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NEURONAL PATHWAY OF THE RECURRENT FACILITATION OF MOTONEURONES

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SUMMARY

1. The recurrent facilitation of motoneurones is a disinhibition, i.e. a release of the motoneurones from a sustained hyperpolarization evoked by tonically active inhibitory interneurones. Only two groups of interneurones are known to receive recurrent inhibition from motor axon collaterals via Renshaw cells; the interneurones mediating the reciprocal Ia inhibition and the Renshaw cells themselves. The properties of these two groups of neurones were studied to determine if they could produce the tonic inhibition of motoneurones removed during recurrent facilitation.

2. It was found that the tonic firing of Ia inhibitory interneurones is sensitive to anaesthetics to the same degree as is recurrent facilitation. The range of frequencies of tonic discharges of Renshaw cells appeared to be similarly low in unanaesthetized and anaesthetized preparations although in individual cells the discharge rates were decreased by anaesthesia.

3. The recurrent inhibition of Ia interneurones inhibiting a given group of motoneurones and the recurrent facilitation of the same group of motoneurones were, as a rule, evoked from the same nerves, although in some cats the origin of the recurrent facilitation was somewhat wider. In contrast no evidence could be found that the Renshaw cells which inhibit a functional group of motoneurones are inhibited by volleys in the nerves from which recurrent facilitation is regularly evoked.

4. It was concluded that the recurrent facilitation is caused mainly by inhibition of the tonic activity of Ia inhibitory interneurones and that it is thus a manifestation of the recurrent control of Ia reciprocal inhibition of motoneurones.

INTRODUCTION

Wilson and his collaborators (Wilson, 1959; Wilson, Diecke & Talbot, 1960*a*; Wilson & Burgess, 1962*a*) have shown that the recurrent facilitation of motoneurones (Renshaw, 1941) is, in fact, a disinhibition, and they proposed that it is due to an inhibition by Renshaw cells of some tonically active inhibitory interneurones (Wilson & Burgess, 1962*b*) which evoke a steady hyperpolarization of the motoneurones. Until recently it was not known to which inhibitory pathways these interneurones might belong.

Recent experiments showing that the interneurones of the Ia inhibitory pathway are effectively inhibited from motor axon collaterals (Hultborn, Jankowska & Lindström, 1971*a*, *b*; Jankowska & Roberts, 1971*a*) and that they are tonically active (Hultborn *et al.* 1971*b*) indicate that they may be mediating recurrent facilitation. Renshaw cells are the other group of neurones found to be inhibited by volleys in recurrent motor axon collaterals and Renshaw cells (Ryall, 1970; Ryall & Pierce, 1970) and it has been suggested (Ryall, 1970) that both the tonic inhibition and the disinhibition of motoneurones can be due to Renshaw cells. These two groups of cells, the Ia inhibitory interneurones and the Renshaw cells are the only ones known which may be involved in recurrent facilitation as the last order interneurones. In the present study their properties were therefore analysed with regard to those required to produce the tonic inhibition of motoneurones removed during recurrent facilitation. The Ia inhibitory interneurones were concluded to be the main source of this inhibition.

METHODS

The experiments were performed on unanaesthetized cats which were anaemically decorticated as described by Andén, Jukes, Lundberg & Vyklický (1966) and in which a pronounced decerebrate rigidity was developed (cf. Hultborn *et al.* 1971*a*). Some of the animals were, in addition, spinalized at a low thoracic level. Recurrent facilitation was evoked by stimulation of hind limb muscle nerves in preparations in which the dorsal roots were cut or by stimulation of the ventral roots in preparations with dorsal roots intact. Recurrent facilitatory potentials (RFPs) were recorded intracellularly in motoneurones in L6 and L7. They were usually averaged using a CAT 1000 or Hewlett-Packard 548 A averaging computer, although records were taken also of single superimposed responses. Spike activity of Ia inhibitory interneurones and Renshaw cells was recorded extracellularly. Pentobarbital sodium (Nembutal, Abbott, 5–10 mg/kg), hexobarbital (Evipan, 5 mg/kg) and chloralose (α-chloralose, 30–60 mg/kg), administered intravenously, were used to test the sensitivity of recurrent facilitation to anaesthesia. In all other respects the preparation and the techniques of recording and stimulation were the same as in the previous experiments (Hultborn *et al.* 1971*a*).

Abbreviations. The following abbreviations are used: anterior biceps and semimembranosus (ABSm) or anterior biceps (AB) and semimembranosus (Sm) separately, adductor femoris and longus (Add), deep peroneus (without cutaneous and extensor

digitorum brevis branches) (DP) or the whole peroneal nerve (Per), flexor digitorum and hallucis longus (FDL), gastrocnemius and soleus (G-S) or medial gastrocnemius (MG) and lateral gastrocnemius and soleus (LG-Sol) separately, gracilis (Grac), posterior biceps and semitendinosus (PBSt) or posterior biceps (PB) and semitendinosus (St) separately, plantaris (Pl), quadriceps (Q), sartorius (Sart), inhibitory post-synaptic potential (IPSP), recurrent inhibitory post-synaptic potential (RIPSP), recurrent facilitatory potential (RFP), ventral spinocerebellar tract (VSCT).

RESULTS

I. *Central latency of recurrent facilitation*

For an analysis of the neuronal pathway of recurrent facilitation it is important to ascertain whether its latency is consistent with one interneurone being interposed between the Renshaw cells and motoneurones, as postulated by Wilson & Burgess (1962*a*) or whether more complex neuronal chains must be considered. If the RFPs are mediated trisynaptically their latencies should exceed the latencies of the disynaptic RIPSPs recorded in motoneurones (1·1–1·8 msec; Eccles, Fatt & Koketsu, 1954) by the sum of the conduction time from the soma to the terminals of the interposed inhibitory neurone and of the synaptic delay of IPSPs evoked by this interneurone. The conduction time is estimated to be 0·10–0·80 msec for Ia inhibitory interneurones (Jankowska & Roberts, 1971*b*) and is unknown but probably similar for Renshaw cells. Values of 0·30–0·40 msec have been found for the synaptic delay (Eccles, 1964; Jankowska & Roberts, 1971*a*). Taking into account the shortest and longest of the above values the expected latencies of the onset of the RFPs would be between 1·50 msec and 3·00 msec. This estimation does not consider other factors which might tend to increase the latencies of the RFPs, e.g. a strong excitation or a weak recurrent inhibition of the Ia interneurones, as a consequence of which they might fire during the early phase of the RIPSPs. The latencies of the recurrent facilitation reported in previous studies were rather longer, 2·8–3·0 msec when antidromic conditioning of monosynaptic reflexes was used (Wilson, 1959), and 5 msec (4–7 msec) for RFPs (Wilson & Burgess, 1962*a*). The latter were, however, measured from the time of peripheral nerve stimulation so that the segmental latencies of the RFPs would be shorter by 1·0–2·0 msec.

