J. Physiol. (1966), **182**, *pp.* 612–648
With 20 text-figures
Printed in Great Britain

A STUDY OF THE INTERACTION BETWEEN MOTONEURONES IN THE FROG SPINAL CORD

By A. D. GRINNELL

From the Biophysics Department, University College London and Department of Zoology, University of California, Los Angeles, U.S.A.*

(*Received* 26 *May* 1965)

SUMMARY

1. A short-latency interaction between motoneurones has been studied with intracellular and root potential recordings from the isolated spinal cord of the frog. Antidromic stimulation of one ventral root causes brief depolarization (VR-EPSP) of the motoneurones of adjacent, non-excited motoneurones. The summed activity of many such VR-EPSPs can be seen as a brief depolarization (VR-VRP) passing out an adjacent ventral root.

2. Both intracellular and root-recorded signs of this interaction are graded in amplitude.

3. It was found that this interaction decreased with increasing temperature. This is in contrast to the behaviour of the ventral root potential resulting from dorsal root stimulation (DR-VRP) or the dorsal root potentials resulting from either dorsal root (DR-DRP) or ventral root (VR-DRP) stimulation, all of which increased in amplitude from below 10 to about 17° C.

4. Pharmacological evidence suggests that the interaction between motoneurones is not chemically mediated. The VR-VRP was not affected by a large variety of transmitter blocking agents, including curare, dihydro-β-erythroidine, atropine, succinylcholine, hexamethonium and DOPA, while the VR-DRP, which probably originates with the release of ACh from an axon collateral, was consistently blocked.

5. Mg^{2+} suppressed the VR-VRP more slowly than the other potentials, and this suppression was increased by adding Ca^{2+}, rather than reversed, as in the case of the other root potentials, which are presumably mediated by chemical transmission.

6. The interaction between motoneurones is strongly facilitated by orthodromic depolarization of the motoneurones being antidromically

* Present address.

stimulated. Extracellular recordings within the cord support the conclusion that this facilitation is a result of the enhancement of antidromic invasion, perhaps especially of the dendrites, by slight depolarization.

7. One VR-VRP (or VR-EPSP) first suppresses response to another (for about 10 msec), then facilitates response to the second, with maximum effect around 20–40 msec. This is the case whether both stimuli go to the same or to different ventral roots, although occlusion is less and facilitation greater in the latter case. Occlusion of the VR-EPSP also results from full excitation of the cell in which recording is being done.

8. The mechanism of this interaction remains uncertain, but it would seem likely that overlapping dendrites of adjacent motoneurones interact with each other electrically through close apposition or specialized contacts. Occlusion would result from the refractoriness of strongly depolarized dendrites, facilitation from the enhancement of invasion of antidromically stimulated motoneurones by the weaker (or residual) depolarization occurring after earlier activity of motoneurones or their dendrites.

INTRODUCTION

Since the classical work of Barron & Matthews (1938), it has been known that in the frog spinal cord antidromic firing of ventral root fibres results in depolarization of dorsal root fibres. This dorsal root potential (DRP) is easily blocked by cholinergic blocking agents (Eccles & Malcolm, 1946; Eccles, 1947; Koketsu, 1956; Kiraly & Phillis, 1961), suggesting that the synaptic pathway leading to dorsal root depolarization begins with the release of ACh by motoneurone axon collaterals, the existence of which has been shown by Sala y Pons (1892) and Silver (1942). This antidromically produced DRP is sometimes large enough to produce firing of the dorsal root fibres, which in turn cause orthodromic depolarization and firing of motoneurones (Katz & Miledi, 1963).

Recently, it has been discovered that antidromic firing of motoneurones has a more direct, short latency effect on adjacent motoneurones. Washizu (1960), working with the excised toad spinal cord, found that 20 % of the motoneurones from which he recorded intracellularly could be fired by stimulation of either of two ventral roots. The responses were not identical, however; there was always a difference in latency of 0·6 msec or more between the two routes (average 1·1 msec at 11–20°). Moreover, there were consistent differences in the rise time of the two, in their behaviour on high repetition rate blockade, in susceptibility to CO_2, and in their response to anelectrotonus. Washizu observed no EPSP-like prepotential and so favoured the conclusion that the later response was the result of an interaction effected through dendritic bridges between motoneurones or, possibly, through some sort of ephaptic transmission.

Kubota & Brookhart (1962) and Katz & Miledi (1963), however, found graded post-synaptic depolarization in frog motoneurones after ventral root stimulation. This ventral root EPSP (VR–EPSP) has been extensively studied by Kubota & Brookhart (1963), who observed transient depolarization following stimulation of an adjacent ventral root in nearly three-quarters of the pelvic motoneurones studied. In about half of these (63 of 138), the transient depolarization gave rise to a spike. The graded potential came with a sharp onset at the average latency (at 15–16° C) of 2·2 msec and reached amplitudes of 6–7 mV in some cases. This potential summed with the depolarization caused by intracellularly passed current to initiate a spike potential at a constant threshold depolarization. The amplitude of antidromically invading spikes superimposed on the VR-EPSP was unaffected, suggesting that no somatic membrane conductance changes occur during the potential. Moreover, hyperpolarization or depolarization of the motoneurone soma by as much as 50 mV had no effect on this VR-EPSP, even though EPSPs elicited by stimulation of the lateral columns were greatly modified in size. These findings, coupled with the preliminary observations that succinylcholine chloride, decamethonium bromide, or curare altered the antidromically produced VR-EPSP, led to the suggestion that the interaction was monosynaptic and mediated by a chemical synapse between recurrent axon collaterals and distal portions of motoneurone dendrites.

The possible implications of having a cholinergic synapse *on* motoneurones, plus the unpublished observation by Katz and Miledi that, contrary to the findings of Kubota and Brookhart (1963), curare does not affect this interaction, have led me to investigate the problem further. In the present experiments, a variety of pharmacological tests favour the conclusion that the interaction between motoneurones is *not* chemically mediated, but more likely electrical in nature. Measurements of extracellular potentials in the cord, coupled with the effects of orthodromic depolarization, suggest that the interaction probably takes place between motoneurone dendrites.

METHODS

The methods employed in these experiments were identical in most respects to those described by Katz & Miledi (1963). *Rana temporaria* were normally used, although on many occasions *R. pipiens* and *R. catesbeiana* were also tried. No physiological differences between the three were observed. In all cases, the cord was removed together with the ventral and dorsal roots and mounted on its side in a groove cut out of the convex top of a Perspex disk. The cord was bathed in Ringer's solution having the following composition (mM): NaCl, 114; KCl, 2; $CaCl_2$, 1·8; $NaHCO_3$, 2; and glucose 1 g/l. It was oxygenated by continuous bubbling of 95 % O_2–5 % CO_2 into a side compartment of the bath. The temperature was maintained at 5–10° C by series connected Peltier elements built into the floor of the bath, as described by Katz & Miledi. The large dorsal and ventral roots 7–9 (8–10 in the nomenclature of Ecker & Wiedersheim, 1899) were then lifted out of

the Ringer's solution and into a layer of paraffin oil, where they were mounted on platinum or chlorided Ag wires for stimulation and recording of root potentials. Root recording was differential between an electrode on the distal end of the root and the bath, i.e. the point on the root where it passed into paraffin oil. This was usually within 1–2 mm of the surface of the cord. In many experiments, especially when the effects of drugs were being investigated, the cord was hemisected sagittally before mounting, medial surface down. Hemisection was found greatly to reduce the penetration time of some drugs into the cord.

For intracellular recording from motoneurones, the cord was often hemisected and mounted with the medial surface exposed. In other cases, the pial membrane covering the lumbar enlargement of the cord was removed in places to make insertion of a micro-electrode possible. The micro-electrodes were filled with either 3 M-KCl or 2 M-K citrate and were usually of 10–20 MΩ resistance. In certain preparations a bridge circuit was used to permit d.c. displacement of the membrane potential during recording. The cathode follower outputs were connected to the two channels of a Tektronix 502 oscilloscope. For intracellular recording, one channel normally displayed the cellular response at high gain, a.c., the other at low gain, d.c., to give an approximate measure of the cell resting potential.

In testing the effects of various pharmacological agents, a small volume of concentrated solution was added to the bath and allowed to diffuse to the final concentration desired, a process that was complete within 10–20 min. The drug was washed out by repeated flushing of the bath with Ringer's solution. Since the interaction between motoneurones proved to be quite labile and especially sensitive to temperature shocks or mechanical disturbances during changes of the bath fluid, care was taken to minimize these causes of trauma, and each drug was tried several times to establish the repeatability of results. Since it is possible that a drug penetrated to the site of one interaction much faster than to that of a second, the drug was normally left in the bath for at least 1 hr after a significant (or even complete) effect on one type of response had been observed.

The following drugs were used: D-tubocurarine chloride (Burroughs Wellcome), dihydro-β-erythroidine HBr, atropine chloride (Burroughs Wellcome), hexamethonium bromide (May and Baker), (2-hydroxyethyl) trimethyl ammonium chloride carbamate (Carbachol, L. Light and Co.), succinylcholine chloride (Burroughs Wellcome), prostigmine bromide (Roche), picrotoxin (British Drug Houses), β-(3,4-dihydroxyphenyl)-L-alanine (DOPA, L. Light and Co.), Pronethalol (ICI), procaine HCl (British Drug Houses), sodium pentobarbitone (Nembutal, Abbott Laboratories), and nicotine (British Drug Houses).

