in generating RIPSPs are thus in good agreement with the segmental locations of their respective motor nuclei.

(iii) There is also a dorso-ventral gradient in the patterns of RIPSP distribution. For example, Fig. 8 is formed by assembling the various motoneurones and their associated motor fibres into four groups that correspond to their dorso-ventral location, the dorso-ventral sequence being: FDL+Pl; MG, LG, Sol; PB, AB. ST; SG. For each group of motoneurones the RIPSPs are plotted as percentages of the aggregate RIPSP.

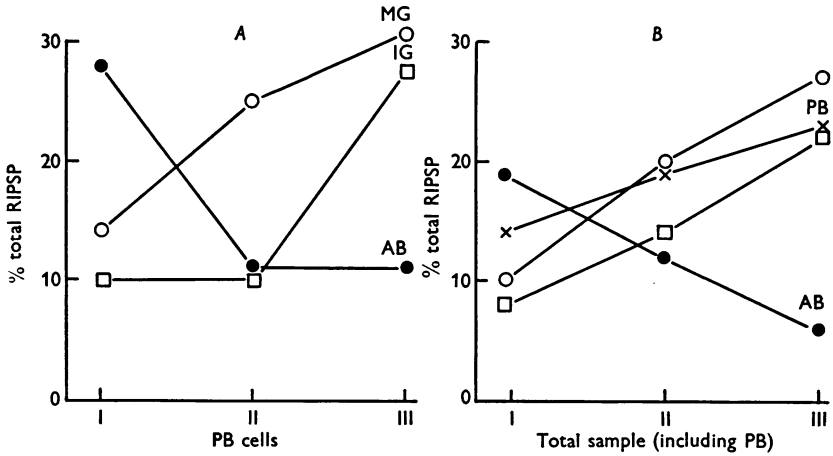

Fig. 7. *A*. In one cat posterior biceps motoneurones were investigated at different segmental levels: (I) in the lower L7 region; (II) 1 mm more caudally; and (III) in the upper S1 region. At each site the means of the RIPSPs from each source (MG, IG and AB) are expressed as percentages of the sum of all RIPSPs received. In *B*, mean RIPSPs in all the motoneurones in that same cat (including the posterior biceps that are alone plotted in *A*) are plotted for the three regions in the same way as in *A*, but RIPSPs produced by PB volleys are added. Crosses refer to PB volleys, otherwise symbols as in Fig. 7*A*.

There is seen to be a general tendency for the more ventrally located motoneurones to develop large RIPSPs in response to antidromic volleys to the corresponding motor nuclei, and in particular FDL and Pl volleys were quite ineffective on the two most ventral groups. Likewise the two most dorsal groups of motoneurones generate very little RIPSP in response to SG volleys. Neither FDL nor Pl, the most dorsal nuclei, give or receive RIPSPs of the same order of magnitude as more ventrally situated nuclei.

In order to compensate as far as possible for the incomplete sampling imposed by the experimental conditions, the results for all the experiments were pooled and the input pattern for each muscle nucleus was calculated.

The average value for the RIPSP from each muscle nerve to the average cell of the particular motoneuronal nucleus was then plotted on a histogram in Fig. 9. The importance of proximity is still evident. The peroneal nucleus at the rostral end of the series is not affected by the volleys in the muscle nerves of the more caudally located nuclei; reciprocally the motoneurones of the three most caudal nuclei (PB, MG and Sol) develop little if any RIPSP in response to volleys to the more rostrally located nuclei.

