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# RECURRENT INHIBITION FROM MOTOR AXON COLLATERALS OF TRANSMISSION IN THE Ia INHIBITORY PATHWAY TO MOTONEURONES

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## SUMMARY

1. The effects of impulses in recurrent motor axon collaterals on reflex transmission from different types of primary afferents to motoneurones were investigated in the cat by conditioning of PSPs evoked in motoneurones.

2. IPSPs evoked by volleys in large muscle spindle (Ia) afferents were effectively decreased when preceded by an antidromic stimulation of ventral roots. Some IPSPs from group II muscle afferents and low threshold cutaneous afferents were also slightly depressed, while other PSPs were unaffected.

3. The depression of the IPSPs could be evoked by antidromic volleys, which produced neither conductance changes in the motoneurones nor depolarization of Ia afferent terminals.

4. The effect on the Ia IPSPs is most likely due to post-synaptic inhibition of the Ia inhibitory interneurones, evoked through $\alpha$-motor axon collaterals and Renshaw cells. The depression of some IPSPs from flexor reflex afferents is explained by a convergence of excitatory effects from these afferents on the Ia inhibitory interneurones.

5. The results indicate a selective recurrent control from motor axon collaterals of the interneurones in the reciprocal Ia inhibitory pathway to motoneurones.

## INTRODUCTION

Impulses in motor axons can give rise to either inhibition or facilitation of motoneurones via recurrent motor axon collaterals and specific interneurones, namely Renshaw cells (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954; Wilson, 1959). The facilitation is caused by the release of motoneurones from a tonic inhibitory bombardment and it has been postulated

that this disinhibition is due to recurrent inhibition of tonically active inhibitory interneurones (Wilson, 1959; Wilson & Burgess, 1962). Cells with the required properties have been found in the ventral horn (for references see Hultborn, Jankowska & Lindström, 1971*a*).

The experiments presented in this and the two following papers (Hultborn, Jankowska & Lindström, 1971*a*, *b*) were prompted by the idea that the interneurones inhibited during recurrent facilitation of motoneurones might be interposed in some inhibitory reflex pathways to motoneurones. The effects of antidromic volleys in motor axons have been investigated on reflex transmission from different types of primary afferents to motoneurones. It will be shown that the reciprocal inhibitory pathway from large muscle spindle (Ia) afferents is susceptible to recurrent inhibition, most likely at the interneuronal level. Preliminary reports of some cf the results have been published (Hultborn, Jankowska & Lindström, 1968*a*, *b*).

## METHODS

*Preparation.* The experiments were carried out on sixteen cats, some partly used for investigations presented in the two following papers. Ether anaesthesia was used during dissection, but discontinued about 3 hr before recording. Under anaesthesia the cats were anaemically decorticated as described by Andén, Jukes, Lundberg & Vyklický (1966). When the resulting rigidity was pronounced the experiments were continued without anaesthesia. In three cases of weak rigidity the animals were kept under light pentobarbitone sodium anaesthesia (Nembutal, Abbott, 25 mg/kg). Seven cats were in addition spinalized at low thoracic level. The results were similar in all three types of preparation except that the recurrent facilitatory potentials were reduced by Nembutal (cf. Fig. 3 and Wilson & Talbot, 1960). All cats were immobilized with gallamine triethiodide (Flaxedil, May and Baker Ltd.) and artificially respired. End tidal $CO_2$ was monitored on a Beckman medical gas analyser and kept within 4·5–5·5 % by adding a $CO_2$–$O_2$ mixture to the inspired air. Arterial blood pressure was recorded continuously. Blood loss was compensated with a mixture of low- and high-molecular-weight Dextran. A drop in blood pressure below 80 mm Hg was counteracted by slow intravenous infusion of noradrenaline (3 $\mu$g/min). Rectal temperature was kept within 37–39° C.

The spinal cord was exposed by laminectomy from L 4 to L 7. In fourteen cats the ventral roots L 5–S 1 were sectioned while in two cats the dorsal roots L 4–S 1 were cut instead. Several peripheral nerves in the left hind limb (cf. Abbreviations, below) were dissected for electrical stimulation. The nerves on the dorsal side and the cut roots were mounted on bipolar silver electrodes, while the ventral nerves were stimulated through buried electrodes. The skin flaps around exposed areas of the cord and the hind limb were sewn to form a pool filled with paraffin oil.

*Recording and stimulation.* Effects of antidromic volleys in motor axons on reflex transmission to motoneurones were analysed by comparing unconditioned and conditioned post-synaptic potentials in motoneurones or monosynaptic reflexes recorded from ventral roots. Different species of $\alpha$-motoneurones in L 5–S 1 spinal segments were sampled intracellularly. The cells were classified according to their Ia receptiveness (Eccles, Eccles & Lundberg, 1957*b*; R. M. Eccles & Lundberg, 1958), except in the two experiments with intact ventral roots where they could be

identified antidromically. The micro-electrodes were routinely filled with 2 M-K-citrate solution but with 3 M-KCl solution when reversal of IPSPs was required. They had broken tips with diameters of 1·5–2·0 $\mu$ and resistance of 2·5–6 MΩ. The recorded potentials were fed in parallel via a cathode follower and an a.c. amplifier, to a modified Tektronix 502 oscillograph having two pairs of beams with independent time bases and to an averaging computer (CAT 1000). Usually twenty unconditioned and twenty conditioned responses were alternated in real time to two halves of the computer memory. The averaged responses were displayed on the oscillograph for photography. The resting membrane potential of the neurones was continuously monitored. The membrane conductance was measured by injecting small current pulses through the micro-electrodes. The device used allowed compensation of the voltage drop across the micro-electrode resistance (Eide, 1968). Dorsal root potentials (DRPs) were recorded from the most caudal rootlet in L6 and the excitability of primary afferent terminals was tested by the technique of Wall (1958). An a.c. amplifier used for DRP recording had a time constant of 1·6 sec.

The nerves were stimulated with square pulses of 100 $\mu$sec duration. The strength of stimulation is expressed in multiples of threshold for the lowest threshold fibres. Nerve volleys were recorded from the surface of the spinal cord with an electrode placed close to the dorsal root entry and an indifferent electrode in the back muscles. Differentiation between afferent fibre groups was done as described by Bradley & Eccles (1953), Eccles, Eccles & Lundberg (1957*a*, *c*) and R. M. Eccles & Lundberg (1959). The test stimulation was, as a rule, submaximal for the group of afferents investigated. If not otherwise stated, conditioning volleys in the ventral roots were evoked by single stimuli supramaximal for $\alpha$-fibres and they preceded the test responses by 6–20 msec. The stimulus repetition rate was about 1/sec.

*Abbreviations.* The following abbreviations are used: anterior biceps and semimembranosus (ABSm), deep peroneus (without cutaneous and extensor digitorum brevis branches) (DP), gastrocnemius and soleus (G-S), posterior biceps and semitendinosus (PBSt), plantaris (Pl), quadriceps (Q), saphenus (Saph), sartorius (Sart), superficial peroneus (only cutaneous part) (SP), suralis (Sur), dorsal root (DR), ventral root (VR), post-synaptic potential (PSP), excitatory post-synaptic potential (EPSP), inhibitory post-synaptic potential (IPSP), recurrent inhibitory post-synaptic potential (RIPSP), recurrent facilitatory potential (RFP), dorsal root potential (DRP).