RESULTS

Ventral and dorsal root potentials and the effect of temperature

Both Katz & Miledi (1963) and Kubota & Brookhart (1963) reported being able to record the interaction between motoneurones as a depolarization electrotonically spreading out a ventral root following stimulation of an adjacent ventral root. This potential proved readily obtainable and quite convenient for the study of the motoneurone interaction, especially when examining the effects of drugs. In early experiments it was discovered that the interaction between motoneurones, as recorded on the ventral roots (hereafter called the VR-VRP), was differently affected by temperature than were the ventral root potentials resulting from stimulation of the dorsal roots (DR-VRP) or the dorsal root potentials following ventral root stimulation (VR-DRP) or the stimulation of an adjacent dorsal root (DR-DRP). As Fig. 1 shows, the amplitude of the VR-VRP

decreases as the temperature is increased, frequently disappearing altogether above 17–18° C, while the other three potentials are maximal at about 17–18° C. Thus, in order to obtain a large VR-VRP, the bath temperature was usually maintained at 5–10° C. In this temperature range, the root recorded responses were typically like those shown in Fig. 2. The orthodromic DR-VRP began 4–6 msec after stimulation of a dorsal root and reached a peak around 30–40 msec. The DR-DRP was somewhat later (10–12 msec latency, 40–60 msec to peak), while the VR-DRP began

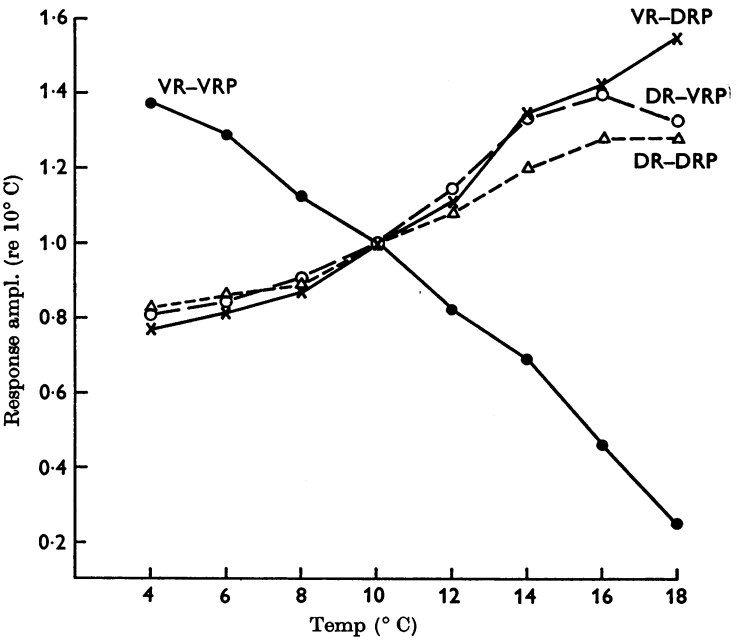

Fig. 1. The effect of temperature on the response amplitude of the four root potentials studied. Relative responses are plotted by taking the amplitude at 10° C ≡ 1. In this and following figures, the first letters designating a potential indicate the root stimulated, while the subsequent letters indicate the root from which the potential is recorded (e.g. DR-VRP: dorsal root stimulation, ventral root recording).

only after 20–25 msec latency, and reached a peak at 70–100 msec. The VR-VRP, on the other hand, came at an average latency of 4·5 msec and reached a peak at 7·5 msec (range 4–12 msec). This value is fully consistent with the 3·3 msec onset latency observed by Kubota & Brookhart (1963) in root recordings made at 16–18° C.

The root-recorded VR-VRP seldom exceeded 0·5–0·6 mV in amplitude, and more often was approximately 0·2 mV. It was occasionally accompanied by firing of a few fibres, which was evidence of the potency of the interaction arising from stimulation of an adjacent ventral root.

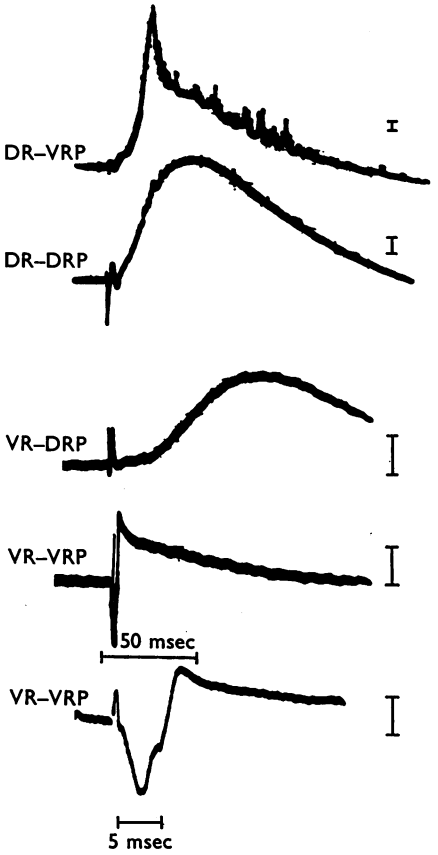

Fig. 2. Sample d.c. records of typical dorsal and ventral root responses to orthodromic (DR-VRP and DR-DRP) and antidromic (VR-VRP and VR-DRP) stimuli. The last trace shows the VR-VRP at higher sweep speed. Temp. 6° C. Amplitude calibration 0·2 mV.

Intracellular responses: latency, form, importance of recording site

Motoneurones were located for intracellular study by the techniques used by Katz & Miledi, i.e. the micro-electrode was inserted through the exposed lateral or medial surface and advanced slowly through areas of the cord where the antidromic invasion potential was largest. Although electrical changes in the electrode tip resulted in considerable uncertainty in the d.c. recording level, penetration of a motoneurone was usually marked by a sudden drop in the potential and the appearance of an antidromic spike. Of the several hundred cells so penetrated, 80–100 had sustained resting potentials of 40–60 mV and antidromic spikes of 40–80 mV, permitting satisfactory study of their behaviour. Of these, approxi-

mately 40 showed clear-cut depolarizations resulting from stimulation of an adjacent ventral root.

The probability of finding the VR-EPSP was clearly dependent on the part of the cord being examined. Where motoneurones from two different ventral roots were mixed (that is, near the junction of segments where the invasion potentials from both roots were large) nearly every cell showed a response to stimulation of the adjacent root. Where nearly all motoneurones belonged to the same ventral root, fewer cells were affected by stimulation of the adjacent root, although stimulation of their own, at intensities subthreshold for invasions of that cell, often gave rise to a typical VR-EPSP. Thus the proximity of antidromically invaded motoneurones is important to the occurrence and amplitude of the interaction.

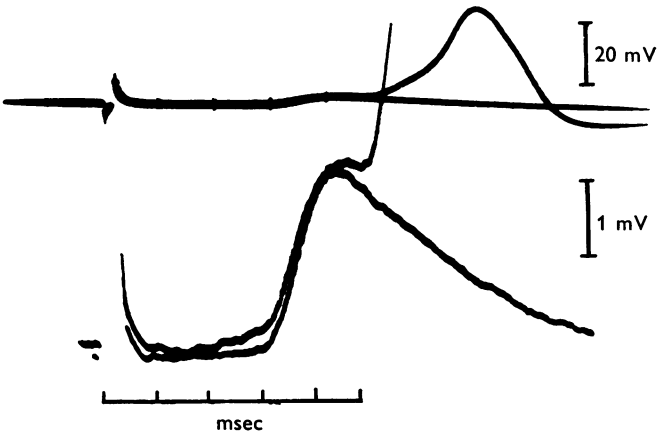

Fig. 3. A representative VR-EPSP, giving rise in one trace to an action potential, recorded in a VR 7 motoneurone following stimulation of VR 8. Temp. 10° C.

The intracellularly recorded interaction came at approximately the same latency as that observed on the ventral roots. With stimulation of an adjacent ventral root, the onset of the potential came at 4·5 msec (range 3–6 msec), reaching a peak at 7·8 msec (range 3·5–12); with stimulation of their own ventral root (subthreshold for the motoneurones involved) the average latency to onset was 4·1 msec (range 3–8), the latency to peak, 6·5 msec (range 4–10). The greater latency following stimulation of an adjacent root presumably reflects the greater conduction time involved. In certain cases, a motoneurone showed a VR-EPSP to stimulation of a ventral root two segments away, in which case the latency was normally 0·5–1·0 msec greater than that from the adjacent root. Root recorded responses were often recorded two segments away, an observation somewhat different from the findings of Kubota & Brookhart (1963).

The amplitude and time course of the VR-EPSPs were highly variable.

The usual maximum amplitude in different cells was 0·5–2 mV, but some reached 6–7 mV. The larger ones commonly gave rise to a spike (or in one case, two spikes) although the percentage that did so was well below that observed by Kubota and Brookhart (1963). Figure 3 shows a characteristic VR-EPSP, in one trace producing a spike. Many maximal VR-EPSPs were almost spike-like in shape, decaying nearly as fast as they rose; others were fast rising with very slow decay, as in the example shown in Fig. 13 and in fig. 3 of Katz & Miledi (1963). Since all these cells were undoubtedly damaged to a certain extent, it is difficult to say which represents the more natural time course.

Pharmacology

When conduction time to and from the presumed site of the VR-VRP is taken into account, it seems quite clear that there is a delay at 10–15° C of 1·2 msec or less, indicating the existence of only one synapse. If transmission were chemically mediated, as Kubota and Brookhart (1963) suggest, then the expected transmitter substance would be ACh. The fact that the antidromically evoked dorsal root depolarization (VR-DRP) is easily blocked by curare, ACh, atropine, hexamethonium, and dihydro-β-erythroidine (Eccles & Malcolm, 1946; Kiraly & Phillis, 1961) further suggests (though it does not prove) that at least one recurrent output from the motoneurone acts by releasing ACh. It is obviously of great importance to determine whether the same blocking agents affect the VR-VR interaction.

Cholinergic blocking agents. D-tubocurarine chloride, as one of the most potent nicotinic neuromuscular blocking agents, was the first tried. All experiments were consistent in showing that, at concentrations of 10^{-4} M or higher, the VR-DRP was severely reduced or abolished, while the VR-VRP was not affected by the drug (in accordance with unpublished observations of Katz & Miledi). Figure 4*a* illustrates the effect of 5×10^{-5} M tubocurarine in a representative experiment. The orthodromic DR-DRP was usually somewhat reduced, especially with high curare concentrations, while the DR-VRP was characteristically increased in amplitude and sometimes prolonged for as much as several seconds by repetitive firing. All the effects of curare were reversible, although recovery of the VR-DRP sometimes required 2 or 3 hr (as in Fig. 4*a*).