The onsets of the RFPs are usually fairly gradual and difficult to determine even in averaged records. Therefore when measuring their latencies it was necessary to compare: (1) the intracellular and extracellular potentials evoked by the antidromic volleys (cf. Fig. 1*A*), (2) the intracellular potentials before and after reversal of RFPs by chloride injection into the motoneurones (cf. Fig. 2*G*), or (3) the intracellular potentials before and after abolishment of the RFPs by anaesthesia (cf. Fig. 3). Without these

precautions the latencies of the RFPs were easily overestimated. Fig. 1*C* shows a histogram of latencies measured under the above circumstances in forty-two motoneurones. The RFPs were evoked from Q in PBSt motoneurones and from G–S in DP motoneurones. No systematic differences were found between these two groups of motoneurones so that all data are shown together. The latencies of the RFPs were measured from the peak of the positive potential (line *a*) signalling arrival of the antidromic

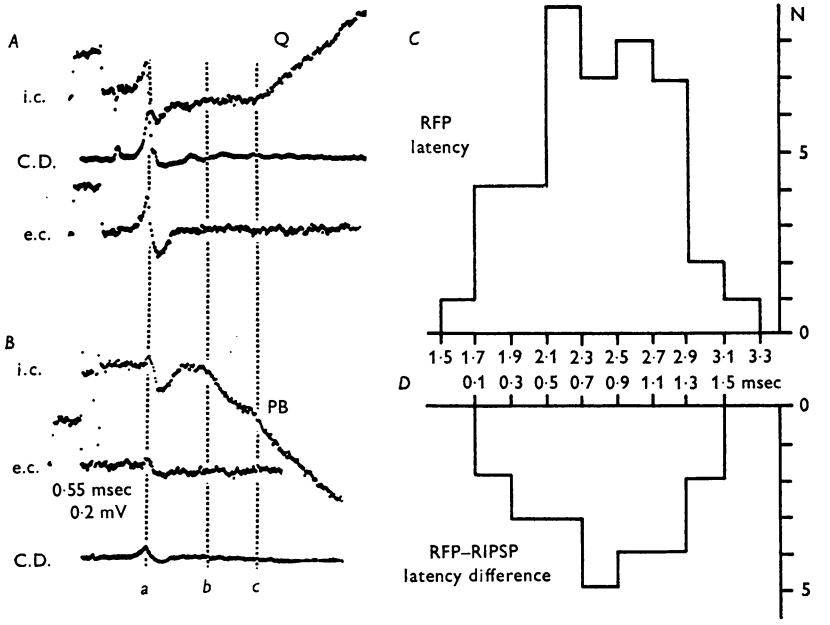


Fig. 1. Latencies of RFPs in relation to RIPSPs. The upper traces in *A* and *B* show the intracellular potentials (i.c.) recorded in a St motoneurone upon stimulation of the Q (*A*) or PB (*B*) nerves. The latency of the RFP in *A* was 2·1 msec and that of the RIPSP in *B* was 1·25 msec, as measured from the positive peak (*a*) of the incoming volley recorded from the surface of the cord (C. D.). The incoming volleys from Q and PB were recorded with the surface electrodes in L5 and L7 respectively. The extracellular records are denoted e.c. *C* is a histogram of the latencies (*a–c*) of RFPs evoked in twenty-two PB or St motoneurones (from Q) and in twenty DP motoneurones (from MG). *D* is a histogram of the latency differences (*b–c*) between the RFPs and RIPSPs in twenty-three of the motoneurones represented in *C*.

volley at the spinal cord, as recorded in L7 for G–S and in L5 for Q, with an estimated accuracy of about ± 0·1 msec. Their mean value was 2·4 msec, and nearly all fell within the predicted limits. A histogram of the differences between the latencies of the RFPs and of the RIPSPs, measured in the same motoneurones, is shown in Fig. 2*D*. The mean difference of 0·8 msec corresponds reasonably well with one synaptic delay (0·3–

0·4 msec) and a mean conduction time from the inhibitory interneurones to motoneurones of about 0·3 msec. It is therefore reasonable to proceed on the Wilson hypothesis that only one interneurone is interposed between the Renshaw cells and the motoneurones in the pathway mediating recurrent facilitation.