## RESULTS

### I. *Recurrent effects on the inhibitory pathway from Ia afferents to motoneurones*

#### (*a*) *Depression of Ia IPSPs*

Test Ia IPSPs were evoked in motoneurones by stimulation of nerves to antagonist muscles with the strength adjusted to excite only the lowest threshold group I afferents (Bradley & Eccles, 1953; Eccles *et al.* 1957*a*). Antidromic volleys in motor axons produced a marked depression of such IPSPs. The effect is exemplified in Fig. 1 with Ia IPSPs in a PBSt motoneurone. The test IPSPs (second column) were evoked at increasing strength of stimulation of the Q nerve. A preceding antidromic volley in the L5 and L6 VRs clearly decreased these IPSPs (third column). The conditioning volley evoked also a small recurrent facilitatory potential

(RFP), but the depression of the IPSPs was not due to the RFPs (cf. next section). In the graphs the amplitudes of the unconditioned and conditioned IPSPs (*G*) and the Ia component of the afferent volleys (*H*) have been plotted against the testing stimulation strength. The IPSP increased with the Ia component and reached its maximum below threshold for the Ib

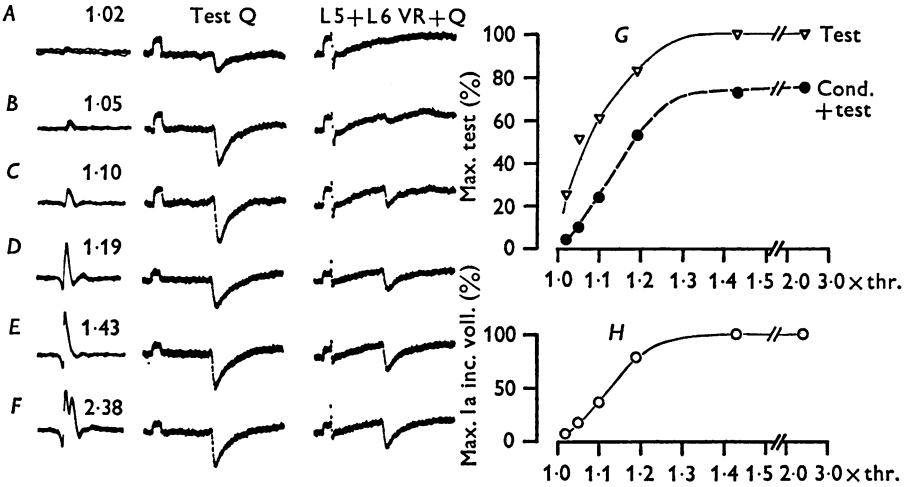


Fig. 1. Depression of Ia IPSPs in a motoneurone by antidromic volleys in motor axons. Records in the left column show afferent volleys recorded at the dorsal root entry zone. Middle and right columns show averaged intracellular records (cf. Methods) from a PBSt motoneurone. The test Ia IPSPs in the middle column were evoked from the Q nerve with the strength of stimulation (in multiples of threshold for Ia fibres) increasing from *A* to *F* as indicated above corresponding afferent volleys in the left column. The right column shows the test IPSPs conditioned by a preceding stimulation of L5 and L6 VRs. In the graph *G* are plotted the amplitudes of the unconditioned (▽) and the conditioned (●) IPSPs expressed as a percentage of the maximal unconditioned test IPSP (ordinate) against the strength of stimulation (abscissa). The amplitudes of the Ia component of the afferent volleys, expressed as a percentage of the maximal Ia component, are similarly plotted in graph *H*. Calibration pulses in each intracellular record are 0·5 mV and have 2 msec duration. Note lower amplification of intracellular records in D–F. In this and the following Figures negativity in cord surface records is signalled upwards and hyperpolarization in intracellular records downwards.

component (cf. incoming volleys in *E* and *F*). This maximal Ia IPSP was decreased to about 75% while, as generally found, the depression of the submaximal IPSPs was even more pronounced; in many cells such IPSPs were virtually abolished.

The effects of antidromic volleys in motor axons on Ia IPSPs were tested in more than 140 motoneurones innervating flexor and extensor muscles at the hip, knee and ankle joints (48 PBSt, 7 Sart, 14 DP, 46 Q,

9 ABSm, 22 G-S and 2 Pl motoneurones). Several other impaled motoneurones, particularly those of ABSm and G-S, could not be used either because the Ia IPSPs were too small to be tested or because all stimulated ventral roots evoked RIPSPs (cf. section *b*).

A clear decrease of the Ia IPSPs was found in all the tested motoneurones except six out of twenty-two G-S cells. Examples of records from different species of motoneurone are shown in Fig. 2. In each pair the upper

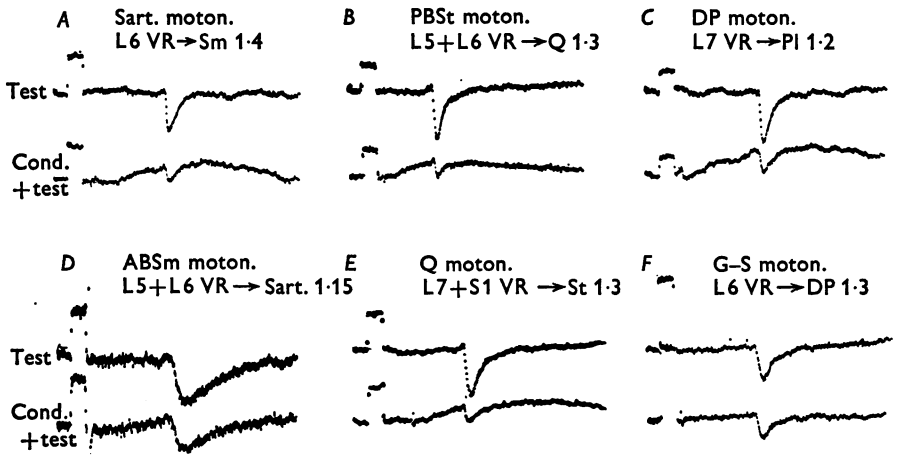


Fig. 2. Depression of Ia IPSPs in different species of motoneurone. Averaged intracellular records: *A–C* from motoneurones innervating the flexor muscles Sart, PBSt and DP; *D–F* from motoneurones supplying the extensors ABSm, Q and G-S. Upper traces in each pair show test Ia IPSPs evoked from nerves of antagonist muscles, lower traces the test IPSPs conditioned by a preceding stimulation of ventral roots. Nerves and ventral roots stimulated are indicated above the records. Duration and amplitude of calibration pulses: 4 msec and 1 mV in *A*, *C*, *E* and *F*; 4 msec and 0·5 mV in *B*; 2 msec and 0·5 mV in *D*.

trace is the unconditioned and the lower trace the conditioned response. *A–C* are from motoneurones to flexors, Sart, PBSt and DP, and *D–F* from motoneurones to extensors, ABSm, Q and G-S respectively. On the average, IPSPs evoked in motoneurones to Q, Sart, PBSt and DP by maximal Ia volleys were decreased to about 70 %. Smaller effects were found in G-S and ABSm motoneurones but the explanation may be that the most effective ventral roots could not be used for conditioning stimulation since large RIPSPs were evoked from them.