Dihydro-β-erythroidine (DHE) is a very potent neuromuscular blocking agent, and has been found to be one of the most effective antagonists of recurrent excitation of Renshaw cells in mammals (Curtis & Eccles, 1958). It is in addition the strongest blocker of the VR-DRP in frogs, totally abolishing the potential at 10^{-5} M concentration (Kiraly & Phillis, 1961). In the present experiments, 10^{-4} M DHE quickly eliminated the VR-DRP but had no influence on the VR-VRP, even after several hours exposure.

Similarly, the amplitude of the orthodromic potentials was unchanged. Figure 4*b* shows the effect of this drug on the VR-DRP and VR-VRP. The blockage of the VR-DRP was only slowly reversible and recovery was not even attempted in the experiment shown.

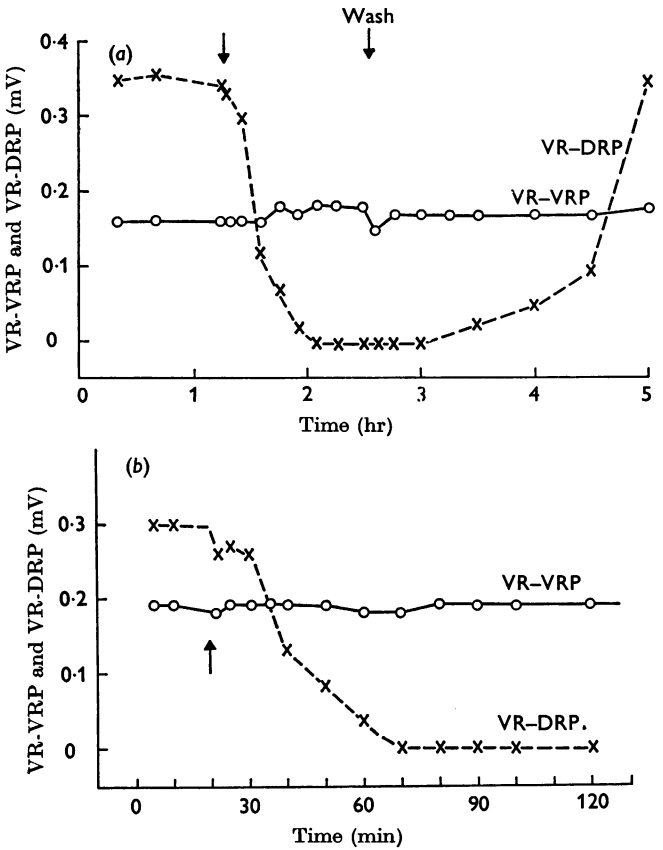

Fig. 4. Effects (*a*), of d-tubocurarine chloride, 5×10^{-5} M at first arrow, and (*b*) of dihydro-β-erythroidine HBr, 10^{-4} M at arrow, on the VR-VRP and VR-DRP.

Nicotine, at a concentration of 10^{-3} M, blocked the VR-DRP but had no apparent effect on the VR-VRP.

The possibility was considered that the transmitter might be ACh but the receptor muscarinic in nature, and therefore atropine and hexamethonium were tested as they are known to block such receptors (Eccles, R. M. & Libet, 1961). In the frog, both substances have been found to reduce or abolish the VR-DRP in low concentrations, atropine being somewhat the more effective (Kiraly & Phillis, 1961). The present results confirm these findings. Atropine 10^{-4} to 2×10^{-4} M always blocked the VR-DRP

within 30–60 min, while 10^{-3} M hexamethonium was necessary for an equivalent rate of reduction of the potential. Neither drug significantly affected the VR-VRP, as is clear in the experiment shown in Fig. 5, in which both drugs were used.

Figure 5 is included also to show the abrupt change in recorded VR-VRP amplitude that often accompanied introduction of a drug or subsequent washing. Since the potential did not decrease progressively with time or with increase in drug concentration, these abrupt changes presumably represent the effects of trauma or change in recording condition due to fluid movement or fluid level change, and are not an effect of the drug.

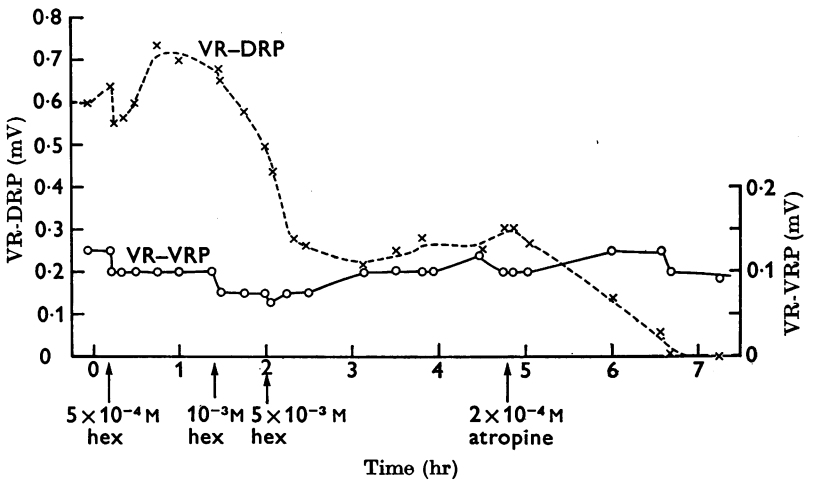

Fig. 5. The effects of hexamethonium (hex) (up to 5×10^{-3} M) and atropine (2×10^{-4} M) on the VR-DRP and VR-VRP. The abrupt changes in response amplitude accompanying introduction of a drug were frequently encountered artifacts (see text).

Cholinomimetic drugs. Cholinomimetic substances were also tried. Eccles (1947) found that ACh severely depressed the VR-DRP and carbachol was shown by Kiraly & Phillis (1961) to be an even more potent blocking agent. In the present experiments, carbachol and succinylcholine were tried and both, in concentrations of about 10^{-3} M, were found to reduce quickly or to abolish the VR-DRP while not affecting the VR-VRP. Figure 6 shows an experiment with succinylcholine followed by picrotoxin (see below).

The anti-cholinesterase prostigmine (5×10^{-4} M) had no clear or consistent effect on any of the root potentials.

Presynaptic blocking agents. Drugs that appear to block presynaptic inhibition were also tried. Picrotoxin, which was shown by Schmidt (1963) to reduce the DR-DRP amplitude, had the same effect in the present experiments. Its blocking effect on the VR-DRP was even stronger,

with complete and only slowly reversible suppression of the potential at concentrations as low as 10^{-5} M. The VR-VRP, however, was still unaffected even after long exposure at 10^{-4} M (see fig. 6).

DOPA (DL-β-(3,4-dihydroxyphenyl) alanine), a very effective blocking agent for the DR-DRP in mammals (Anden, Lundberg, Rosengren & Vyklicky, 1963), has a similar effect in frogs, reducing the DR-DRP and VR-DRP at concentrations of 5×10^{-5} M and higher. It had no effect on the VR-VRP, even after several hours in 5×10^{-5} M.

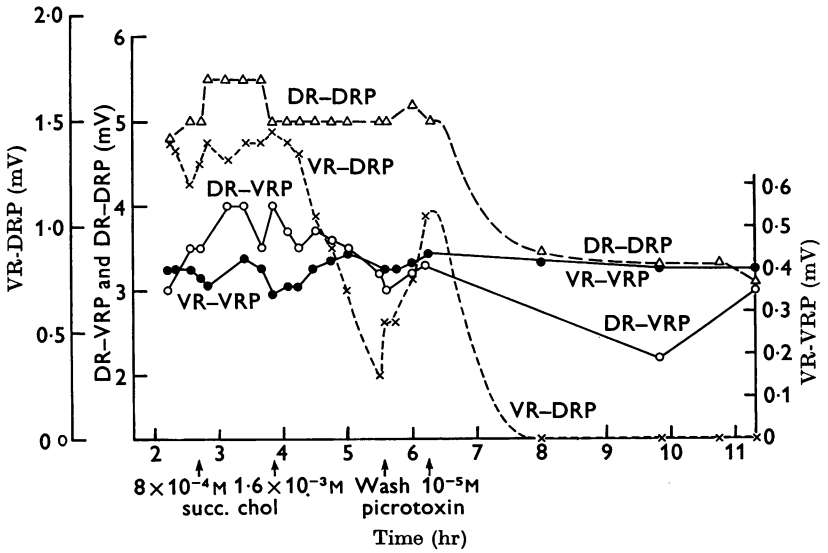

Fig. 6. The effects of succinylcholine and picrotoxin on all four root potentials. Note that 8×10^{-4} M succinylcholine was apparently ineffective in this experiment.

A single experiment with the β adrenergic blocking agent pronethalol suggests that this drug, too, is ineffective against the VR-VRP, although at approximately 5×10^{-5} M it easily blocked the VR-DRP and reduced the orthodromic DR-VRP and DR-DRP.

Non-specific anaesthetics. Since cholinergic and adrenergic blocking agents and cholinomimetic substances proved uniformly ineffective against the VR-VRP, some nonspecific anaesthetics were tried. Procaine, in addition to its local anaesthetic action, is capable of blocking neuromuscular transmission in very low concentrations (del Castillo & Katz, 1957). In the present experiments, however, it had no such specific action. At concentrations greater than 5×10^{-4} M it reduced all activity, but the VR-VRP less rapidly than any of the other root potentials (50 % in 5 hr). The VR-DRP was totally eliminated in this length of time, and recovered much more slowly than the other potentials after removal of the drug.

Nembutal, which is routinely employed as a C.N.S. anaesthetic and can be used preferentially to block interneurone activity in the frog spinal cord (Brookhart & Fadiga, 1960), was also tried. At 10^{-3} M it caused sharp reduction in the size of the DR-VRP, the DR-DRP and the VR-DRP, as had previously been observed in frogs by Eccles & Malcolm (1946) and in the toad spinal cord by Schmidt (1963). The VR-VRP, however, decreased in amplitude much more slowly, sometimes after a long period of no effect or slight increase in amplitude. Moreover, unlike the other potentials, which lengthened in duration as they fell in amplitude, the VR-VRP was considerably shortened. It was possibly an increase in synchrony that led to the transient increase in amplitude shown in the sample experiment of Fig. 7.