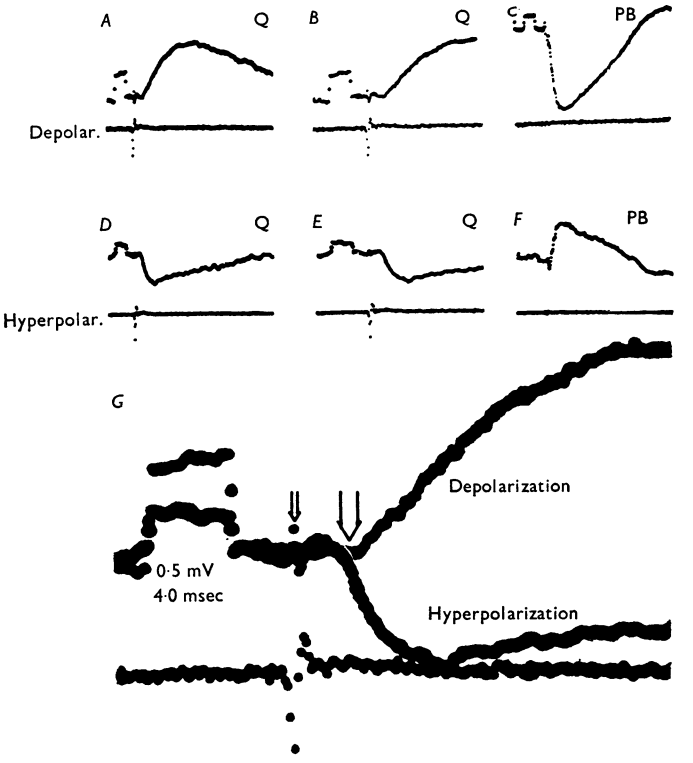


Fig. 2. Reversal of a RFP. Upper traces are averaged intracellular potentials from a St motoneurone. Lower traces are cord dorsum potentials. The motoneurone was either depolarized (*A–C*) or hyperpolarized (*D–F*) by passing currents through the KCl-filled recording electrode. Records *B* and *E* show the expanded early parts of *A* and *D* and are superimposed in *G*. In *G* the small arrow indicates the peak positivity of the incoming volley and the large arrow the onset of the RFP. The calibration pulse has an amplitude 0·5 mV and a duration of 4 msec. Note the lower amplification in records *D–F*. In this and the following Figures a negativity is signalled downwards in microelectrode recording and upwards in records from the cord surface.

II. *Evidence that the inhibition of Ia inhibitory interneurones is responsible for recurrent facilitation*

Two lines of evidence will be presented: first, that the tonic activity of Ia inhibitory interneurones is present only in those preparations in which

recurrent facilitation can be evoked, and second, that the recurrent facilitation of any group of motoneurones (e.g. Q, PB, etc.) and the recurrent inhibition of the Ia interneurones which inhibit those motoneurones are evoked from the same nerves. Additional observations will be presented which suggest that the lack of the recurrent facilitation in anaesthetized preparations is due primarily to the absence of tonic inhibition of motoneurones.

1. *Recurrent facilitation and the tonic activity of Ia inhibitory interneurones*

The recurrent facilitation of motoneurones was found in unanaesthetized or lightly anaesthetized decerebrated animals (Renshaw, 1941) and in unanaesthetized, high spinal preparations (Wilson, 1959; Wilson & Burgess, 1962*a*). No RFPs were, as a rule, seen in low spinal cats under Nembutal anaesthesia (Eccles, Eccles, Iggo & Ito, 1961). In the present experiments RFPs were found also in unanaesthetized, low spinal cats and their amplitudes appeared to be of the same order of magnitude before and after spinalization. The strong depressive effect of Nembutal on recurrent facilitation (Wilson & Talbot, 1960) was confirmed and compared with the effects of other anaesthetics.

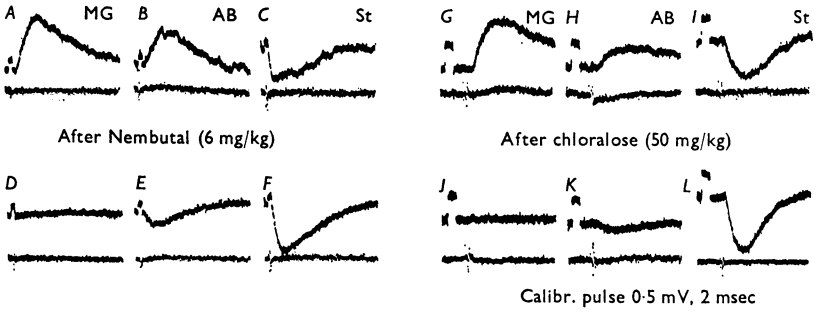


Fig. 3. Effects of Nembutal and chloralose on RFPs. The upper and lower traces are averaged intracellular and cord dorsum potentials respectively. *A–F* and *G–L* are from two DP motoneurones, recorded in separate experiments, the latter in a spinal preparation. The corresponding records in the upper and lower rows were taken before and after injection of Nembutal or chloralose as indicated. The membrane potential was 10 mV higher for records *A–C* than records *D–F*, and 5 mV higher for records *G–I* than *J–L*. The calibration pulse is 0·5 mV and 2 msec. Note the slower time scale in *A–F*.

RFPs could not be evoked for about $\frac{1}{2}$ hr after 5–7 mg/kg of Nembutal and for about 4–5 hr after a dose of 30–35 mg/kg. Hexobarbital (Evipan) abolished them as effectively as Nembutal (the smallest effective dose being 5 mg/kg) although the effect lasted for a shorter period of time.

After 50–60 mg/kg of chloralose RFPs were usually completely abolished and reappeared only after 5–8 hr. Nembutal and chloralose had similar effects on RFPs as illustrated in Fig. 3 for two DP motoneurones. In both cases the RFPs from MG and AB were suppressed and the RIPSPs from AB, previously masked by the RFPs, were disclosed. In no cases were the RIPSPs reduced after these doses of Nembutal or chloralose.

The interneurones mediating reciprocal Ia inhibition (Hultborn *et al.* 1971*b*; Jankowska & Roberts, 1971 *a*, *b*) are tonically active in unanaesthetized preparations. They fire with a frequency of 20–130/sec or higher in decorticate and high or low spinal cats (Wilson & Burgess, 1962*b*; Hultborn *et al.* 1971*b*) and below 20/sec under very light Nembutal anaesthesia (Smerdlow & Maksimova, 1965). The present experiments have shown that the resting activity of these interneurones is abolished by the doses of barbiturates (Nembutal, Evipan) and chloralose which suppress recurrent facilitation. The durations of the effects on the Ia interneurones are also similar to those on recurrent facilitation.

2. *The contribution from different nerves to the recurrent facilitation of motoneurones and to the recurrent inhibition of Ia inhibitory interneurones*

The pattern of distribution of recurrent facilitation from some muscle nerves to different motor nuclei was established by Renshaw (1941) and Wilson, Talbot & Diecke (1960*b*). However, not all combinations needed for a comparison with the pattern of recurrent inhibition of Ia interneurones were tested in these studies and those missing had to be investigated. The origin of the RFPs was analysed in more than 200 motoneurones in which at least one nerve gave a RFP larger than 0·1 mV. Unanaesthetized cats were used except in a few experiments in which small doses of Nembutal were given a few hours before recording.