Besides the decrease in amplitude, a clear increase was found in central latency of the Ia IPSPs conditioned by stimulation of the ventral roots. In four cells analysed more extensively the average increase was about 0·2 msec. There was also a greater variability in central latency of the test

responses to consecutive shocks and a small increase in time to peak of the conditioned test IPSPs.

(*b*) *Depression of Ia IPSPs in relation to permeability changes in the motoneuronal membrane*

Before the depression of Ia IPSPs could be ascribed to interference with the reflex transmission to motoneurones conductance changes in the

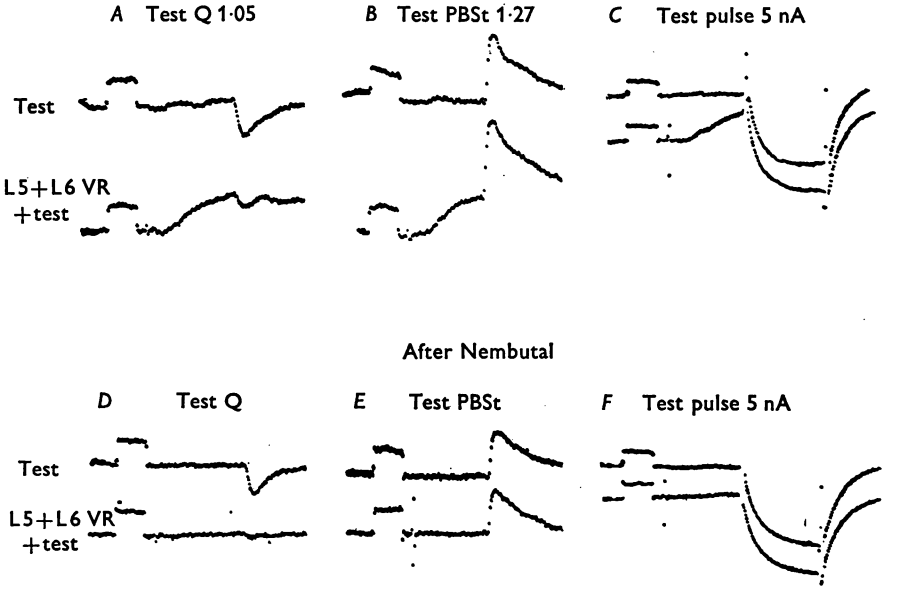


Fig. 3. Effects of Nembutal on RFPs and on the depression of Ia IPSPs. Averaged intracellular records from a PBSt motoneurone. Upper traces in each pair show the test responses: in *A* and *D* Ia IPSPs from the Q nerve, in *B* and *E* homonymous Ia EPSPs, in *C* and *F* conductance measurement pulses (5 nA). Lower traces in each pair show these test responses conditioned by a preceding stimulation of L5 and L6 VRs. Nembutal (9 mg/kg) was given intravenously a few minutes before the records in *D–F* were taken. Note that the amplitudes of the synaptic responses before and after Nembutal are not comparable because of the change in threshold of the responses caused by the anaesthetic. Calibration pulses: 0·5 mV and 4 msec.

motoneuronal membrane had to be excluded. The hyperpolarization and the increased conductance during an RIPSP can in itself reduce the amplitude of IPSPs. Thus when the antidromic volley evoked an RIPSP it was usually impossible to judge if the decrease of the test IPSP was due only to the RIPSP or to interference with the transmission in the Ia inhibitory pathway as well. However, in many motor nuclei the RIPSPs and the depression of Ia IPSPs were evoked from different ventral roots (see section (*c*) below). It was thus possible to obtain a depression of Ia IPSPs by ventral root volleys which either had no effect on the motoneurones them-

selves or evoked RFPs. The latter should not cause any decrease of Ia IPSPs but rather an increase, since the RFP is due to a release of the motoneurone from a tonic inhibitory bombardment and is associated with a decreased conductance of the motoneuronal membrane (Wilson & Burgess, 1962).

As the recurrent facilitation is very sensitive to barbiturate anaesthesia (Wilson & Talbot, 1960) an injection of Nembutal was used to show that the depression of Ia IPSPs can be dissociated from RFPs. Fig. 3 illustrates the effect of a small dose of Nembutal (9 mg/kg) administered intravenously while recording from a PBSt motoneurone. The test responses (upper

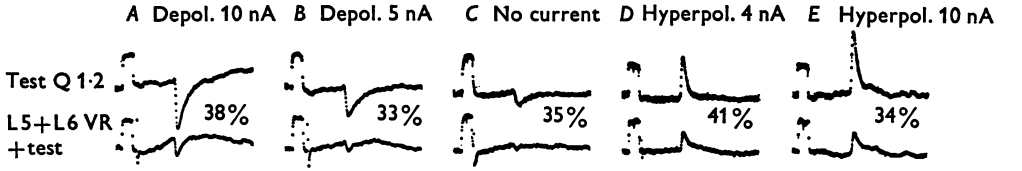


Fig. 4. Depression of normal and reversed Ia IPSPs. Averaged intracellular records from a PBSt motoneurone. Upper traces show test Ia IPSPs evoked from the Q nerve, lower traces the test IPSPs conditioned by a preceding stimulation of L5 and L6 VRs. The amplitude and the polarity of the test IPSP was successively changed from *A* to *E* by current injection through the recording KCl electrode, as indicated above records. The percentage depression is given between the traces in each pair. Calibration pulses: 0·5 mV and 4 msec.

traces) were the Ia IPSP (*A* and *D*), the homonymous Ia EPSP (*B* and *E*) and a small hyperpolarizing current pulse injected through the microelectrode (*C* and *F*). These tests were conditioned by a preceding antidromic volley in the L5 and L6 VRs (lower traces). Without anaesthesia the antidromic volley evoked a large RFP (*A–C*) which was completely abolished by Nembutal (*D–F*). During the RFP there was a conductance decrease of about 10% as compared with the resting conductance (*C*) which can fully explain the increase in amplitude of the conditioned Ia EPSP in *B*. When the RFP was abolished there was no change in amplitude of the Ia EPSP (*E*) or the test pulse (*F*), indicating that the conditioning volley did not evoke any conductance change in the motoneuronal membrane, detectable from the soma. The Ia IPSP was, however, still effectively depressed (*D*).

In motoneurones which were progressively depolarized the ratio between the unconditioned and the conditioned IPSPs was the same at different membrane potential levels. This was the case also when the Ia IPSPs were reversed in depolarizing direction as illustrated in Fig. 4. The upper records show Ia IPSPs evoked in a PBSt motoneurone by stimulation of the Q nerve with the same strength throughout. A conditioning anti-

dromic volley in the L5 and L6 VRs markedly decreased these IPSPs (lower records). Passage of depolarizing and hyperpolarizing current through the KCl-filled micro-electrode changed the amplitude and the polarity of the Ia IPSP. The ratio between the unconditioned and the conditioned IPSPs remained constant, however, throughout the series. Note that the small RFP seen in *A–C* is clearly reversed in hyperpolarizing direction in *E*.