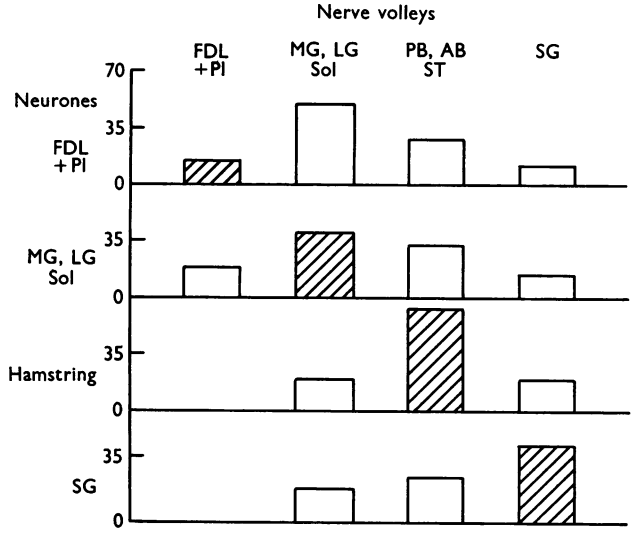

Fig. 8. The amounts of the RIPSPs from different sources are plotted as percentages of the sums of all the RIPSPs. The motor nuclei are arranged in horizontal rows (in relation to their dorso-ventral position): the most dorsal nuclei on the uppermost line; the most ventral on the lowermost. The corresponding nerve volleys are arranged in the vertical columns as indicated by the symbols. The height of each block is a measure of the sum of the RIPSPs which are produced by the antidromic volleys of its column, this sum being expressed as a percentage of the total RIPSPs of the motoneurones belonging to that row. With the hatched areas the RIPSP was produced in motoneurones by volleys of the same group.

The RIPSPs produced in Sol motoneurones provide an illustration of the patterns displayed in Fig. 9. The largest RIPSPs are produced by Pl, LG, AB, PB, MG volleys. The respective nuclei are either intermingled with Sol (LG and MG) or lie immediately dorsal (Pl) or ventral (AB, PB). The least effective volleys belong to nuclei more rostrally located (SM, SG, FDL, Per). An exceptional position is, however, occupied by ST which produces a very small RIPSP despite the proximity of the ST and Sol motor nuclei.

In order to display the pattern of distribution, the mean RIPSPs have been calculated for each type of antidromic volley acting on each type of

motoneurone. In Table 2 the volleys are arranged in columns and the motoneurones in rows. The antidromic volleys which were least effective were Per and FDL, which belong to the nuclei situated most dorsolaterally, and hence furthest from the location of most of the Renshaw cells (Eccles, Fatt & Koketsu, 1954). These motoneurones were also amongst the lowest recipients of RIPSP. Thus it seems that promixity

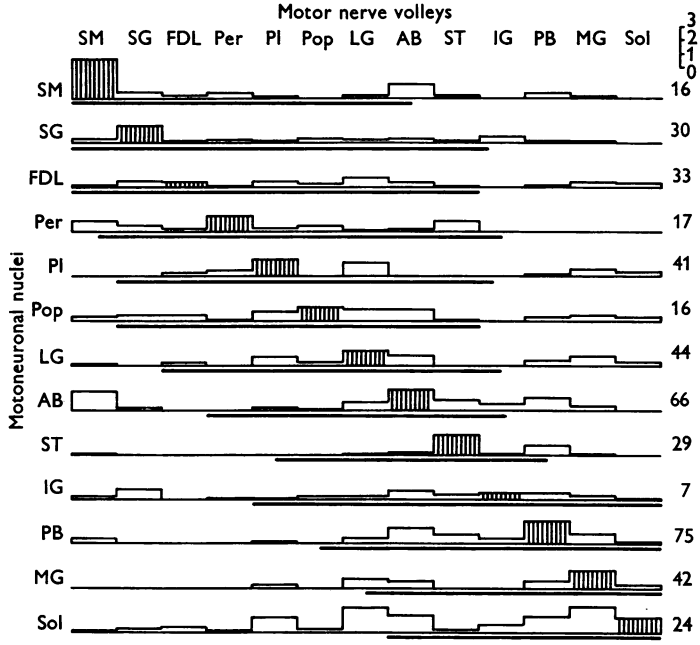

Fig. 9. The mean amounts of the RIPSPs received by all types of motoneurones are plotted for each type of antidromic volley in horizontal rows as indicated by the symbols. The motoneurones are arranged in vertical sequence on the left, the descending order representing relative rostro-caudal position. The RIPSPs produced by the various nerve volleys are arranged in vertical columns as indicated by the symbols above. Numbers to the right indicate the number of motoneurones in each category; the voltage scale applies to all potentials. The approximate longitudinal extent of each nucleus is indicated by the thick lines under each entry.

of the motoneuronal nuclei to the location of the Renshaw cells is an important factor in determining the size of the RIPSP. The poverty of action of Per volleys is presumably related to the extreme lateral course of their axons in the spinal cord (Balthasar, 1952), the motor axon collaterals consequently having a particularly long developmental path in order to reach the Renshaw cells.