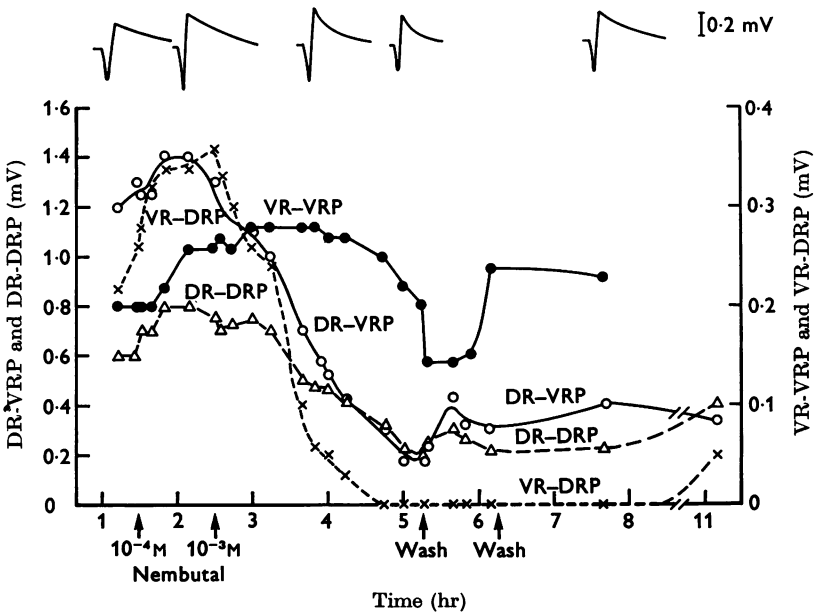

Fig. 7. Effects of Nembutal on the four root responses. Note the change in time course of the VR-VRP (upper traces).

Table 1 summarizes the effects of the various drugs tried on all four root potentials: VR-DRP, VR-VRP, DR-DRP and DR-VRP.

The effect of any given concentration of a drug varied greatly from one experiment to another, reflecting variability in the rate and extent of penetration. Moreover, since some drugs undoubtedly penetrated the cord more quickly and completely than others, it is unsafe to compare even the average effects of given concentrations. Nevertheless, since it seems most likely that the site of the VR-VRP was reached in essentially

the same concentrations that reached and blocked the sites of the VR-DRP or other interactions, it must be concluded that the VR-VRP does not behave like a cholinergic synapse, or, indeed, like any of a number of other chemical synapses. On the other hand, as Table 1 shows, there was usually no striking difference in the effects of a given drug on the VR-VRP and the DR-VRP. Conceivably a similar chemical mechanism of transmission is involved in both.

TABLE 1. Effects of various drugs on the four root potentials recorded in the frog spinal cord, at the concentration shown. Effects ranged from complete block of the response (− − −) to considerable (− −) or slight (−) suppression to no effect (0) or even enhancement (+) of the potential

Drug	Concentration	VR-DRP	VR-VRP	DR-DRP	DR-VRP
Curare	2×10^{-4} M	− − −	0	−	0 to +
DHE	2×10^{-4} M	− − −	0	0	0
Nicotine	10^{-3} M	− −	0	0	0 to +
Atropine	10^{-4} M	− − −	0	0	0
Hexamethonium	10^{-3} M	− − −	0	0	0
Succinylcholine	2×10^{-3} M	− − −	0	0	0
Carbachol	10^{-3} M	− −	0	0	0
Prostigmine	5×10^{-4} M	0 to +	0	0 to +	0
Picrotoxin	10^{-4} M	− − −	0	− −	0
DOPA	10^{-4} M	−	0	−	0
Pronethalol	5×10^{-5} M	− − −	0	−	−
Procaine	5×10^{-4} M	− − −	−	− −	− −
Nembutal	10^{-3} M	− −	−*	− −	− −

* Sometimes preceded by +. See text.

Effects of Mg and Ca. A further test *did* clearly establish a difference between the VR-VRP and the DR-VRP. This test was the effect of Mg^{2+} and Ca^{2+} on the potentials. A wide variety of synapses involving many different transmitters (e.g. the stellate ganglion of squid (Takeuchi & Takeuchi, 1962), the *Onchidium* ganglion (Kusano & Hagiwara, 1961) the vertebrate sympathetic ganglion (Hutter & Kostial, 1954), and the frog spinal cord (Katz & Miledi, 1963)) are quickly blocked by moderate concentrations of Mg^{2+}, presumably by means of a reduction in transmitter released, as is known to occur at the vertebrate neuromuscular junction (del Castillo & Engbaek, 1954). This block by Mg^{2+} is reversed by increased concentrations of Ca^{2+}. The effects of Mg^{2+} and Ca^{2+} on nerve and muscle excitability are the same, however; both raise the threshold.

Consequently the effects of Mg^{2+} and Ca^{2+} were tested on the VR-VRP in the frog spinal cord. Figure 8 shows the results in a typical experiment. As in all cases, when low Ca^{2+} Ringer's solution was used initially and a split cord preparation was employed to facilitate ion penetration, 3–5 mM-Mg^{2+} sharply reduced the DR-VRP and DR-DRP and virtually abolished the VR-DRP. The VR-VRP was only slowly reduced. In some experiments it was totally unaffected until the Mg^{2+} concentration reached 10 mM or more. Addition of 10 mM or even 20 mM Ca^{2+} had no clear

effect on any of the other three potentials in the presence of this Mg^{2+} concentration, but further reduced the VR-VRP. At higher concentrations (30–40 mM), Ca^{2+} continued to reduce the VR-VRP, but always began to reverse the effects of Mg^{2+} on the DR-VRP, the DR-DRP, and the VR-DRP. Washing with Ringer's solution would then bring about complete recovery of all the potentials. The fact that washing initiated recovery of

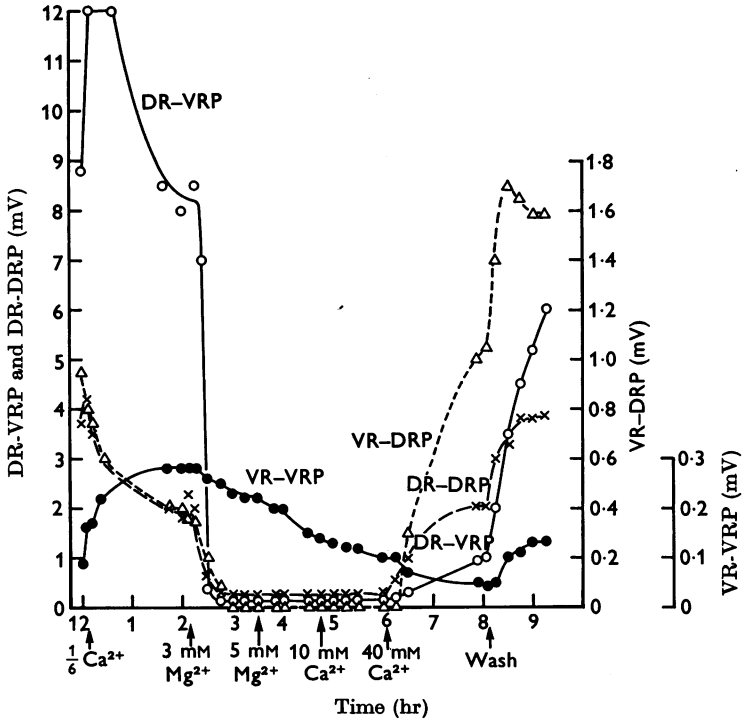

Fig. 8. Effect of Mg^{2+} and Ca^{2+} on the four root potentials. Low Ca^{2+} enhanced the VR-VRP while reducing the other potentials. 3 mM-Mg^{2+} essentially blocked all potentials except the VR-VRP, which slowly declined in amplitude, even with 5 mM-Mg^{2+}. 40 mM-Ca^{2+} dramatically counteracted the Mg^{2+} block in the DR-VRP, DR-DRP, and VR-DRP, but only further blocked the VR-VRP. All recovered on washing.

the VR-VRP essentially as rapidly as the other root potentials seems to rule out the possibility that the small and slow effect of Mg^{2+} and the absence of an antagonistic effect of added Ca^{2+} can be explained by their needing much more time to reach the site of the Mg^{2+} action.

Thus Mg^{2+} had less blocking effect on the VR-VRP than on the other root potentials, and the blocking effect that it did have was not reversed by Ca^{2+}, as in the case of the other potentials, but rather, if anything, increased. Indeed, the VR-VRP was clearly very susceptible to high Ca^{2+}

concentrations and improved on lowering the Ca^{2+}, as is apparent in Fig. 8. It was observed that this effect of low Ca^{2+} was counteracted by increasing the Mg^{2+} concentration, providing further evidence that Mg^{2+} does reach the site of the interaction. In Ca^{2+}-free solution, the VR-VRP was often the only potential that could be recorded, since it disappeared much more slowly than the others. Figure 9 shows the results of increasing the Ca^{2+} concentration above that in normal Ringer's solution.

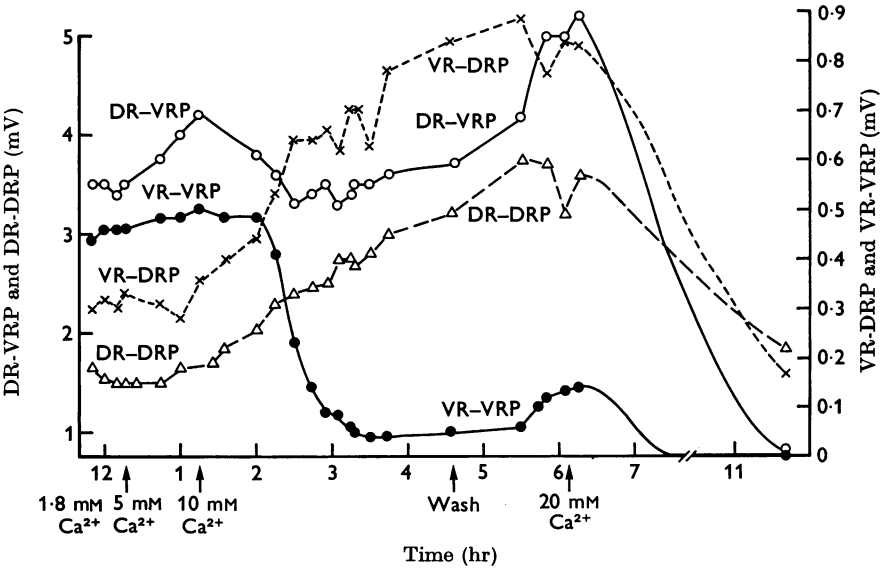

Fig. 9. Effects of different high Ca^{2+} concentrations on the four root potentials.