(*c*) *Effective ventral roots*

Volleys in different ventral roots were not equally effective in depressing Ia IPSPs in a given species of motoneurone. In most cells it was, however, difficult to compare quantitatively the effects from all the ventral roots since considerable RIPSPs were evoked from some of them. This could be performed in only a few cells without RIPSPs as shown in Fig. 5 for a

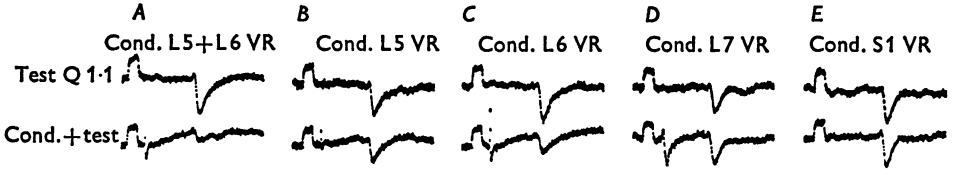


Fig. 5. Comparison of Ia IPSP depression evoked from different ventral roots. Averaged intracellular records from a PBSt motoneurone located in caudal L7. Upper traces show test Ia IPSPs evoked from the Q nerve, lower traces the test IPSPs conditioned by stimulation of different ventral roots as indicated above each set of records. The strength of the ventral root stimulation was supramaximal for $\alpha$-fibres, except in *D* where it was just subthreshold for antidromic invasion of the motoneurone investigated. Note that the stimulation in *D* was strong enough to activate several other motoneurones as indicated by the field potential close to the calibration pulse in the lower trace. Calibration pulses: 0·5 mV and 2 msec.

PBSt motoneurone located in the caudal part of the L7 segment. The test Ia IPSPs were evoked by a submaximal group Ia stimulation of the Q nerve. In the upper row are the unconditioned test responses while the corresponding records in the lower row show the effect of antidromic volleys in different ventral roots. The conditioning stimulation was supramaximal for $\alpha$-fibres, except in the case of the L7 VR which was stimulated just subthreshold for antidromic invasion of the cell. The latter stimulation was, however, strong enough to fire several neighbouring motoneurones as indicated by the antidromic field potential (lower trace in *D*). Fig. 5 illustrates the typical finding that the Ia IPSPs in PBSt motoneurones were depressed by volleys in the L5 and L6 VRs while volleys in the L7 and S1 VRs were without significant effect. In Q motoneurones, on the other hand, the Ia IPSPs from the PBSt nerve were

reduced from the L7 and S1 VRs. Observe also in Fig. 5 that combined stimulation of the L5 and L6 VRs was more effective (record *A*) than separate stimulation of either of them (records *B* and *C*).

In unanaesthetized animals RFPs were evoked in Q and PBSt motoneurones by volleys in the same ventral roots which depressed the Ia IPSPs. The recurrent inhibition was mainly evoked from the other roots (L7 and S1 VRs in PBSt motoneurones and L5 and L6 VRs in Q motoneurones). Different segmental origin of the RFPs and the effects on Ia IPSPs, on one hand, and of the RIPSPs on the other, was seen also in the other species of motoneurones, though less clearly in motoneurones to ABSm and G-S.

*(d) Contribution from α- and γ-efferents to the depression of Ia IPSPs*

The recurrent inhibition of both α- and γ-motoneurones is caused by impulses in α-motor axons (Renshaw, 1941; Ellaway, 1968; Brown, Lawrence & Matthews, 1968). To test if α-efferents are also responsible for the depression of Ia IPSPs, the motor axons were stimulated in the peripheral nerves. It was then possible to control the threshold separation between α- and γ-fibres by recording from a cut ventral rootlet (cf. Leksell, 1945). These experiments were performed on preparations with transected dorsal roots in which the test Ia IPSPs were evoked by very weak stimulation of dorsal roots containing Ia afferents from antagonists (cf. Hultborn *et al.* 1971*b*).

An example of a disynaptic IPSP evoked in a PBSt motoneurone by very weak stimulation of the L5 DR is shown in Fig. 6*B*. Records *C–F* and graph *A* illustrate the effect on this IPSP of conditioning antidromic stimulation of the Q nerve with increasing strengths. Graph *G* gives the relation between the strength of stimulation and the size of the volleys in α- and γ-fibres, recorded from a cut rootlet of the L5 VR. The two graphs show that the conditioned Ia IPSP decreased approximately in parallel with the increase of the α-volley and reached a plateau at a stimulus strength just maximal for α-fibres. Similar results were obtained from six other motoneurones of different types.

The finding that no additional effect was produced when the stimulus strength was increased to activate γ-fibres does not exclude that γ-efferents might contribute to the recurrent effects. If they have collaterals which converge on the same Renshaw cells as the α-motor-axon collaterals the plateau of the depression could be due to occlusion. However, this explanation is very unlikely in view of the marked parallelism between the depression of the Ia IPSPs and the increase of the α-volleys.

*(e) Time course of the recurrent depression of Ia IPSPs*

Recurrent inhibition and facilitation of motoneurones by single antidromic volleys have a typical prolonged time course which has been attributed to the repetitive firing of Renshaw cells (Eccles *et al.* 1954).

The central latencies of the effects indicate a disynaptic pathway for the recurrent inhibition (Eccles *et al.* 1954) and a trisynaptic path for the recurrent facilitation (Wilson, 1959; H. Hultborn, E. Jankowska &