Functional relationship of the motoneurones. In Table 2 the mean RIPSPs evoked by antidromic volleys in 387 motoneurones producing

extension and flexion at the various limb joints are tabulated with respect to functional relationship. Unfortunately the Table is incomplete: there were no hip flexors and the number of knee extensor (Q) motoneurones was so small that their RIPSPs were not included in the Table. The Q antidromic volleys were tested on all motoneurones, but uniformly had no action.

TABLE 2. The relation between functional groups of motoneuronal nuclei and the size of the RIPSPs generated in them by antidromic volleys in muscle nerves. The motoneurones producing extension and those producing flexion at hind-limb joints have been grouped and arranged vertically, while the muscle nerves stimulated have been arranged horizontally in the same order. Synergic groups are in bold figures and antagonists are in italic figures

		Nerve volleys										
		Extensors							Flexors			
		Hip		Ankle			Digits		Knee		Ankle	
Motoneurones	No.	AB (mV)	SM (mV)	MG (mV)	LG (mV)	Sol (mV)	Pl (mV)	FDL (mV)	PB (mV)	ST (mV)	Per (mV)	Total (mV)
AB	66	**1·9**	**1·4**	0·42	0·66	0	0·22	0	0·96	0·82	0	6·38
SM	16	**1·07**	**3·0**	0·2	0·2	0	0·1	0·2	0·5	0·2	0·4	5·87
MG	42	0·55	0	**1·3**	**0·68**	**0·23**	0·23	0	0·52	0	*0*	3·51
LG	44	0·84	0·12	**0·74**	**1·2**	**9·3**	0·71	0·2	0·4	0	*0*	4·51
Sol	24	1·3	0·1	**2·0**	**1·9**	**1·1**	1·1	0·37	1·2	0·22	*0·1*	9·39
Pl	41	0	0	0·52	1·0	0·3	**1·2**	**0·24**	0·23	0	*0·41*	3·9
FDL	33	0·37	0·1	0·44	0·7	0·32	**0·5**	**0·4**	0·16	0·12	*0·1*	3·21
PB	75	1·15	0·4	0·78	0·48	0·1	0·15	0	**1·7**	**0·6**	0	5·36
ST	29	0·20	0·12	0·1	0·1	0	0	0	**0·75**	**1·5**	0	2·77
Per	17	0·2	0·7	*0*	*0·1*	*0*	*0·2*	*0·2*	0	0·7	1·1	3·20
Totals		7·58	5·94	6·50	7·02	2·35	4·41	1·61	6·42	4·16	2·11	—

RIPSPs are seen to be particularly large within synergic groups, which are in bold figures in the Table. For example, with the synergic group of hip extensors the AB and SM volleys are particularly powerful on AB and SM motoneurones. Other examples are the ankle extensors (Sol, MG and LG), and the knee flexors (PB and ST). Evidently the large autogenous RIPSPs are just special examples of the large RIPSPs regularly occurring in the interaction between members of synergic groups. In all these examples the large RIPSPs could be attributable to proximity of the respective motoneuronal nuclei; there may be no significance in the functional relationship.

The RIPSPs produced in motoneurones by antidromic volleys from antagonist muscles at the same joint are shown in italic figures. These RIPSPs are always very small and are sometimes absent. It can be stated that RIPSPs between antagonist motoneurones are negligible. Again, however, this may not depend on their opposed functional relationship. In many cases there is a considerable distance between the antagonist motoneuronal nuclei. An exception would be the FDL, Pl and Per motor nuclei; the latter normally lies just lateral to the former two throughout almost the whole L7 segment.

Apart from the synergists at a particular joint, there is a tendency for the largest RIPSPs to be evoked by antidromic volleys from extensors on to extensor motoneurones. However, this relates to the most effective antidromic volleys, AB, MG and LG, rather than to all extensor antidromic volleys, and AB, MG and LG volleys are also effective on flexor motoneurones. Another relatively powerful antidromic volley is from the knee flexor, PB. On the other hand the knee extensor, Q, has little or no action on the extensors at other joints (Figs. 2, 10), an exception that is presumably attributable to the extensive segmental separation between Q motor nucleus in L5 and upper L6 segments, and the nuclei of all the other extensor muscles (in Table 2). Another general statement is that, apart from synergists, flexor antidromic volleys produce little or no RIPSP in flexor motoneurones.