It is obvious that 10 mM Ca^{2+} very much reduced the VR-VRP, while greatly increasing the amplitude of the other three potentials. In the absence of excess Mg^{2+}, on the other hand, 20 mM Ca^{2+} was sufficient to reduce and eventually eliminate all of them, as can be seen in Fig. 9. The increase in response amplitude of the DR-DRP, DR-VRP, and VR-DRP can be interpreted as a result of increased transmitter release, while the reduction of the VR-VRP amplitude and of all the responses at higher Ca^{2+} concentration is probably due to failure of activation of some or all of the presynaptic terminals, as is known to occur with high Ca^{2+} at the neuromuscular junction (Miledi, 1961).

The evidence from experiments with Mg^{2+} and Ca^{2+} thus adds strong support to earlier indications that the VR-VRP does not result from a chemical form of synaptic transmission. The alternative, an electrical interaction, poses several questions of its own, e.g. where does the interaction take place? Which antidromically invaded cells are involved? What is the nature of the contact between cells? What is the explanation of the

relatively long 'synaptic delay'? And what is the functional significance of the interaction?

Evidence concerning the site of the interaction

The presynaptic cells involved. The identity of the presynaptic cells in this interaction requires consideration. In addition to motoneurone axons, frog ventral roots contain the axons of sympathetic fibres and perhaps even occasional sensory fibres, anatomical evidence for which has been reported by Dunn (1914). Thus it is not immediately obvious that the important result of antidromic stimulation is the activation of motoneurone axons. Nevertheless, the possibility that sympathetic fibres form the presynaptic elements in the interaction has been eliminated by establishing that the VR-VRP is undiminished if stimulation is done on the sciatic nerve, well peripheral to the point at which sympathetic fibres exit. Moreover, both the VR-VRPs and the VR-EPSPs are finely graded in amplitude, a condition probably requiring the presynaptic influence of large numbers of cells. This argues against the postulate that the small number of aberrant sensory fibres that might be present in the ventral root could be responsible for these potentials. Additional evidence for the involvement of antidromically activated motoneurones in this interaction is seen in the clear correlation between the size of the VR-VRP and the amount of activity in the ventral horn (see below).

Thus antidromic invasion of motoneurones is apparently responsible both for the VR-VRP and the VR-DRP. Further specificity within motoneurone populations has not been explored. There is one observation, however, that indicates either that the antidromically fired cells involved in the VR-VRP and the VR-DRP belong to different populations, or that the same motoneurones are involved, but a larger percentage must be activated to produce a detectable VR-VRP. This is the finding that the threshold for the VR-DRP (the lowest intensity eliciting a detectable response) was uniformly lower than that for VR-VRP. Figure 10 shows a typical example. The average ratio of thresholds was approximately two-fold, even when the VR-VRP was recorded in a separated bundle of the stimulated root. The VR-DRP first appeared at approximately the intensity at which a nerve action potential could first be detected in the stimulated root. It often reached a maximum before the nerve action potential became maximal, at approximately twice the threshold stimulus intensity. The VR-VRP, on the other hand, appeared only as the nerve action potential approached 80–100 % of full amplitude, and continued to grow with a two to five times stimulus increase beyond this level, when the only changes in the root action potential were a slight increase in synchrony and decrease in latency.

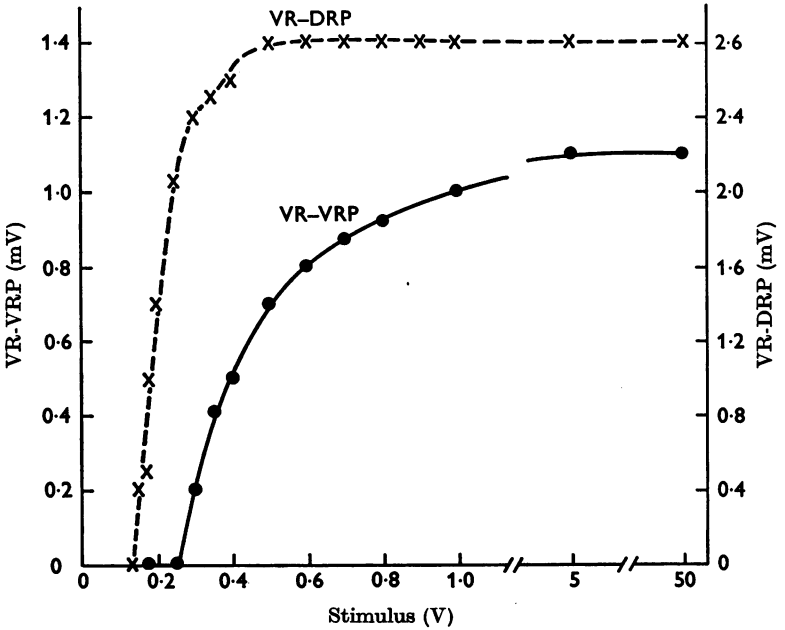

Fig. 10. Typical pattern of response amplitude of the VR-DRP and VR-VRP as a function of stimulus intensity. VR 8 was stimulated, recordings made on VR 9 and DR 8. The nerve action potential of the stimulated ventral root grew at approximately the same rate as the VR-DRP, reaching its maximum amplitude at about 0·6 V stimulus strength. With stronger stimuli, the VR nerve action potential shortened slightly in latency. (See text for further discussion of this relation).

Orthodromic depolarization and the importance of antidromic soma invasion. As was implied above, these differences might be explained by postulating that different fibres, having different thresholds, are involved in the two interactions; perhaps the axons going to 'fast' and 'slow' muscle fibres. Other evidence, however, indicates that separate populations need not be involved, and that there is in fact very little difference in threshold for the two interactions. Most important is the observation, first made by Katz & Miledi (unpublished) that the VR-VRP (or VR-EPSP) can be greatly facilitated by an orthodromic volley. This phenomenon has been examined here in more detail. In many cases, with both intracellular and root recording, a preceding orthodromic stimulus made possible a large VR-EPSP or root potential where none had been detectable to the antidromic stimulus alone. In all cases, following a dorsal root volley, the VR-VRP threshold was much reduced and the interaction appeared at nearly the same stimulus level at which an antidromic nerve action potential was

first detected. Since this facilitation occurred even when the stimulus was applied to the sciatic nerve several centimetres from the cord surface, where no DR-VRP could be recorded, it cannot be argued that the lowering of threshold of the VR-VRP was a result of electrotonic spread of depolarization from the roots lowering the threshold of a population of high-threshold axons.

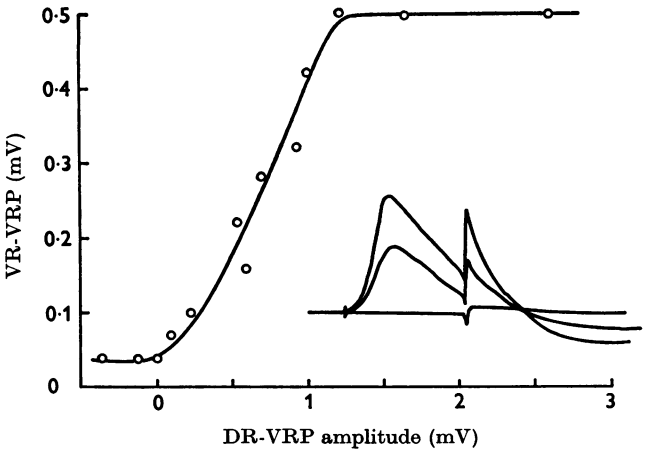

Fig. 11. Facilitation of the VR-VRP by orthodromic depolarization (DR-VRP). Inset shows sample records. A VR 9 stimulus of constant intensity was presented 65 msec following a DR 8 stimulus of two different intensities. Recording was from VR 8. The points preceding the DR-VRP amplitude scale indicate the response to a VR 9 stimulus alone.

Further study established that over a wide range, the larger the orthodromic depolarization (DR-VRP), the larger was the VR-VRP occurring at that time. Figure 11 shows this relation in one preparation in which the stimulus interval was kept constant (65 msec). As would be expected from this relation, the time course of facilitation reflects that of the DR-VRP, reaching a maximum, on the average, 20–30 msec after the dorsal root stimulus and declining as the DR-VRP declined, as may be seen in the example of Fig. 12. This was observed in all but the exceptional cases in which a large orthodromic stimulus resulted in prolonged asynchronous reflex firing of motoneurones, when there could be a maximally facilitated and highly synchronous VR-VRP several seconds after the DR-VRP.

The degree of facilitation could be very great (200 % or more) but the facilitated VR-VRP usually reached a maximum at about 0·5–0·6 mV, and a larger DR-VRP caused no further facilitation. Some preparations were found in which the VR-VRP was initially as large as this, and a

DR-VRP effected little or no facilitation. Intracellularly recorded VR-EPSPs were often facilitated much more dramatically, often by 500% or more to maximum amplitudes of 5–7 mV, when the facilitated potential looked almost spike-like (but still graded) as in the motoneurone of Fig. 13. Very commonly the facilitated VR-EPSPs gave rise to an all-or-none firing of the cell.