In the unanaesthetized preparations RFPs were seen in almost all motoneurones innervating Sart, ABSm, Q, PBSt and Per muscles, while they were only rarely found in motoneurones supplying ankle and toe extensor muscles (Wilson *et al.* 1960*b*). The largest RFPs (mean 0·8 mV) were evoked in Per motoneurones by antidromic volleys in G–S. The data are summarized in Table 1, and only new findings and discrepancies with the results of earlier investigations will be pointed out in the following.

(i) In motoneurones to the Sart muscle the recurrent facilitation was evoked mainly from the nerves to its strict antagonists, i.e. muscles working as antagonists at the same joint, Add, Sm and AB (cf. Wilson *et al.* 1960*b*). Smaller RFPs were seen from Q and G–S. Recurrent facilitation was sometimes observed also from Grac but the mean amplitude of RFPs was below 0·05 mV and is thus indicated as zero in Table 1. The effects

from Grac and Q could be underestimated since large RIPSPs were often evoked from these nerves (Hultborn *et al.* 1971*c*).

(ii) The recurrent facilitation of motoneurones to hip extensors, Sm and AB, was evoked from the same nerves. The largest RFPs were evoked from Q and only smaller ones from hip flexor Sart and knee flexor Grac. RFPs from Add (in Sm the mean was below 0·05 and is not indicated in Table 1) were probably masked by the RIPSPs which are often evoked in this combination (Hultborn *et al.* 1971*c*).

TABLE 1. The mean amplitudes of RFPs generated in lumbar motoneurones by antidromic volleys in hind limb muscle nerves. The species of motoneurone investigated are listed to the left and the figures in parentheses indicate the numbers of cells tested. The amplitudes of RFPs (in mV) recorded in different motoneurones are given in corresponding horizontal rows. Mean values below 0·05 mV are given as zero. When St and PB were stimulated separately the resulting RFPs are shown from each of them separately.

Moto-neurones	Nerves stimulated: Sart	Add	Sm	AB	Grac	St	PB	Q	Per	G–S	Pl	FDL
Sart (22)	—	0·40	0·29	0·22	0	0		0·11	0	0·06	0	0
Sm (19)	0·19	0	—	0	0·07	0		0·31	0	0	0	0
AB (18)	0·11	0·06	0	—	0·11	0		0·15	0	0	0	0
St (21)	0·07	0·36	0·07	0	0	—	0	0·57	0	0	0	0·07
PB (14)	0·07	0·33	0·18	0	0	0	—	0·44	0	0·28	0·31	0·25
Q (37)	0	0	0·05	0·12	0·09	0·38		—	0·14	0·05	0	0
Per (71)	0	0	0·08	0·43	0	0·08	0·16	0·06	—	0·75	0·35	0·09

(iii) In agreement with Wilson *et al.* (1960*b*) the largest RFPs in PBSt motoneurones were evoked by antidromic volleys in the Q nerve. Stimulation of Sart, Add and Sm nerves was followed by RFPs but the amplitude of those from Sm was probably underestimated since RIPSPs are often evoked from this nerve (Hultborn *et al.* 1971*c*). In a few cats there were also quite large RFPs from the ankle extensors G–S and Pl and the toe extensor FDL, the effect being more pronounced in PB than in St motoneurones. However, greater numbers of PB than of St motoneurones were sampled in these few cats and the difference may be insignificant.

(iv) In the knee extensor Q motoneurones the largest RFPs were evoked by antidromic volleys in nerves to the antagonist muscles PB and St. Smaller, although regular effects were seen from Grac, AB and Per. The RFPs from Sm and G–S were fairly small, but possibly underestimated since recurrent inhibition is frequently evoked from these nerves (especially from Sm; see Hultborn *et al.* 1971*c*). This may explain why Wilson *et al.* (1960*b*) failed to detect any recurrent facilitation from Sm and G–S to Q.

(v) In Per motoneurones strong recurrent facilitation was evoked not only from nerves to the antagonist muscles (ankle and toe extensors, particularly G–S and Pl but also from nerves to AB and, to some extent, PB muscles (cf. Fig. 4). Volleys in Sm, St and Q nerves gave rise to small or infrequent RFPs; the most pronounced effects from Sm and St consisted of recurrent inhibition, which was the only effect reported by Wilson *et al.* (1960*b*).

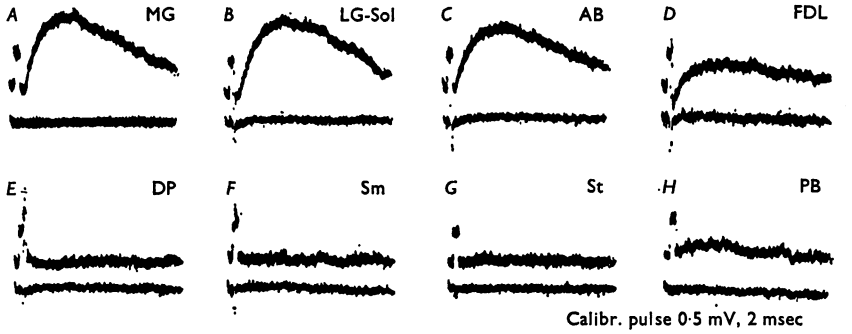


Fig. 4. RFPs in a DP motoneurone in which no RIPSPs were evoked. The upper traces are averaged intracellular potentials, and the lower traces are averaged cord dorsum potentials. The nerves stimulated are indicated above each pair of records. The antidromic invasion of the spike was blocked at the moment of recording so that also the DP nerve (*E*) could be stimulated with a strength supramaximal for α-fibres. The calibration pulses are 0·5 mV and 2 msec.