Though there is much evidence that proximity of motoneuronal nuclei has an important influence on the size of the RIPSP, there is also evidence that other factors must be envisaged. For example, in Table 2 ST volleys are very poor at producing RIPSPs in Sol and G motoneurones. Reciprocally Sol and G antidromic volleys are also very poor in producing RIPSPs in ST motoneurones, yet the respective nuclei are in close apposition in the lower L7 and upper S1 segments, the ST being just ventral to the G and Sol motoneurones.

Excitatory post-synaptic potentials generated by antidromic volleys

In both anaesthetized and decerebrate cats, Renshaw (1941) regularly found a facilitation of monosynaptic reflex discharge when the conditioning antidromic volley entered the cord at a different segmental level from the tested muscle nucleus. Since Wilson (1959) has shown that the time course, as measured by changes in the size of the monosynaptic reflex, is longer for recurrent facilitation than for the inhibition, recurrent facilitation might be expected to be evident as a later depolarization. The latency of the facilitation was about 1 msec longer than for the inhibition, which Wilson (1959) suggested was due to at least one more interneurone in the facilitatory path.

Depolarization was never large in the present experiments though occasionally a small late depolarization was recorded (Fig. 10*K*). In order to see if a depolarization was being concealed by a larger hyperpolarization, the membrane was shifted to the equilibrium potential for the RIPSP (approximately -80 mV) by passing a steady current through the intracellular electrode and across the cell membrane, but no additional recurrent excitatory post-synaptic potentials (REPSPs) were revealed by this procedure. Except for one unusually large REPSP of 0·4 mV, all REPSPs were 0·1 mV or less. The total number of times that such a trace

as that of Fig. 10*K* was detected was 32 in a total of about 6000 trials. On 14 of these occasions a flexor motoneurone was depolarized, a peroneal motoneurone being involved ten times; which corresponds to the findings of Wilson, Talbot & Diecke (1960). The next most frequent examples were the extensor motoneurones, AB (8) and FDL (6).

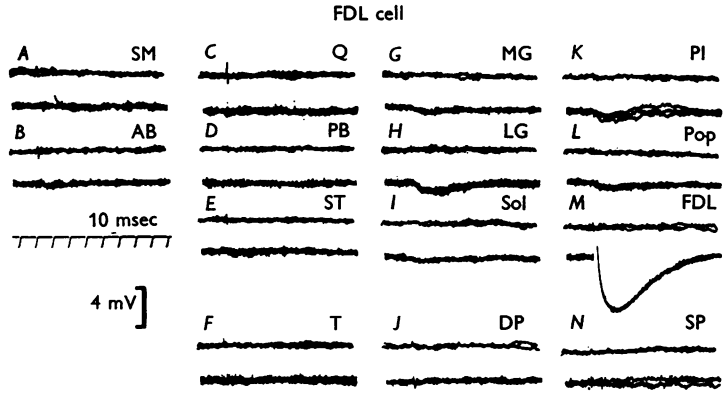

Fig. 10. The RIPSPs from all sources are shown for an FDL motoneurone as in Fig. 1. Note the small REPSP from plantaris (*K*). The antidromic spike was 58 mV; duration of the after-hyperpolarization was 110 msec and the resting potential was −54 mV.

DISCUSSION

In attempting to correlate the present results on the distribution of the RIPSP with the distributions reported by Wilson, Talbot & Diecke (1960), account must be taken of several differences between the two investigations. Recurrent facilitations were far more prominent in the investigation of Wilson *et al.* than were REPSPs in the present investigation, a difference readily explicable by the selective depression exerted by barbiturates on recurrent facilitation (Wilson & Talbot, 1960), although Renshaw (1946) observed large facilitations in cats anaesthetized with Nembutal. However, the present investigation has the advantage that detailed quantitative information is provided for many hundreds of motoneurones. Furthermore, the autogenous RIPSP was regularly measured, whereas autogenous recurrent inhibition was not investigated by Wilson, Talbot & Diecke (1960). Thus, the present investigation is much more comprehensive in respect of recurrent inhibition, but makes virtually no contribution on recurrent facilitation. It may be noted that there is on the whole very good agreement between Table 1 and the preliminary results of Eccles, Fatt & Koktesu (1954, Table 1) with relatively few motoneurones. The low values there reported for most autogenous RIPSPs arise because

in many cases they were submaximal, the stimuli being just below the threshold of the motoneurone's axon.