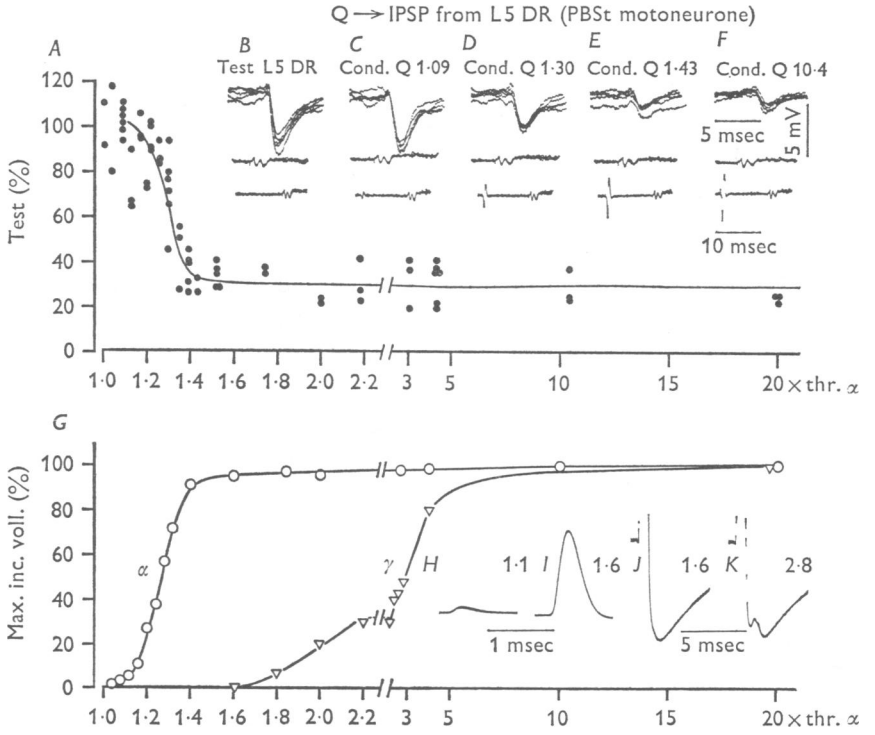


Fig. 6. Effects from $\alpha$- and $\gamma$-efferents. Graph *A* and sample records in *B–F* show the depression of Ia IPSPs in a PBSt motoneurone by conditioning stimulation of efferent fibres with increasing strength. Upper traces in *B–F* show intracellular potentials. Middle and lower traces are from the dorsal-root entry zone; the lower recorded with a slower sweep speed. The test Ia IPSP (*B*) was evoked by very weak stimulation of the L5 DR. The conditioning stimulation was applied to the Q nerve (*C–F*; conditioning–testing interval constant). The stimulus strength is expressed in multiples of threshold for $\alpha$-fibres. In the graph *A* are plotted the amplitudes of the conditioned IPSPs expressed as a percentage of the unconditioned test IPSPs (ordinate) against the strength of conditioning stimulation (abscissa). Each point represents the average amplitude of five responses. Graph *G* shows the relation between the strength of conditioning stimulation (abscissa) and the size of the antidromic volleys in $\alpha$- (○) and $\gamma$- (△) efferents expressed as a percentage of the maximal $\alpha$- and $\gamma$-volleys respectively (ordinate). The volleys were recorded from a dissected rootlet of the L5 VR shortly after recording the series in *A*. The inserted records show the $\alpha$-volleys (*H* and *I*) and the $\gamma$-volleys (*J* and *K*) at indicated strengths of stimulation. Note higher amplification and slower sweep speed in *J–K*.

S. Lindström, unpublished). The latency and time course of the recurrent depression of Ia IPSPs in motoneurones are shown in the graphs in Fig. 7. A disynaptic test IPSP was evoked in a Sart motoneurone by very weak stimulation of the L6 DR (*C*). The conditioning stimulation, supramaximal for $\alpha$-fibres, was applied to the Sm nerve (*D–F*). The conditioning volley had to precede the test volley by at least 0·6 msec to evoke the depression. The effect was maximal at an interval of about 8 msec and

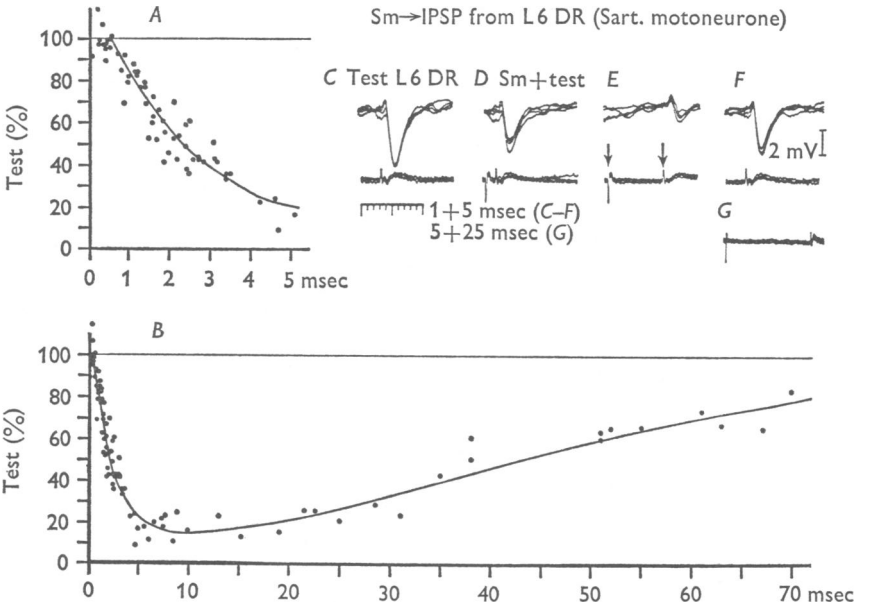


Fig. 7. Time course of the recurrent depression of Ia IPSPs. Upper traces in records *C–F* show intracellular potentials in a Sart motoneurone. Lower traces are from the dorsal root entry zone in L5. Record *C* shows a test Ia IPSP evoked by weak stimulation of the L6 DR. *D–F* show the test IPSP conditioned at different time intervals by an antidromic volley in the Sm nerve (the conditioning–testing interval of *F* is shown in *G*, recorded with a slower sweep speed). In graphs *A* and *B* the amplitudes of the conditioned IPSPs expressed as a percentage of the unconditioned test IPSPs (ordinates) are plotted against the time interval between the conditioning and the testing volleys (abscissae). The time intervals were measured as indicated by arrows in *E*. Each point represents the average amplitude of five responses.

lasted for some 80 msec. Similar results were obtained with the test Ia IPSPs evoked by stimulation of peripheral nerves and conditioning stimulation of ventral roots. In sixteen motoneurones the minimal effective interval between the antidromic and the Ia volleys ranged between 0·3 and 0·9 msec (average 0·5 msec). The time to peak of the depression varied between 5 and 12 msec (average 8 msec) and the duration between 50 and 100 msec (average 75 msec).

The depression of the Ia inhibition of motoneurones could be demonstrated also when tested on the Ia inhibition of monosynaptic reflexes although care had to be taken that the results were not obscured by recurrent facilitation. The time course of the recurrent effects found in this way is illustrated in Fig. 8. The records in *C* show the unconditioned PBSt

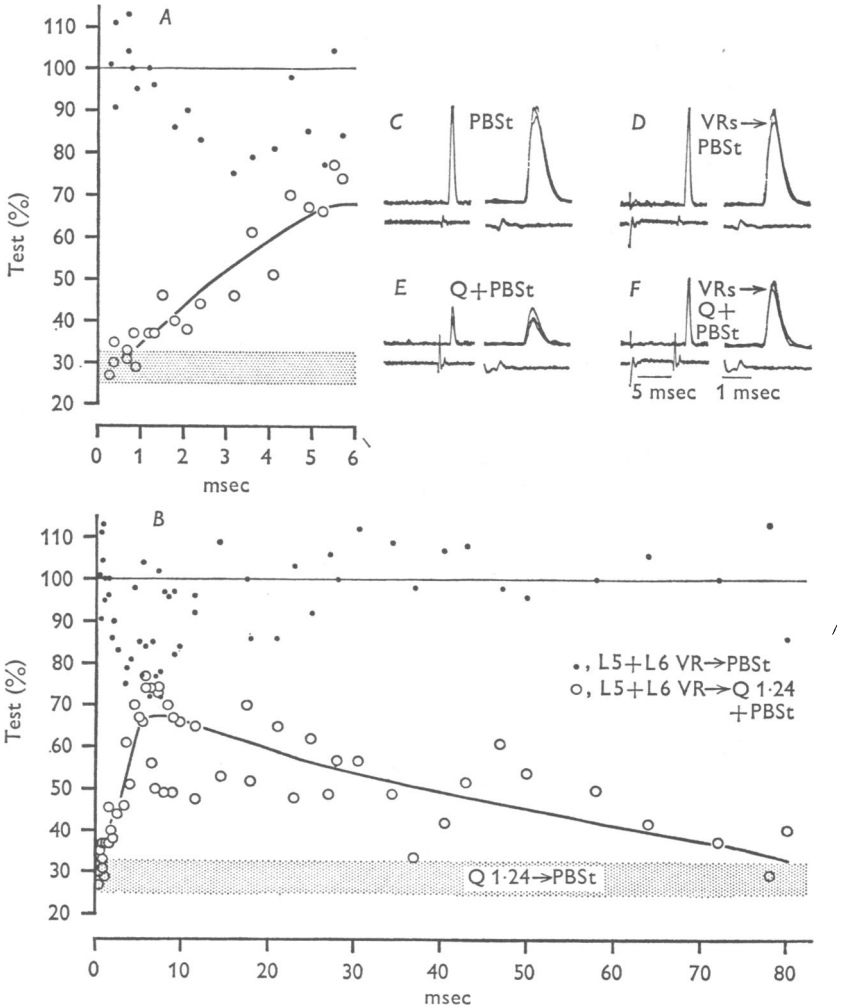


Fig. 8. For legend see opposite page.

test reflex at two different sweep speeds. In *E* and *F* this test is conditioned by a Ia volley in the nerve to the antagonist Q. In *F* the inhibition is much weaker because of a preceding antidromic volley in the L5 and L6 VRs. The latter stimulation alone did not modify the test reflex in a significant way (*D*). Records *C*–*F* are samples from the series plotted in the graphs.