A comparison of the pattern of distribution of recurrent facilitation and of recurrent inhibition (cf. Tables 3 and 5 in Hultborn *et al.* 1971*c*) is beyond the aims of this study. However, for the sake of further discussion a few points must be mentioned. Our results show that recurrent facilitation and recurrent inhibition in a given motor nucleus are evoked mainly from different nerves although both those effects are sometimes seen from the same nerve, even in individual motoneurones as illustrated in Fig. 3. RFPs were seen also in motoneurones which did not receive RIPSPs from any nerves. The records from one such motoneurone, a DP motoneurone, are shown in Fig. 4. Large RFPs were evoked in it from the MG, LG-Sol, AB and FDL nerves (*A–D*), whereas volleys in the DP, Sm and St nerves, which usually produce recurrent inhibition of DP motoneurones, were completely ineffective in the cell (*E–G*). These findings indicate that recurrent facilitation can be evoked independently of recurrent inhibition.

Wilson & Talbot (1960) suggested that recurrent inhibition is regularly followed by recurrent facilitation. This suggestion was based on their observation that recurrent inhibition is invariably lengthened by meprobamate and Nembutal and

is often followed by a facilitatory overshoot. However, the late facilitatory overshoot cannot automatically be considered as a RFP, defined as a removal of a steady hyperpolarization of motoneurones, because there are indications that its mechanism may be different from that of recurrent facilitation; for example, the positive overshoot following large RIPSPs from the homonymous nerves is not likely to be a RFP because facilitatory potentials were never observed from these nerves in motoneurones lacking the RIPSPs. Furthermore, Larsson & Major (1970) gave evidence that the lengthening of RIPSPs by anaesthesia is not due to a removal of tonic inhibition from Renshaw cells. The duration of the late overshoot also seems to be too short to be accounted for by the pause in tonic activity of Renshaw cells following their activation by an antidromic volley in motor axons (Ryall, 1970).

Table 2 gives a comparison of the patterns of recurrent facilitation and of the recurrent depression of Ia IPSPs. The motoneurone-nerve combinations in which recurrent facilitation was found (cf. Table 1) are hatched. Those in which a depression of Ia IPSPs was produced (cf. Table 5 in Hultborn *et al.* 1971*c*) are indicated by dots. The patterns of the two phenomena were roughly overlapping although there were some exceptions. For instance, there were some motoneurone-nerve combinations in which the antidromic volleys depressed the Ia IPSPs without evoking clear RFPs. In all of these combinations, however, the depression of the Ia IPSPs was rather weak (Hultborn *et al.* 1971*c*), and only very small RFPs could be expected. In addition these RFPs might have been masked by the RIPSPs which are sometimes evoked in the same combinations. Therefore the slightly wider origin of the depression of the Ia IPSPs may be due largely to difficulties in detection of the RFPs and does not necessarily indicate a difference in the two patterns. In other combinations the origin of RFPs was wider than that of the depression of Ia IPSPs. This was found in PB, St and Per motoneurones, Fig. 5 showing the records from one of the PB motoneurones in which this difference was

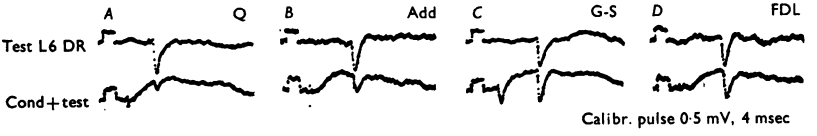
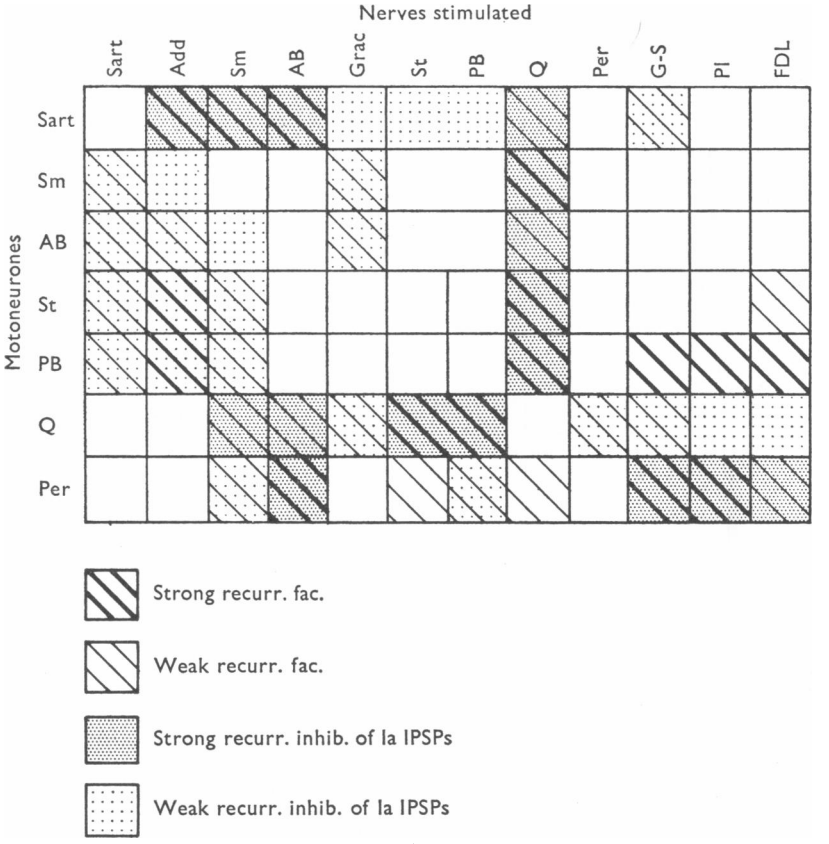


Fig. 5. Effects of antidromic volleys from motor nerves on a Ia IPSP in a PB motoneurone. The upper traces (test) are averaged intracellular records of an IPSP evoked by weak stimulation of L 6 DR (cf. Hultborn *et al.* 1971*c*). The lower traces (cond + test) show the IPSPs preceded by conditioning stimulation of four motor nerves which evoked the RFPs in the motoneurone recorded from. Note decrease of the test IPSP in *A* and *B* and its increase in *C* and *D*. The calibration pulses are 0·5 mV and 4 msec.

most pronounced. Antidromic volleys in Q and Add nerves evoked in it RFPs and a depression of the test Ia IPSP (*A*, *B*), while antidromic volleys in the G–S and FDL nerves evoked RFPs without corresponding

effects on the Ia IPSPs (*C*, *D*). The wider pattern of RFPs, however, was found only in a few preparations. It seems thus that only the RFPs evoked in the motoneurone-nerve combinations showing no depression of the Ia IPSPs may constitute an exception to the parallellism in the origin of the two phenomena.