In the intracellular recording, also, the degree of facilitation closely followed the DR-VRP in time course, and was clearly unrelated to the size of the EPSP (or even IPSP) of the motoneurone in question. This indicates that facilitation depends not on the state of the post-synaptic membrane, but on that of the presynaptic motoneurones. Specifically, the magnitude of the VR-VR interaction appears to depend on the extent of invasion of the whole antidromically stimulated population.

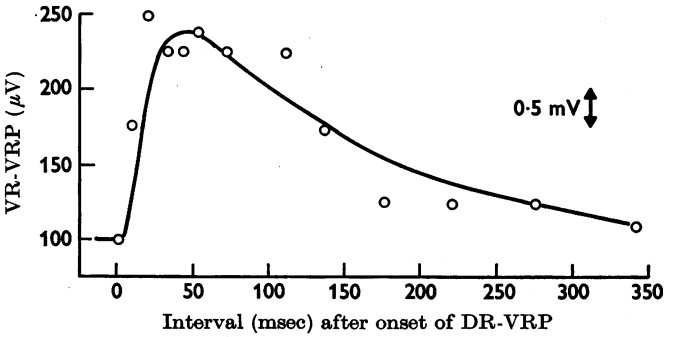

Fig. 12. Time course and magnitude of facilitation of VR-VRP following a dorsal root volley (points), compared with the time course of the DR-VRP (curve, with 0·5 mV amplitude calibration on right). VR 9 and DR 8 were stimulated, recording from VR 8.

Renshaw (1942) first demonstrated the now familiar phenomenon that orthodromic depolarization leads to enhanced antidromic invasion of the motoneurone pool. In the present experiments intracellular recordings from frog motoneurones commonly showed that a subthreshold EPSP would permit an antidromically evoked SD spike where previously the impulse had been blocked at the initial segment, and extracellularly recorded field potentials in a motoneurone pool were greatly increased when antidromic activation followed orthodromic stimulation, even though the antidromic stimulus alone was maximal, and presumably all of the axons were activated.

In several experiments d.c. current was passed between an electrode on the distal (stimulated) end of a ventral root and the bath. When the polarity and current were such that the point of entry of the root into the bath, near the surface of the cord, was slightly depolarized, the interaction

was enhanced. More intense depolarization blocked the nerve action potential and the VR-VRP. In this case, limited depolarization near the somas presumably increased the ease of invasion and so enhanced the interaction. More intense depolarization could have blocked conduction, but the block could also have resulted from the simultaneous hyperpolarization of the ventral root fibres near the polarizing electrode located distally on the root, near the point of stimulation. Even slight hyperpolarization at the point of stimulation would be expected to have decreased

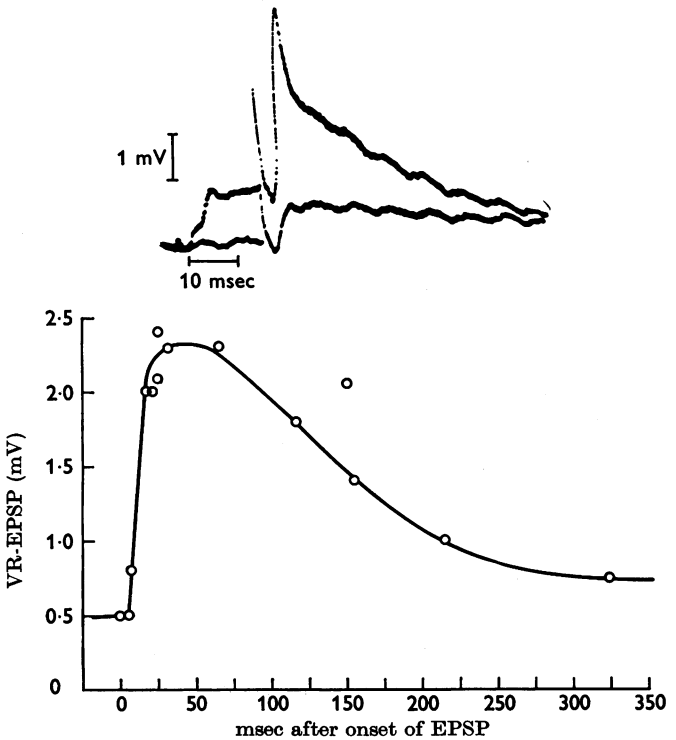

Fig. 13. Facilitation of a VR9-EPSP in a VR8 motoneurone following stimulation of DR9. Record (top) shows VR-EPSP alone (lower trace) and on a small orthodromic EPSP. Even the spike-like initial portion of the facilitated VR-EPSP was graded in amplitude, as could be shown by reducing either the VR or DR stimulus. The orthodromic EPSP was of approximately constant amplitude from 5 to 50 msec and had declined to resting level within 80 msec after the DR stimulus, quite unlike the time course of facilitation of the VR-EPSP. In other cells, similar facilitation was observed even during large IPSPs.

the likelihood of firing of the highest-threshold fibres, so the observed enhancement of the VR-VRP under this condition provides additional evidence that a separate high-threshold population is not predominantly responsible. Depolarization near the distal stimulated end of a ventral

root, with hyperpolarization near its point of entry into the cord, simply reduced or blocked the VR-VR interaction.

In another series of experiments, similar to ones described by Katz & Miledi (1963), chloride-free Ringer's solution (78 mM-$NaSO_4$, 1·6 mM-K_2SO_4, 2 mM-$NaHCO_3$ saturated with $CaSO_4$) was used. Under these conditions, Katz & Miledi found that an orthodromic volley caused repetitive firing of motoneurones followed by prolonged depolarization (40 mV or more for several seconds) during which few if any motoneurone somas could be invaded antidromically. In the present experiments it was found that the VR-VRP was much reduced or eliminated during this time, and reappeared only as the motoneurones became partially repolarized.

Facilitation therefore appears to be accomplished by overcoming an invasion block, probably between the first node and the initial segment of the motoneurones, or between the initial segment and the soma. The fact that the VR-DRP is normally so prominent at low stimulus intensities and without dorsal root stimulation suggests that a common site of the block is between any axon collaterals and the soma, and also suggests that the VR-VRP is not mediated through axon collaterals.

It is not clear why failure of invasion was so common in these preparations, or whether this condition exists in the intact cord. It seems quite likely that this represents a healthy condition at this temperature (6–10° C), since widespread partial depolarization of spinal neurones due to damage or anoxia would tend to increased antidromic invasion. It is also puzzling why in most preparations the VR-VRP continued to grow in amplitude with increasing stimulus intensity after the nerve action potential on the stimulated root had reached a maximum, and was only changing slightly in latency and apparent synchrony of response. It was often observed, however, that the focal potential recorded in the motoneurone pool increased significantly under these conditions, so perhaps an increase in synchrony of activation of motoneurone axons can somehow enhance soma invasion.

Extracellular potentials in the cord

Although interpretation of extracellular potentials evoked in large populations of cells is difficult, careful comparison of the potentials recorded on the antidromically stimulated roots, on adjacent ventral roots, extracellularly within the motoneurone pool, and intracellularly in motoneurones suggests that invasion of the dendritic trees of the stimulated cells may be critical to the interaction. Figure 14 shows examples of typical records taken in these different locations. Records from the antidromically stimulated root (*a*) show an initial triphasic spike resulting from propagation of the action potential toward the cord, followed by a slow potential characteristically having an inflexion on the rising phase. Most of this slower potential disappears when the root is crushed or cut near its point of entry into the cord. Since it is not noticeably changed in form or amplitude by a preceding dorsal root volley, and persists even

when soma invasion is poor, its seems probable that this slower potential represents predominantly the electrotonic spread out from the root of the initial segment (IS) potential of the invaded motoneurones.

Extracellular recordings in the pool of invaded motoneurones show finely graded negative potentials decreasing in latency and increasing in amplitude from the cord surface (or the medial edge of a hemisected cord) to a point in the middle of the ventral horn. Sample records taken at the surface of the cord and in the middle of the pool (about 0·5 mm deep) are superimposed in Fig. 14*b*. The maximal response normally comes about 0·5 msec after the peak of depolarization recorded by an electrode on the stimulated root (trace *a*). This maximal negative potential, which usually

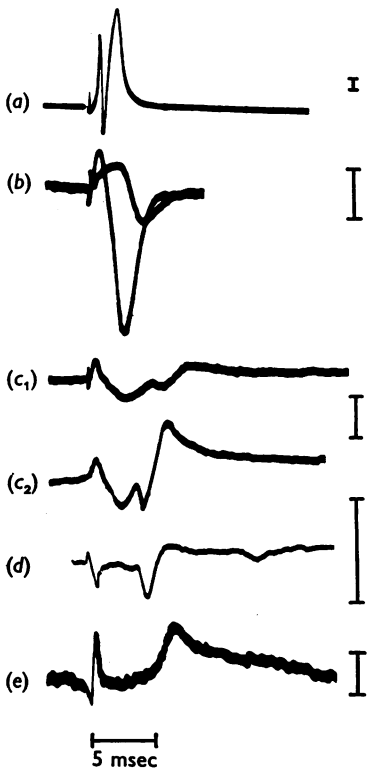

Fig. 14. Sample records of responses to antidromic stimulation made (*a*) on the stimulated ventral root; (*b*) extracellularly in the motoneurone pool of the stimulated ventral root, showing the focal potential near the surface (smaller) and at the centre of the pool (larger); (c_1) on an adjacent ventral root and (c_2) from the same location following dorsal root stimulation; (*d*) on a different ventral root, located caudal to the stimulated root; and (*e*) intracellularly in a motoneurone of an adjacent root. (*a*) and (*b*) are from the same preparation, (*c*), (*d*), and (*e*) from others. All were made at 6–8° C. Amplitude calibration, 0·5 mV in all cases.

can be enhanced and shortened in latency by as much as 0·5 msec by prior orthodromic stimulation, is probably recorded just outside the somas of the invaded cells, while the smaller, later potentials, which are even more enhanced by orthodromic stimulation and shortened in latency by as much as 1–1·5 msec, reflect the activity of motoneurones near the edge of the pool and the depolarization—passive or active—of the dendrites at progressively greater distances from the somas. The initial positive phase in the extracellular records presumably reflects the flow of current from the recording site to the active area deeper in the motoneurone pool. This positive component is particularly prominent in surface recordings and becomes shorter in duration with penetration centrally.