The antidromic stimulation alone seemed to produce some early recurrent inhibition of the PBSt motoneurones but no recurrent facilitation. The increase of the Ia inhibited test by the antidromic volley therefore demonstrates the decrease of the Ia inhibitory action.

### II. *Recurrent effects on other reflex pathways to motoneurones*

#### (*a*) *Effects on Ib IPSPs*

The predominant effects encountered from Ib afferents were inhibition of extensor motoneurones and facilitation of flexors, both evoked from extensor nerves (cf. Eccles *et al.* 1957*c*). Accordingly the recurrent effects on Ib IPSPs were investigated only in extensor motoneurones. The test IPSPs were evoked by stimulation of high-threshold group I afferents in different extensor nerves known not to give Ia inhibition of the motoneurones under investigation (R. M. Eccles & Lundberg, 1958; Hongo, Jankowska & Lundberg, 1969). Effects on Ib IPSPs were tested in forty-three cells, most of them Q motoneurones (twenty-six cells), in which the Ia IPSPs were effectively depressed. The Ib IPSPs were conditioned by antidromic stimulation of the ventral roots used to condition the Ia IPSPs in the cells. As mentioned above, the other ventral roots could not as a rule be used because they produced RIPSPs in the motoneurones. In no case could any clear decrease of Ib IPSPs be demonstrated. One example of effects on Ib IPSPs evoked in a Q motoneurone is given in Fig. 9. The Ib IPSPs in *B* and *C* were evoked by stimulation of the G-S nerve at two different strengths. These IPSPs were not decreased when conditioned by

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Legend to Fig. 8.

Fig. 8. Effects on the Ia inhibition of monosynaptic reflexes. Upper traces in records *C–F* show monosynaptic reflexes recorded in L7 VR, lower traces are from the L6 dorsal root entry zone. The same responses are shown with two different sweep speeds (cf. calibrations in *F*). The test reflex (*C*) was evoked by stimulation of the PBSt nerve. This test was inhibited by a preceding volley in Ia afferents from Q (*E*). Following a stimulation of L5 and L6 VRs the Ia inhibition of the test reflex was less pronounced (*F*). The antidromic volley alone had no influence on the test reflex (*D*). In the graphs *A* and *B* the amplitudes of monosynaptic reflexes conditioned by stimulation of ventral roots (ordinates) are plotted against conditioning–testing intervals (abscissae). The amplitudes of the conditioned responses are expressed as a percentage of the unconditioned test reflex. Dotted area shows the mean amplitude (with a 95 % confidence interval) of the Ia inhibited test reflex (as in *E*). ●, Test reflex conditioned by ventral root stimulation (as in *D*); ○, the Ia inhibited test reflex conditioned by ventral root stimulation (as in *F*). Time intervals were measured between the arrival at the spinal cord of the ventral root volley and the PBSt volley (●) or the Q volley (○). Each point represents the average amplitude of three test reflexes.

volleys in the L7 and S1 VRs but slightly increased due to the small RFP. As shown in *A*, antidromic stimulation of the same ventral roots effectively decreased the Ia IPSP from the St nerve.

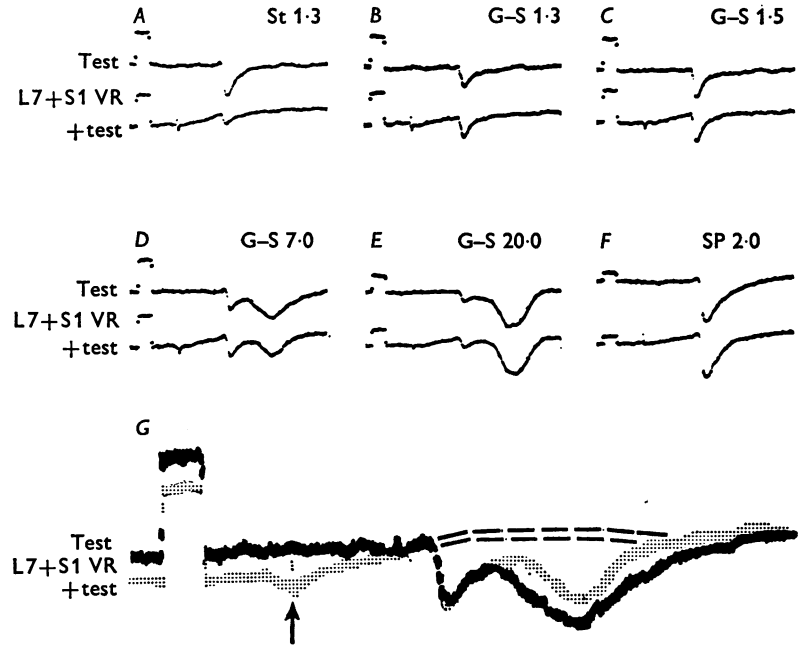


Fig. 9. Effects on IPSPs from different groups of afferents. Averaged intracellular records from a Q motoneurone. Upper traces in records *A–F* show test IPSPs evoked from different groups of afferents: *A*, group Ia afferents in the St nerve; *B* and *C*, group Ib afferents in the G-S nerve, stimulated with two different strengths; *D*, group Ib and II afferents in the G-S nerve; *E*, the latter plus group III afferents; *F*, low-threshold cutaneous afferents in the SP nerve. Lower traces show these IPSPs conditioned by an antidromic volley in L7 and S1 VRs. *G* shows the two traces of record *D* superimposed. The response to ventral root stimulation alone has been added as a double interrupted line. Further details in the text. Calibration pulses: 1 mV and 4 msec.

### (*b*) *Effects on FRA IPSPs*

From the point of view of their reflex actions group II and III muscle afferents, cutaneous afferents and high-threshold joint afferents are often grouped together as flexor reflex afferents (FRA; R. M. Eccles & Lundberg, 1959). In the acute spinal cat their principal effects to motoneurones are excitation of flexors and inhibition of extensors. Under certain conditions the FRA may instead evoke IPSPs in flexor motoneurones and EPSPs in extensor motoneurones, indicating the existence of alternative pathways from the FRA to motoneurones (R. M. Eccles & Lundberg,

1959; Holmqvist & Lundberg, 1961). Recurrent effects on FRA IPSPs were investigated mainly in extensor motoneurones, but also in some flexor motoneurones (total fifty-seven motoneurones). The IPSPs were evoked from different muscle and cutaneous nerves and from the posterior nerve to the knee joint of the ipsilateral hind limb. In most cells the antidromic volleys were without any effect on the FRA IPSPs (Fig. 9*F*). However, a small depression was found of some IPSPs evoked from group II muscle afferents and/or low-threshold cutaneous afferents. The depression was less pronounced than that of Ia IPSPs even if the submaximal FRA IPSPs were smaller than the Ia IPSPs. The effect was not due to a conductance change in the motoneuronal membrane.