TABLE 2. Comparison of the origin of RFPs in motoneurones and of recurrent depression of Ia IPSPs. The species of motoneurone investigated are listed to the left, while the nerves used for antidromic stimulation are indicated above. The contribution from different nerves to the recurrent depression of Ia IPSPs is based on earlier results (Hultborn *et al.* 1971*c*). The recurrent facilitation was arbitrarily divided into three groups, strong (> 0·2 mV, dense dotted), weak (0·05–0·2 mV, sparsely dotted) and absent or very weak (< 0·05 mV, without dots).



III. *Renshaw cell activity and the occurrence of RFPs*

If recurrent facilitation is due to inhibitory interactions between Renshaw cells (Ryall, 1970), then their tonic discharge should be dependent on the level of anaesthesia in the same manner as is recurrent

facilitation, and the pattern of their mutual inhibition should correspond to the pattern of origin of recurrent facilitation.

The frequencies of tonic discharges of Renshaw cells do not seem to differ significantly in anaesthetized and in unanaesthetized preparations. According to Ryall (1970) in the anaesthetized cats the rate of their spontaneous firing is usually about 1–2/sec but it can be as high as 20–25/sec (Curtis & Ryall, 1966). The highest discharge rates found in the present experiments in unanaesthetized animals were 15–25/sec, i.e. within the same limits. No tonic firing was observed in thirteen of twenty-eight recorded Renshaw cells. Discharge rates of less than 10/sec and between 10 and 25/sec were seen in eleven and four cells respectively. These rates refer to cells in which no obvious injury was produced by the recording electrode. Usually when the electrode tip approached the cell, the discharge rates increased considerably. The effect of Evipan (5–10 mg/kg) was tested in four cells among those with the highest firing frequency, two of which increased their discharge rates after adjustment of the electrode position. In all of them a reduction of the frequency of firing was found: from 8 to 0, 24 to 4, 31 to 13, and 43 to 2 spikes/sec respectively. It thus appeared that the anaesthesia can reduce the frequency of firing in individual cells although the ranges of discharge rates of Renshaw cells in anaesthetized and unanaesthized preparations are similar. It remains, however, an open question whether the small proportion of cells with a higher discharge rate would be capable of producing the required steady hyperpolarization of motoneurones which is removed during the recurrent facilitation.

A comparison of the pattern of inhibition from motor axons into Renshaw cells (Ryall, 1970) with the pattern of recurrent facilitation of motoneurones is much more difficult than in case of the Ia inhibitory interneurones since individual Renshaw cells cannot easily be defined with respect to their target motoneurones (Eccles, Eccles, Iggo & Lundberg, 1961). If recurrent facilitation is due to inhibitory interaction between Renshaw cells, one would, however, expect that it should be possible to depress RIPSPs by antidromic volleys which can evoke RFPs in the same motoneurones. This was tested in two experiments, mainly with PBSt motoneurones in which RFPs are evoked regularly from the Q nerve and in some preparations also from G–S, Pl and FDL. These occasional RFPs cannot be explained by inhibition of the Ia inhibitory interneurones which are known to inhibit the PBSt motoneurones (see above) and thus seemed to be the most likely combination for demonstrating effects via Renshaw cells. The experiments were done on anaesthetized animals in which the RFPs were abolished since the conductance decrease associated with the RFPs might otherwise obscure a small depression of

the RIPSPs. The conditioning-testing intervals were varied so that either the peak or the decay phase of the test RIPSP would correspond in time with the expected peak of the conditioning effects. The test RIPSPs were evoked by weak stimulation of homonymous and/or heteronymous nerves and were always only a small fraction of the maximal RIPSPs.

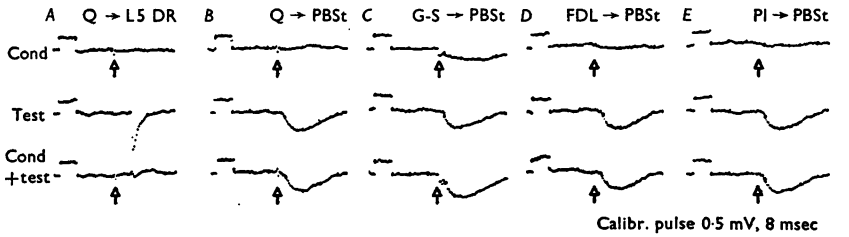


Fig. 6. Recurrent effects on Ia IPSP and a RIPSP evoked in a PBSt motoneurone. All records are averaged intracellular potentials. The upper row shows the effects of the conditioning (cond) volleys alone, the middle row the test responses and the lower row the conditioned test (cond + test) responses. The test stimuli used are an almost maximal Ia IPSP from the L 5 dorsal root (*A*) and a small RIPSP (about 25 % of the maximal) evoked from the PBSt nerve (*B–E*). The conditioning antidromic volleys (arrows) were maximal for α-fibres and were evoked from Q (*A–B*), G–S (*C*), FDL (*D*), and Pl (*E*) nerves. The calibration pulses are 0·5 mV and 8 msec.

In the PB motoneurone illustrated in Fig. 6 the test RIPSP was evoked from PBSt (*B–E*) and was preceded by stimulation of a number of nerves. Neither in this nor in other motoneurones tested in a similar way were we able to demonstrate any depressive effect of the conditioning volleys (decrease of peak amplitude or a faster decay). The Ia IPSPs evoked in these motoneurones were effectively depressed (Fig. 6*A*) even when their amplitudes were nearly maximal and much larger than those of test RIPSPs. A small depressive effect on the RIPSPs has, however, been observed in a few cells in experiments in which the conditioning antidromic volley was produced by ventral root stimulation. The above results indicate that Renshaw cells terminating on a group of motoneurones are either unaffected by antidromic volleys which evoke RFPs in these motoneurones or else that the inhibition of the Renshaw cells is very weak compared to the excitation, even when the latter is submaximal (cf. Ryall, 1970 and Fig. 7).