There is a very satisfactory degree of agreement between the present results on the distribution of RIPSP and those of Wilson, Talbot & Diecke (1960) on recurrent inhibition, particularly if allowance is made for the occasions when recurrent inhibition was masked by recurrent facilitation. For example, in agreement with Wilson, Talbot & Diecke (1960), there is in Table 2 virtually no RIPSP between the ankle extensors and flexors in either direction (G and Sol on the one hand and Per on the other); but there is a considerable RIPSP within each synergic group. There is similar agreement between the two series of investigations on the motoneurones responsible for extension and flexion of the digits, where likewise there is a negligible RIPSP between antagonistic motoneurones, which contrasts with the fairly large RIPSPs within each synergic group. Finally, there is agreement on additional interactions that occur between flexor and extensor motoneurones independently of functional grouping; G+Sol volleys produce an RIPSP in PB and ST motoneurones, which in Table 2 is seen also to be reciprocal; PB and ST volleys produce an RIPSP in AB motoneurones, which also is reciprocal in Table 2, at least for PB; finally Wilson, Talbot & Diecke (1960) reported that SM inhibits and AB facilitates Per motoneurones, though in Table 2 both actions were weakly inhibitory.

These last examples were reported by Wilson, Talbot & Diecke (1960) as being exceptions to their postulated general pattern of distribution of recurrent inhibition and facilitation. According to this pattern antidromic volleys from extensor muscles inhibit extensor motoneurones and facilitate flexors, while flexor antidromic volleys inhibit flexors and have little or no action on extensors. But in Table 2 there are several instances in which extensor volleys produce quite large RIPSPs in flexor motoneurones (AB → PG; G → BP; SM → Per); and similarly with flexors to extensors (PB → AB; ST → AB; PB → Sol). In fact, if the synergic groups be deleted from Table 2, there is on the average little more RIPSP from extensors to extensors (mean −0·35 mV) than from extensors to flexors (mean −0·28 mV) or flexors to extensors (mean −0·30 mV). Possibly, Wilson, Talbot & Diecke (1960) underestimated such types of recurrent inhibition because they were masked by recurrent facilitation. It can be concluded that the results documented in Table 2 provide many examples of distribution of RIPSP that do not conform with the postulates of Wilson, Talbot & Diecke (1960). In Table 2 there is no support for their proposal that the RIPSP has a distribution corresponding to that of Ib inhibition, which is very largely restricted to extensor motoneurones (Eccles *et al.* 1957*b*).

On the other hand, in Table 2 there is much support for the postulate that the distribution of RIPSP is related to the proximity of motoneuronal nuclei regardless of function. For example, AB and PB motoneurones are in the same neuronal column and have a considerable segmental overlap, but they have quite different functions, hip extension and knee flexion respectively. Yet in Table 2 there is a remarkable parallelism between the two motoneuronal types in respect both of action of the antidromic volleys, and of generation of RIPSPs by the motoneurones. However, there are several exceptions to the simple proximity hypothesis that was proposed by Eccles, Fatt & Koketsu (1954): ST motoneurones lie in a column just ventral to G and Sol motoneurones, and are at the same segmental level, yet there is virtually no interaction in either direction; the very poor interaction between the contiguous Per and FDL cell columns is another example. This latter case may arise on account of the lateral trajectory of the Per motor axons, as mentioned above, but the former case seems to require some functional discriminatory factor between flexor and extensor motoneurones in respect of RIPSP connexions.

The patterns of Renshaw cell connexions give an opportunity for investigating problems relating to the manner in which inhibitory connexions are established in development and to the possibility of changing the connexions in response to altered motoneurone function consequent on cross-union. Certainly the patterns are much less discriminative than with the Ia inhibitory action, which very largely operates between antagonists at a joint (Laporte & Lloyd, 1952; Hunt & Perl, 1960). Yet mere random growth and connexion seems inadequate to account for some of the observed specification of connexion or lack of connexion. Undoubtedly, a major factor governing the pattern of distribution is that the linkage by recurrent inhibition can be established only over short distances, regardless of the functional significance of the connexion if it could be established. The absence of inhibitory interconnexion between the motoneurones of knee flexors and extensors has been mentioned above. Instead there is strong recurrent facilitation in both directions (Wilson, Talbot & Diecke, 1960). This recurrent facilitation can operate over longer distances, which is presumably attributable to the one or more additional interneurones in the pathway (see Wilson, Talbot & Diecke, 1960; Wilson, Diecke & Talbot, 1960).