Fig. 9*D* and *G* exemplifies the decrease of an IPSP evoked from high-threshold muscle afferents. The records are from the same Q motoneurone in which the Ib IPSPs (*B–C*) were investigated. In record *D* the stimulation of the G-S nerve was increased to activate also group II afferents. The conditioning volley did not change the early Ib component of the IPSP (cf. records *B* and *C*), but the second component evoked from group II afferents was delayed and decreased. This is best seen in *G* with superimposed traces of the unconditioned and conditioned tests. Record *E* shows the effect of the same conditioning volley when the testing stimulus was increased to activate group III muscle afferents. The early part of the FRA IPSP (evoked from group II afferents) was still delayed by the conditioning volley but there was no depression of the main component. In fact the amplitude of the conditioned FRA IPSP in *E* was even slightly larger than of the unconditioned test, presumably due to the small RFP. Depression of group II IPSPs was found in eight Q, two AB and three PBSt motoneurones of twenty-eight tested. When group II IPSPs were evoked in the same Q cells from several nerves only some of them were decreased while the others were unaffected.

Decrease of IPSPs from low-threshold cutaneous afferents was found in six Q and two PBSt motoneurones of twenty-seven tested cells. All the affected IPSPs were evoked from the Sur nerve, while IPSPs from the SP and Saph nerves were not significantly depressed. Likewise no depression was found of IPSPs from high-threshold cutaneous afferents. The IPSPs evoked from the Sur nerve were depressed in Q motoneurones from the L7 and S1 VRs but in PBSt motoneurones from the L5 and L6 VRs. This indicates that in a given motoneurone FRA IPSPs can be depressed from the same ventral roots as the Ia IPSPs.

(c) *Recurrent effects on EPSPs*

Recurrent effects on EPSPs from different afferent systems were studied less extensively than the effects on IPSPs. Only effects from ventral roots

which did not evoke RIPSPs in the motoneurones could be analysed. Fig. 3*E* illustrates that the amplitude of monosynaptic Ia EPSPs was unchanged by antidromic volleys which effectively depressed the Ia IPSPs in the same cells, provided the conditioning stimulation did not evoke RFPs (as in *B*) or RIPSPs.

The excitatory pathways from Ib afferents and the FRA include one or more interneurones which might be influenced by volleys in motor axon collaterals. No effect was found on Ib EPSPs tested in a few motoneurones.

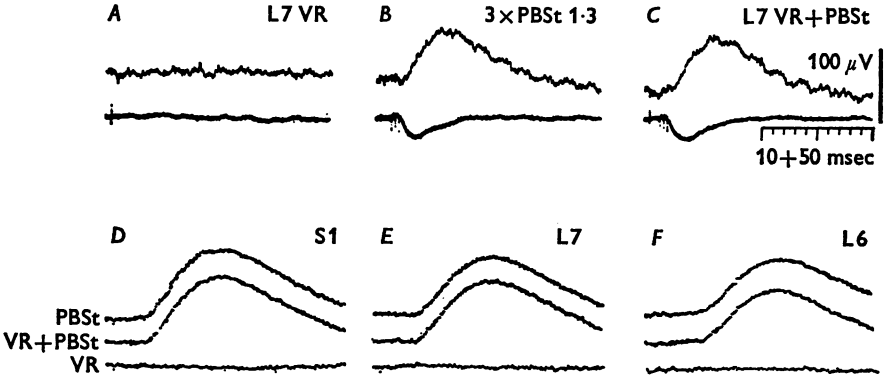


Fig. 10. Effects on DRPs evoked by Ia volleys. Upper traces in *A–C* are single sweep records from the most caudal rootlet in L6, lower traces are from the dorsal root entry zone. *A* demonstrates that no DRP was evoked by single antidromic stimulation of the L7 VR. The DRP in *B* was evoked by three Ia volleys in the nerve to the flexor PBSt. *C* shows this DRP conditioned by a preceding single stimulation of the L7 VR. *D–F* are averaged records from the same rootlet. Upper traces show DRPs evoked by PBSt volleys as in *B*. Middle traces show these DRPs conditioned by a single antidromic volley in the S1, L7 and L6 VRs respectively, and the lower traces the effects of the antidromic volleys alone.

Neither were EPSPs evoked from the FRA decreased. In a few cells a small increase in amplitude of FRA EPSPs was found, but this effect was probably due to small RFPs.

### III. *Recurrent effects on primary afferent terminals*

The observed depression of group Ia IPSPs by impulses in motor axon collaterals could be due to presynaptic inhibition of the transmission from Ia primary afferents to the inhibitory interneurones. This seemed unlikely as in the cat antidromic stimulation of ventral roots neither evokes a 'P-wave' in the cord dorsum recording (Gasser & Graham, 1933) nor DRPs (Barron & Matthews, 1938). Further, Wall (1958) was unable to demonstrate any change in excitability of G-S group I afferent terminals in the ventral horn after antidromic stimulation of the S1 VR.

We have confirmed that DRPs are not evoked by antidromic stimulation of ventral roots (Fig. 10*A* and lower traces in *D–F*). The finding that ventral root volleys do not depress Ia EPSPs (Fig. 3*E*) also indicates that such volleys do not depolarize Ia terminals on motoneurones. However, selective effects on Ia terminals on the Ia inhibitory interneurones cannot be entirely excluded since the electrotonic spread of the terminal depolarization of Ia afferents might be limited by the length or small diameter of the preterminal part. The excitability of Ia afferents was therefore tested as described by Wall (1958) with the stimulating electrode placed dorsomedially to the motor nuclei in a region where Ia collaterals terminate (Szentágothai, 1967) and interneurones supposedly mediating Ia inhibition have been found (Hultborn *et al.* 1971*a*). Neither in this case could any effect of ventral root stimulation be demonstrated on Ia terminals.

The influence of volleys in motor axon collaterals on the transmission in the pathway from Ia afferents to Ia afferent terminals was also tested. This was done by conditioning DRPs evoked by a train of group Ia volleys in a flexor nerve, which are known to evoke depolarization of Ia afferent terminals (Eccles, Magni & Willis, 1962). In no case were these DRPs decreased by single shock or repetitive stimulation of the ventral roots. This is exemplified in Fig. 10 (*B*, *C* and upper and middle traces in *D–F*) with DRPs evoked by Ia volleys in the PBSt nerve. DRPs evoked by volleys in group Ib muscle afferents or low-threshold cutaneous afferents were likewise unaffected.

## DISCUSSION

The present results demonstrate that impulses in recurrent motor axon collaterals, besides the well-known inhibitory effects on motoneurones, also affect transmission of synaptic actions to motoneurones. The effect is probably restricted to inhibition of the interneurone in the reciprocal Ia inhibitory pathway to motoneurones.