In relation to individual Renshaw cells we have confirmed Ryall's (1970) finding that they can be inhibited by antidromic volleys in motor axons. The cell in Fig. 7 was activated from the PBSt nerve (*A*) but not from any other dissected nerve. When the conditioning stimulation was applied (interrupted line) near the end of a series of spikes evoked by test stimulation of PBSt it was found that the subsequent number of spikes

was reduced by antidromic volleys in G–S (C), FDL (D) and Pl nerves (not illustrated). The Q (B) and SmAB nerves (not illustrated) had no appreciable effect. If such Renshaw cells (cf. also Table 1 in Ryall, 1970) terminated on PBSt motoneurones and tonically inhibited them the removal of this inhibition by volleys in nerves to ankle extensors would

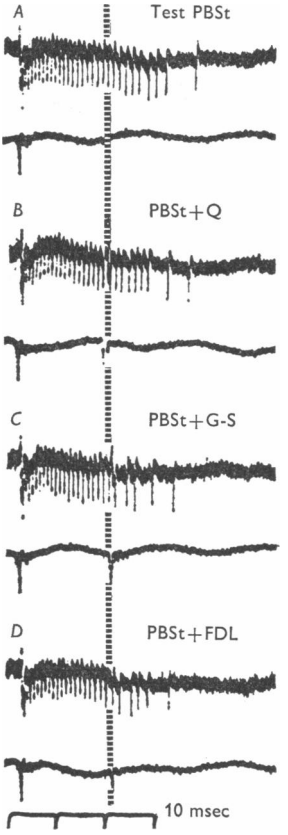


Fig. 7. Recurrent inhibition of a Renshaw cell. The upper traces in each pair show the extracellularly recorded potentials from a Renshaw cell. The lower traces are cord dorsum potentials. The test response (*A*) was the train of spikes evoked by an antidromic volley in the PBSt nerve. The conditioning volleys in the Q, G–S and FDL nerve (*B*, *C* and *D* respectively) were put in late during the train of test spikes as approximately indicated by the interrupted line. The time calibration is 10 msec.

explain the recurrent facilitation found from them in some PBSt motoneurones. However, the disinhibition from Q, which is the main source of recurrent facilitation in PBSt motoneurones, must have involved other inhibitory interneurones.

DISCUSSION

The latencies of the recurrent facilitation found in this study are fully consistent with the proposed trisynaptic linkage. This pathway should thus include only two interneurones: an inhibitory interneurone which produces a sustained hyperpolarization of the motoneurones, and a Renshaw cell which inhibits the tonic activity of the former and thereby causes a disinhibition of the motoneurones (Wilson, 1959; Wilson & Burgess, 1962*a*).

Only two groups of interneurones which exert an inhibitory influence on motoneurones have been shown to be inhibited via motor axon collaterals and Renshaw cells: the interneurones in the Ia inhibitory pathway (Hultborn *et al.* 1971*a*, *b*, *c*; Jankowska & Roberts, 1971*a*) and Renshaw cells (Ryall & Pierce, 1970; Ryall, 1970). Both of them have been considered as the last order interneurones in the pathway of the recurrent facilitation (Hultborn *et al.* 1968, 1971*a*; Ryall, 1970). The aim of the present study was to analyse whether removal of their respective inhibitory effects on motoneurones might be responsible for recurrent facilitation. Two main requirements were considered. The first was that the cells responsible for the tonic inhibition of motoneurones must fire with higher frequencies in unanaesthetized preparations, in which RFPs are present, and have less or no tonic activity under anaesthesia, when the RFPs are absent. This must be the case since recurrent facilitation is abolished by anaesthetic doses which do not decrease the recurrent inhibition evoked by Renshaw cell activity (Fig. 3, Wilson & Talbot, 1960; Larsson & Major, 1970; Biscoe & Krnjević, 1963; see, however, Haase & van der Meulen, 1961). Hence the abolishment of recurrent facilitation must be due to a loss of tonic inhibition rather than the blockage of the Renshaw inhibitory effects. This conclusion is supported also by the observation that the resting membrane conductance of motoneurones is lowered by the doses of anaesthesia which abolish the RFPs (cf. upper traces in *C* and *F* in Fig. 3 in Hultborn *et al.* 1971*a*).

The second requirement was that the recurrent facilitation in a given group of motoneurones and the inhibition of interneurones producing the tonic hyperpolarization of these motoneurones should be evoked from the same nerves.

The evidence implicating the Ia inhibitory interneurones is very strong. They have high resting discharge frequencies in the unanaesthetized preparations (Hultborn *et al.* 1971*b*; cf. Wilson & Burgess, 1962*b*) in which the recurrent facilitation of moitoneurones is most pronounced. Their spontaneous firing is also abolished by the same doses of barbiturates and chloralose as the RFPs. The recurrent facilitation and the depression of

the Ia IPSPs in the same group of motoneurones are in most cases evoked from the same nerves. However, the somewhat wider origin of the RFPs in some preparates (e.g. in PBSt motoneurones) indicates that in these cases the Ia inhibitory interneurones are not the only ones responsible for the tonic inhibition of motoneurones.

There are several indications that Renshaw cells do not have any major role as the last order interneurones in the pathway of the recurrent facilitation. Large RFPs can be produced in some cells (cf. Fig. 5) in which apparently no recurrent inhibition is evoked from any of the nerves. The absence of recurrent inhibition following synchronous stimulation of these nerves is hardly compatible with a hypothetical tonic bombardment by Renshaw cells, potent enough to produce a steady hyperpolarization of 0·5–1·0 mV. Furthermore, there is no positive correlation between the amplitudes of RIPSPs and RFPs evoked in other motoneurones. Large RFPs are evoked both in motoneurones with weak (e.g. in DP) and strong (e.g. in PB and St) recurrent inhibition, while the smallest RFPs are seen in motoneurones in which the recurrent inhibition is strong (e.g. G–S and AB). On the other hand there seems to be a reasonably good correlation between the amplitudes of the Ia IPSPs and RFPs. In motoneurones with the strongest recurrent facilitation (DP, PB, St, Sart) the Ia IPSPs are consistently large (Eccles & Lundberg, 1958), while in those to ankle and toe extensors (G–S, Pl, FDL), in which the recurrent facilitation is rare, the reciprocal inhibition is weak (Eccles & Lundberg, 1959).