Recordings made between a point on an adjacent ventral root and the bath often pick up significant signs of these field potentials, possibly because of spread of the field through the root. These field potential components are frequently larger than the VR-VRP immediately following them, as in trace c_1 of Fig. 14 (see also Fig. 2). In virtually all such records, there are two distinguishable peaks of negativity, the first of which approximately coincides with the maximal (and shortest latency) extracellularly recorded potential in the motoneurone pool (*b*), while the later comes at a time closer to that of the maximum negativity recorded with a micro-electrode far lateral to the centre of the pool of invaded motoneurones or near the cord surface, where most negativity probably represents dendritic depolarization. The second root-recorded peak of negativity is clearly of great interest, since it immediately precedes the VR-VRP and is always much more dramatically facilitated by an orthodromic volley than is the earlier peak. Figure 14c_2 shows the effect of orthodromic excitation on the response of trace c_1. In many cases the earlier field potential peak was increased only 5–10 % by orthodromic stimulation, while the later peak was enlarged several times in magnitude and shortened by 1 msec or more in latency, and the VR-VRP, which had not been detectable to an antidromic stimulus alone, appeared and reached maximum amplitude. The later peak was not blocked by curare or other cholinergic blocking agents, and so presumably cannot represent the activity of cells post-synaptic to axon collaterals of antidromically activated fibres. Moreover, it was absent when antidromic invasion of somas was poor.

As Fig. 14*d* shows, it was possible to obtain preparations in which only the later of the two negative peaks was recorded on an adjacent root, apparently because of spatial separation of the centres of activity producing the earlier and later field potentials.

When only the later peak was obtained, the recording electrode was invariably on a root caudal to that being stimulated or on one or two segments rostral. This is consistent with the

observation that, although an influence critical to the VR-VRP obviously does pass caudally, the vast majority of the antidromically activated motoneurones are located rostral to the base of the stimulated root. Thus there is a large population of discharging axons and motoneurone somas near the base of the rostrally located root, where an early field potential is usually prominent, and probably mostly dendritic processes near the caudally located root, where usually only the later field potential is prominent. On the other hand, it should be noted that although the late field potential occurs at approximately the same time as maximal dendritic activity, it is normally much more sharply synchronized than the extracellular potential recorded near the surface of the cord or near the caudal adjacent root.

Trace *e* of Fig. 14 shows a typical intracellular VR-EPSP occurring at essentially the same time as the VR-VRPs. Field potentials were often recorded intracellularly as well as on the roots, and would invariably persist at approximately the same amplitude when the electrode was withdrawn to a point just outside the cell.

In conclusion, despite uncertainties introduced by the large number of active elements involved, their unknown distribution, and the unknown paths of different length from source of activity to recording site in the different cases, these records, nevertheless, show distinct signs of activity that seem best interpretable in the following way. Antidromic invasion of motoneurones is in most cases incomplete. Hence an orthodromic stimulus, which partly depolarizes the somas and dendrites, greatly facilitates invasion, permitting greater penetration of the dendritic trees, perhaps through propagated impulses, and increasing the amplitude of the extracellular focal potential recorded in the motoneurone pool. Facilitation of dendritic invasion by orthodromic stimulation has recently been inferred from work with single antidromically stimulated cat motoneurones by Nelson & Frank (1964). The extent of dendritic depolarization of the whole population of invaded motoneurones would then determine the size of the VR-EPSP recorded in adjacent motoneurones or of the VR-VRP on an adjacent root.

Interaction between successive VR-VRPs

Two stimuli to the same ventral root. The effect of one VR-VRP on subsequent ones also provides useful information about the interaction. When pairs of stimuli (each about 0·2 msec in duration) are given at different intervals to the same ventral root, the effect of the first VR-VRP on the second is as shown in Fig. 15. When maximal stimuli are used, there is total occlusion of the second response for 8–10 msec, followed by fast recovery to normal size in 12–25 msec and then facilitation of the second by as much as 50 %, lasting 100–200 msec. In the example shown, the second VR-VRP reached a maximum facilitated value at approximately 20 msec interval, but there was normally a peak of facilitation at about 40 msec. If antidromic invasion was extremely good, or if the

VR-VRP was already facilitated by an orthodromic stimulus, the response to a ventral root stimulus sometimes could not be facilitated by an earlier VR-VRP. In these cases only occlusion was observed, with rapid

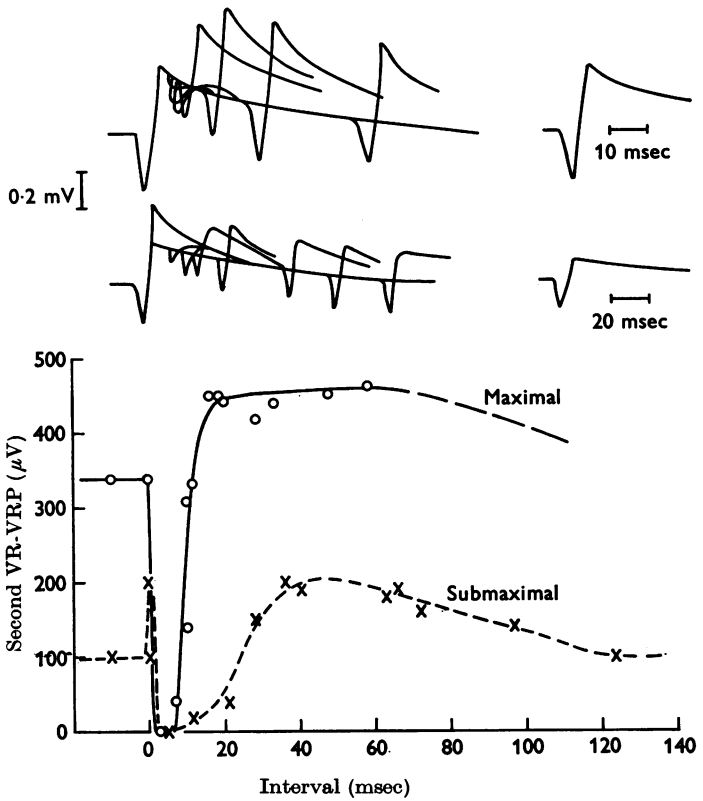

Fig. 15. Top: Traces showing the interaction between VR-VRPs resulting from pairs of stimuli to the same adjacent root, when both stimuli were maximal and both were submaximal. The traces to the right show responses to the second stimulus alone. Bottom: Amplitude of response to the second stimulus as a function of interval after the first, showing occlusion and facilitation by the earlier VR-VRP.

recovery to 100 % response amplitude. This was presumably the case in the intracellular records obtained by Kubota & Brookhart (1963) who observed no such facilitation.

Submaximal responses were not only initially additive but mutually facilitatory. With more than about 1 msec of separation, however, the second was severely or totally occluded. Recovery normally took about 20 msec and was followed by somewhat more facilitation than was seen with maximal stimuli, but reaching a peak at about the same interval.

Final return to normal came after approximately 100–150 msec. (At the same time, the VR-DRP was totally inhibited for 20–50 msec and only 50–60 % recovered in 150 msec. There was no sign of facilitation.)

The facilitation observed in these experiments probably arises in the same way as the facilitation after a dorsal root stimulus, i.e. by short-term residual depolarization of soma and dendrites, allowing more complete invasion by a subsequent antidromic spike. The occlusion can easily be explained by refractoriness of a part of the invaded motoneurones necessary for the interaction.

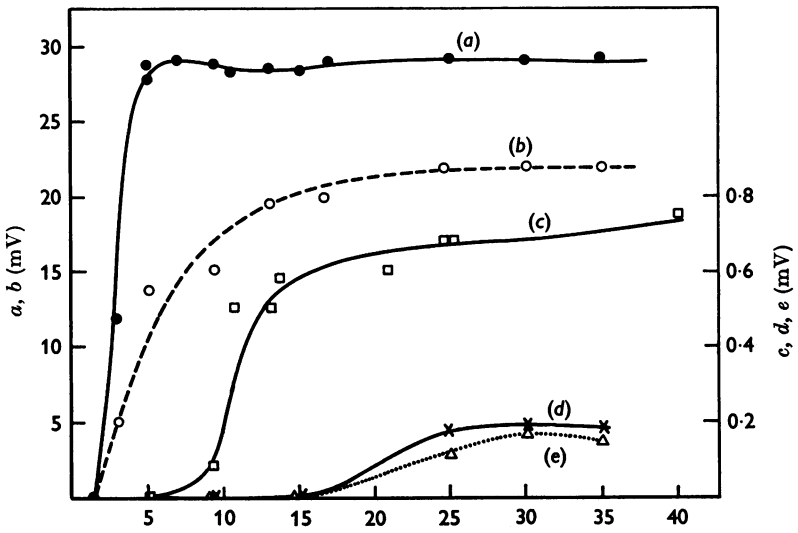

Fig. 16. Recovery curves of several responses to ventral root stimulation: (*a*) action potential on stimulated ventral root; (*b*) electrotonic spread of motoneurone (probably initial segment) depolarization on the stimulated VR; (*c*) extracellular focal potential in the motoneurone pool; (*d*) VR-VRP; and (*e*) later of two negative field potential peaks on adjacent VR (see text).

It is perhaps significant that, as Fig. 16 shows, the ventral root axon action potential typically recovers completely within about 5 msec at these temperatures, while the electrotonically spreading (probably IS) depolarization recorded on the antidromically stimulated root requires 10–20 msec. The negative focal potential recorded extracellularly in the middle of the pool of invaded motoneurones shows recovery beginning at about 5 msec, but even this potential is at least 50 % recovered at 10–15 msec, when the VR-VRP first reappears on an adjacent root. The later of the two peaks of negativity recorded on the adjacent root, however, always recovers with approximately the same time course as the VR-VRP, and sometimes

even exhibits marked facilitation at intervals greater than 25–30 msec. A refractoriness of motoneurone dendrites long after recovery of the block between axon and soma was found by Lloyd (1951), who attributed this refractoriness to the flow of after-currents.