The present results agree with those of Kuno (1959) in fully confirming the important postulate of Granit *et al.* (1957) and Granit & Rutledge (1960) that tonic alpha motoneurones receive much more recurrent inhibition than phasic alpha motoneurones (cf. Holmgren & Merton, 1954). The tonic motoneurones were identified by the long duration of their after-hyperpolarization (Eccles *et al.* 1958; Kuno, 1959) and it was shown (Fig. 5)

that there was almost a linear relationship between the duration of after-hyperpolarization and the amount of RIPSP that a motoneurone received. On the basis of these two criteria it must be concluded that there are not two discrete categories of fast and slow motoneurones, but that all transitions exist (cf. Kuno, 1959). The specially large RIPSPs of motoneurones with long after-hyperpolarizations are further evidence that some specific factors control the development of recurrent inhibitory pathways in addition to mere proximity.

The general functional significance of recurrent inhibition is still an open question. Perhaps, as suggested by Wilson, Talbot & Diecke (1960), there are several functions. (i) There can be no doubt that the very wide distribution cutting across all functional classification must give recurrent inhibition a general suppressor action on motoneurones of diverse type, such as was originally suggested by Eccles, Fatt & Koketsu (1954). (ii) The high level of RIPSP distribution to tonic motoneurones would also act to stabilize the frequency of discharge during the maintenance of postures as proposed by Granit *et al.* (1957). (iii) Recurrent inhibition certainly would sharpen the operation of the gamma-loop activation of muscle as implied by Brooks & Wilson (1959); but it has yet to be demonstrated that such an action is functionally important in enhancing the precision of movement. (iv) Wilson, Talbot & Diecke (1960) incorporate recurrent facilitation in developing their concept that recurrent actions mediated through Renshaw cells tend to heighten the excitation of flexor motoneurones and depress the extensors, so helping to maintain a balance which otherwise would be weighted in favour of the more powerfully excited extensors. (v) Since tonic alpha motoneurones are special targets for recurrent inhibition, the intensive motoneuronal discharge subserving rapid movements would inhibit specifically the tonic motoneurones. This action would be functionally desirable, else the slowly contracting and relaxing muscles would impede the rapid movements. Thus recurrent inhibition would have the important function of suppressing all discharges from tonic motoneurones during the rapid movements of running or jumping. The desirability of this suppression was pointed out by Denny-Brown (1928) in his pioneer investigations on fast and slow muscles, and he observed suppression of discharges to soleus under such conditions.

There is experimental evidence for all these proposed actions of the recurrent pathways through Renshaw cells. It remains for further investigation to determine their relative importance in the control of posture and movement.

SUMMARY

1. The technique of intracellular recording has been employed in investigating the recurrent or Renshaw inhibitory post-synaptic potentials (RIPSPs) that are produced by a wide variety of antidromic motor volleys in the motoneurones of the seventh lumbar and first sacral regions of the cat spinal cord.

2. The total amount of the recurrent inhibition was measured as the aggregate of all the RIPSPs received by motoneurones of each type. It was much larger for motoneurones with long after-hyperpolarization, particularly for soleus motoneurones. For the whole population of motoneurones there was a significant relationship between the duration of the after-hyperpolarization and the size of the aggregate RIPSP.

3. The position of any motoneurone in the cord, whether in the rostral-caudal or in the dorsal-ventral dimensions, was related to the size and origin of the RIPSPs that it received. In general the closer the proximity of the motoneuronal nuclei the larger the size of the RIPSP that an antidromic volley in the axons of one produced in the motoneurones of the other.

4. Antidromic volleys in the nerves to flexor and extensor muscles exhibited approximately the same effectiveness in generating RIPSPs. Extensor motoneurones, however, received a larger aggregate RIPSP than did flexor motoneurones.

5. Occasionally an antidromic volley produced a small excitatory post-synaptic potential (the recurrent or Renshaw EPSP).

6. There is a general discussion of the various suggestions that have been made regarding the functional significance of the Renshaw or recurrent inhibition.

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