A comparison of the patterns of excitation and inhibition of the Renshaw cells (Ryall, 1970) does not allow any definite conclusions since it is unknown to which motor nuclei the individual Renshaw cells project. The available data do not seem, however, to be consistent with the hypothesis that the recurrent facilitation is caused primarily by inhibitory interactions between the Renshaw cells. The most pronounced depression of tonic activity of Renshaw cells is seen after their excitation by an antidromic volley (Ryall, 1970). Therefore if Ryall's suggestion that this depression is due primarily to inhibition by other Renshaw cells is correct, the most pronounced RFPs should be seen after large RIPSPs, which is certainly not the case. Further, we could not demonstrate that stimulation of nerves which evoke RFPs in a given species of motoneurone produces a depression of the RIPSPs evoked in them. Nevertheless it cannot be excluded that Renshaw cells contribute to some extent to the recurrent facilitation as the last order interneurones since they display some tonic activity. They discharge within the same low frequency range both in unanaesthetized preparations and under chloralose and barbiturate anaesthesia (cf. Ryall, 1970; Curtis & Ryall, 1966) but the average frequency of tonic discharge of the whole population of Renshaw cells might be

lower in anaesthetized than in unanaesthetized preparations since the firing frequency of individual Renshaw cells can be reduced by anaesthesia. They might thus contribute to a certain degree to the background hyperpolarization of motoneurones removed during recurrent facilitation. The inhibitory interactions between the Renshaw cells might thus explain some observations which would be difficult to explain if the tonic hyperpolarization of motoneurones were due only to Ia inhibitory interneurones (e.g. RFPs evoked from ankle extensors in PBSt motoneurones in some preparations).

It is thus concluded that *recurrent facilitation is caused mainly by inhibition of the tonic activity of the interneurones which mediate the reciprocal Ia inhibition of motoneurones.* This conclusion is supported also by two other facts: (i) that the recurrent facilitation is present not only in motoneurones but also in some cells of origin of the ventral spinocerebellar tract (VSCT) (B. Gustafsson & S. Lindström, unpublished) and (ii) that it is absent in the Ia inhibitory interneurones (Hultborn *et al.* 1971*b*). The recurrent facilitation of VSCT cells is found in unanaesthetized preparations in cells which receive disynaptic IPSPs from Ia afferents. These Ia IPSPs are reduced by conditioning stimulation of motor axon collaterals as are the Ia IPSPs in motoneurones and are likely to be evoked by the same interneurones (Gustafsson & Lindström, 1970). Therefore the recurrent facilitation of VSCT cells can be ascribed to the inhibition of Ia inhibitory interneurones but not by the inhibitory interactions between Renshaw cells since no trace of recurrent inhibition from motor axon collaterals had been found in VSCT cells. In Ia interneurones the situation is the opposite. They are inhibited via motor axon collaterals and Renshaw cells, as are the motoneurones, and it is likely that the same Renshaw cells inhibit both the motoneurones and Ia interneurones (Hultborn *et al.* 1971*c*). If so, the inhibition produced by tonically firing Renshaw cells should be present both in the motoneurones and in Ia interneurones, and it should be removed from them to a similar degree when these Renshaw cells are inhibited by other Renshaw cells. No recurrent facilitation (no RFPs nor increase in firing index) was, however, seen in the Ia inhibitory interneurones.

The conclusion that the recurrent facilitation is caused mainly by inhibition of the Ia inhibitory interneurones leads to a different concept of its role in spinal reflex activities than that suggested by Wilson *et al.* (1960*b*), who recognized a meaningful pattern in the distribution of recurrent actions in the cat spinal cord and concluded that it resembles that of group Ib actions. However, now the recurrent facilitation must be considered as another manifestation of the recurrent depression of transmission in the Ia inhibitory pathway to motoneurones. It is unknown if

and to what extent the Ia interneurones are tonically active in intact animals independently of activity in Ia afferents from muscle spindles, as was observed under our experimental conditions. In view of their apparent high excitability, however, it is likely that they can be easily activated by an inflow of Ia impulses from stretched muscles, or spindles excited by the γ system, and can shift the membrane potential of the motoneurones in a hyperpolarizing direction. This would have an opposing effect to the tonic excitatory action of homonymous Ia impulses on motoneurones (Granit, 1955) and might be involved in a system of α–γ linked reciprocal inhibition (Hongo, Jankowska & Lundberg, 1969; Lundberg, 1970). The two actions would keep the membrane potential of the motoneurones in a state which can be moved in an excitatory or an inhibitory direction by blocking (via Renshaw cells) or by facilitating (e.g. via descending supraspinal actions) transmission from Ia inhibitory interneurones to motoneurones (cf. Hultborn & Udo, 1972). Blocking the inhibitory action of a continuous inflow of Ia impulses would correspond to recurrent facilitation.

The finding that the recurrent facilitation is primarily due to a recurrent control of the interneurones in the reciprocal Ia inhibitory pathway, and, as previously shown by Hultborn *et al.* (1971 *c*), that the origin of recurrent inhibition of α-motoneurones and Ia inhibitory interneurones supplied by the same Ia afferents are similar, leads in addition to the conclusion that the recurrent facilitation and inhibition of motoneurones should not be regarded as positive and negative equivalents of the same reflex action but rather as a manifestation of a similarly organized recurrent control of motoneurones and Ia inhibitory interneurones. The functional significance of this recurrent organization is discussed elsewhere (Hultborn *et al.* 1971*c*; Hultborn & Udo, 1972).

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