The effects of impulses in recurrent motor axon collaterals on reflex transmission to motoneurones were deduced from changes of PSPs in motoneurones after antidromic stimulation of ventral roots. Various evidence has been brought to establish that the observed depression of Ia IPSPs is not due to post-synaptic effects in the motoneurones. In most cells the depression could be produced from ventral roots which did not evoke any detectable RIPSPs. In unanaesthetized animals the effective antidromic volleys instead often evoked RFPs. The depression of the IPSPs, however, did not depend on these RFPs, as the effect could be evoked also when the RFPs were abolished by barbiturate. The effective

antidromic volleys did not then produce any detectable conductance change in the motoneurone when tested with small current pulses. Conductance changes in more remote parts of the dendrites, not revealed by micro-electrodes placed in the soma, are not likely to be of importance, since the synapses of the Ia inhibitory interneurones are most probably localized close to the soma of the motoneurones (Curtis & Eccles, 1959; Smith, Wuerker & Frank, 1967; Burke, Fedina & Lundberg, 1968). Monosynaptic Ia EPSPs and IPSPs from other afferent systems were also unaffected by the ventral root stimuli which effectively decreased the Ia IPSPs. The constant ratio between the unconditioned and the conditioned Ia IPSPs during injury depolarization of the motoneurones or reversal of the IPSPs by injection of chloride ions further demonstrates that the depression of the Ia IPSPs is independent of the state of the motoneurones. The remote possibility that the IPSP depression represented summation of the IPSP with an EPSP in some released disynaptic pathway from Ia afferents is excluded by the observation that the hyperpolarizing and the reversed Ia IPSPs were depressed to the same extent. If an EPSP was present the test response should have been increased on conditioning by addition of an EPSP to the reversed IPSPs. All the evidence thus leads to the conclusion that the depression of Ia IPSPs is due to inhibition of the transmission in the Ia inhibitory pathway, either presynaptically by depolarization of Ia afferent terminals, or at the interneuronal level.

Antidromic volleys in ventral roots are known to evoke primary afferent depolarization in the frog (Barron & Matthews, 1938) and it has recently been reported that such volleys may also give some discharges in primary afferents also in the cat (Decima, 1969; Decima & Goldberg, 1970). However, in cats this effect seems to be very marginal and is probably unrelated to presynaptic inhibition. It may be caused by ephaptic excitation of a few depolarized presynaptic terminals by motoneuronal action currents. Observe that neither P-waves nor DRPs are evoked by antidromic stimulation of ventral roots in the cat (Gasser & Graham, 1933; Barron & Matthews, 1938). Furthermore, conditioning ventral-root stimuli did not give any change in the excitability of Ia afferent terminals stimulated in the region where the presumed Ia inhibitory interneurones have been found. There are therefore no indications that the recurrent depression of Ia IPSPs in motoneurones is caused by depolarization of group Ia afferent terminals. It follows that the effect must be exerted on the interneurone interposed in the Ia inhibitory pathway either by post-synaptic inhibition or by presynaptic inhibition of the transmission from their terminals to motoneurones.

The duration of the recurrent depression of the Ia IPSPs is shorter than

that generally found for presynaptic inhibition and corresponds more with the time course of RIPSPs in motoneurones. The Ia IPSPs are depressed when the antidromic volleys arrive at the spinal cord only 0·3–0·9 msec in advance of the Ia volleys. This small difference in the central latency of the effects allows for only one more synapse in the recurrent inhibitory path to the interneurones than in the excitatory path from Ia afferents. Thus the recurrent inhibitory pathway to the interneurones must be disynaptic like that to the motoneurones. Most likely the recurrent depression of the Ia IPSPs in motoneurones is mediated through motor axon collaterals and Renshaw cells, which evoke post-synaptic inhibition of the Ia inhibitory interneurones. This suggestion is supported by the finding that the central latency and the time to peak of the Ia IPSPs in motoneurones are increased by conditioning volleys in motor axons, indicating an interference with the spike generation in the interneurones. Moreover, interneurones with convergence of monosynaptic Ia EPSPs and disynaptic IPSPs from motor axon collaterals have been identified and will be reported in a following paper (Hultborn *et al.* 1971*a*).

In all cases in which the motor nuclei to antagonists were located at different segmental levels the motor axons giving RIPSPs in one species of the motoneurone and those producing depression of Ia IPSPs in the same cells seemed to have different segmental distribution. The RIPSPs were mainly evoked from ventral roots of the segments in which the motor nuclei investigated were located, which confirms earlier findings that the recurrent inhibition is most pronounced between the motor nuclei within the same segment (Renshaw, 1941; Eccles *et al.* 1954). The Ia IPSPs, on the other hand, were decreased after stimulation of ventral roots from the segments where the Ia afferents, giving rise to the test IPSPs, entered the spinal cord. This finding raised the question whether the depression of the Ia IPSPs in a given species of motoneurones is mediated via axon collaterals of motoneurones supplying antagonists rather than of those innervating synergists. That this is indeed the case will be shown in a following paper (Hultborn *et al.* 1971*b*).

In all motor nuclei investigated the Ia IPSPs could be depressed by antidromic volleys in motor axons. No effect was found on PSPs from other groups of afferents tested with the exception of some IPSPs from group II muscle afferents and low-threshold cutaneous afferents. Because of the scanty effects on such IPSPs a more detailed analysis of the effects could not be performed. Using the same criteria as for the Ia IPSPs it could be shown, however, that the decrease of these FRA IPSPs was due to recurrent inhibition exerted at an interneuronal level. It is therefore of great interest that Ia IPSPs in motoneurones can be facilitated by weak FRA stimulation, due to a spatial summation on the Ia inhibitory inter-

neurones (cf. Lundberg, 1970). Furthermore, the interneurones with convergence of monosynaptic Ia excitation and disynaptic inhibition from motor axon collaterals are also excited by the FRA (Hultborn *et al.* 1971*a*). It is thus likely that volleys in the FRA may evoke inhibition in motoneurones at least partly by activation of Ia inhibitory interneurones. If so, the IPSPs evoked via this route in a given motoneurone should be susceptible to recurrent depression from the same ventral roots as the Ia IPSPs, and this was found to be the case. There is now evidence for a 'private' inhibitory pathway from the FRA to motoneurones in which the interneurones are not shared with Ia afferents (cf. Lundberg, 1970). Our results are well explained if it is assumed that the interneurones of the latter pathway do not receive recurrent inhibition from motor axon collaterals. The inconsistency of the effects on FRA IPSPs may be explained by the fact that the IPSPs to different extent are mediated by one or the other of these two pathways from the FRA. When the FRA IPSPs are not depressed they may be mediated largely by the 'private' FRA pathway while in other cases both interneuronal pathways may contribute. A coactivation of the two pathways may explain why in the same cells we found a smaller depression of submaximal group II IPSPs than of Ia IPSPs.

The present results thus suggest that *the recurrent effects on segmental reflex pathways to motoneurones are restricted to inhibition of the interneurones in the reciprocal Ia inhibitory pathway.* The high selectivity of the recurrent control of the Ia inhibitory interneurones suggests that recurrent inhibition has a very specific function in motor control and this is discussed in a following paper (Hultborn *et al.* 1971*b*).

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