Effect of tetanus. The effect of tetanic stimulation of a ventral root also suggests the existence of a separate dendritic refractoriness. During a 2 min tetanus (20/sec or higher stimulus rate, 5–10° C), the successive VR-VRPs sum to reach a peak at about 100–200 msec, then quickly fall to a slowly declining plateau at 1 to 3 times the size of the initial maximal response to a single stimulus. Immediately after such a tetanus single maximal stimuli elicit responses very much reduced in amplitude, if detectable at all, and recovery of the original single response amplitude sometimes requires 5–10 min. There has never been any sign of post-tetanic potentiation. The VR-VRP resulting from stimulation of a different ventral root is unaffected by the tetanus, so the fatigue or adaptation observed must be arising presynaptically. Control recordings show that the antidromic action potential on the ventral root axons is fully recovered within 20–30 sec, and the potential spreading electrotonically out from the initial segments recovers in approximately the same time. The extracellular focal potentials in the centre of the motoneurone pool recovered more slowly, but reached full amplitude well before the VR-VRP. This suggests that at least part of the long-term refractoriness probably arises in the motoneurone dendrites.

Two stimuli to different ventral roots. The interaction between VR-VRPs resulting from stimulation of two different adjacent ventral roots was surprisingly similar to the case above in which the same root was stimulated twice. Again, there was a period of occlusion, albeit somewhat shorter in duration (average 10–15 msec) and relatively less pronounced (never total), followed by facilitation that was maximal somewhat earlier (20–30 msec) and was usually considerably greater in magnitude than when the same root was doubly stimulated. Figure 17, showing the interaction between VR-VRPs from VR 7 and VR 9 as recorded on VR 8, was typical of these experiments.

In general, the extent of occlusion and facilitation was dependent on the size of the first response. The larger this response was, the deeper was the following occlusion and the greater the subsequent facilitatory effect on a second VR-VRP. Nevertheless, there was some occlusion even when the first response was near threshold. Facilitation was greatest when the second stimulus was near threshold, and became progressively less as the second response approached its maximal size. As with the doubly stimulated single root, two stimuli given approximately simultaneously to separate roots were mutually facilitatory. An example of this relation

is shown in Fig. 18. In this experiment, if a constant submaximal stimulus was presented to VR 8 and, simultaneously, a stimulus of increasing intensity was presented to VR 9, the combined response measured on VR 7 nearly doubled even before the VR 9 stimulus alone resulted in a detectable response.

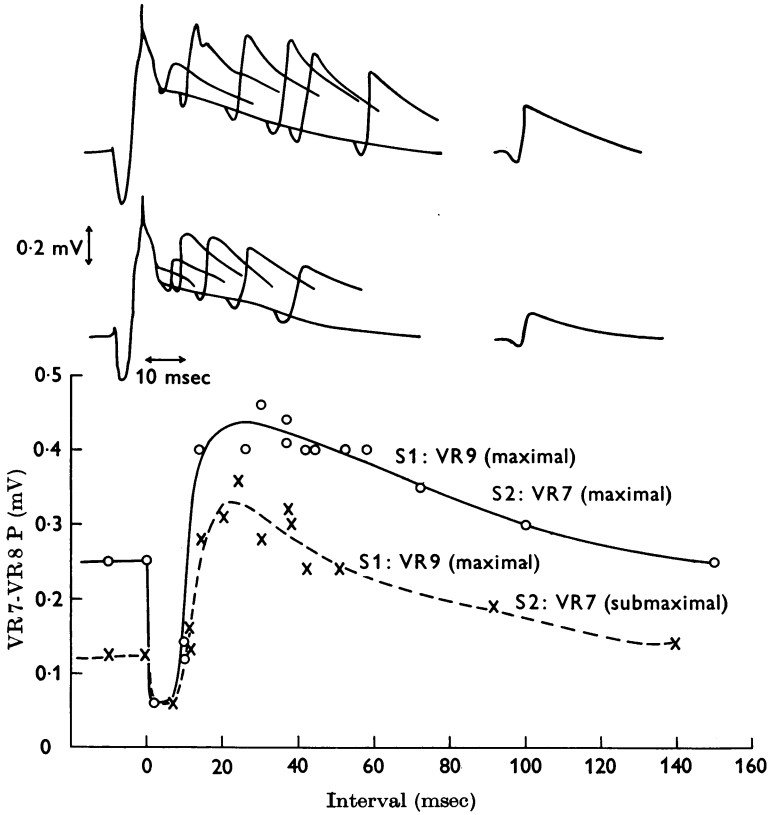

Fig. 17. Occlusion and facilitation of a VR 7-VR 8 P as a function of interval after a VR 9-VR 8 P when both stimuli were maximal and when the second stimulus was submaximal. Traces of actual records are shown at top, with response to VR 7 stimulus alone shown to right.

It is noteworthy that essentially the same pattern of occlusion and facilitation of one VR-VRP by another was seen whether the stimulated roots were to either side of the recording root, as in Fig. 17 or both on the same side.

The findings from root recordings were verified by a small number of intracellular recordings. Very few cells had large VR-EPSPs from two different ventral roots, but those that did consistently showed that one

such VR-EPSP inhibited a second for 10–20 msec, followed by full recovery and sometimes as much as 100–200 % facilitation.

The facilitation of one VR-VRP (or EPSP) by previous stimulation of another ventral root can be explained by the following, clearly tentative hypothesis: the dendrites from motoneurones of all three ventral roots (one used for recording, two for stimulation) are overlapping; hence the in-

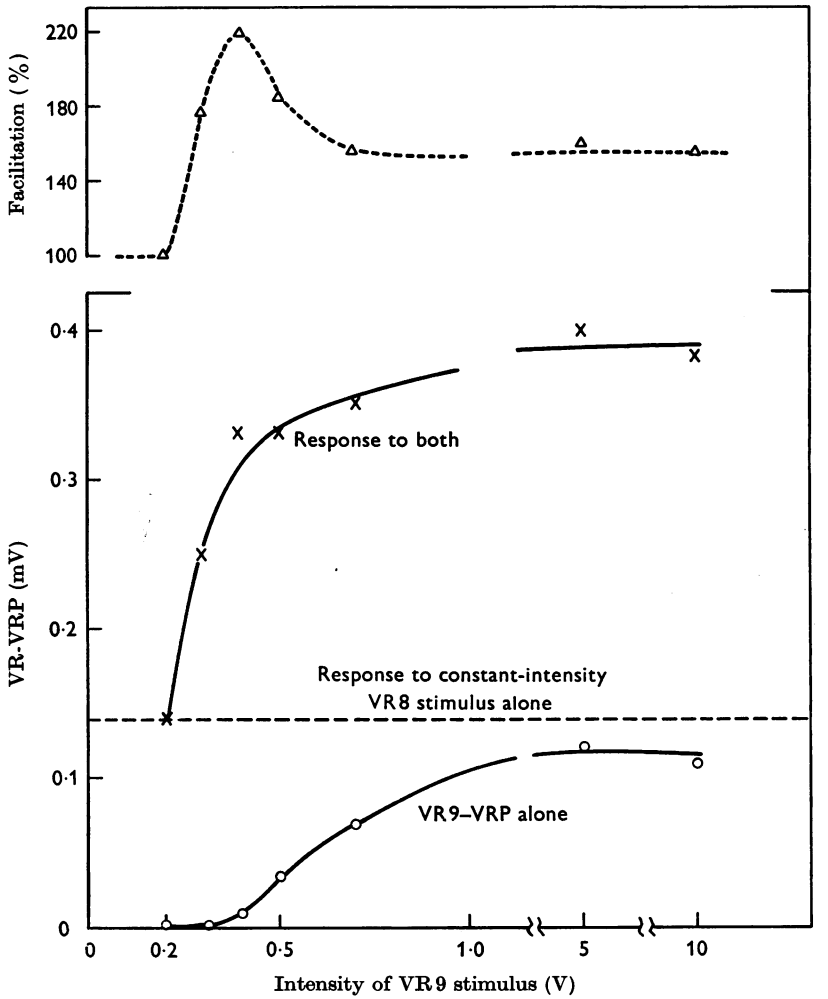

Fig. 18. Curves showing the mutual facilitation of VR-VRPs recorded on VR7 from stimuli presented simultaneously to different ventral roots. The stimulus to VR8 was constant, while that to VR9 was increased in intensity. Note that facilitation was greatest when the smaller response was near threshold. Essentially the same curves were obtained when one VR-VRP facilitated another arriving 20 to 100 msec later.

vasion of part of the pool by stimulation of one root causes the depolarization of large numbers of adjacent dendrites, perhaps some of them only second or third hand. On one adjacent root this is recorded as a VR-VRP. On the other, the same depolarizing event briefly reduces the threshold for antidromic invasion of somas and dendrites, facilitating invasion and causing an enhancement of the second VR-VRP. This facilitation can be considered directly comparable to that following orthodromic depolarization or the antecedent firing of the same ventral root.

Preceding this facilitation, however, is a period of occlusion that must also apparently be explained by dendritic depolarization. This occlusion of the second VR-VRP when two ventral roots are stimulated possibly arises in two ways. Some of it may be presynaptic, the result of intense depolarization and refractoriness of the dendrites of the motoneurones being antidromically excited by the second stimulus. If this depolarization is great enough, it might reduce invasion in the same way that invasion fails in doubly stimulated motoneurones (see Fig. 16). This type of electrical 'presynaptic inhibition' might explain all the occlusion observed in these experiments. A significant part of the VR-VR interaction, however, especially in the case in which roots are stimulated on either side of the recording site, may not involve a final common presynaptic pathway. In this case, some of the occlusion may be post-synaptic, and could result from the same refractoriness, during intense depolarization, that reduces or blocks the *antidromic* invasion of the second of two impulses into the dendrites. Perhaps the post-synaptic depolarization of dendrites is so great at certain points that activity at other sites on the dendrites, at least more distal from the soma than the first, is ineffective.

Additional evidence that occlusion can take place post-synaptically is found in the effect of an action potential on the VR-EPSP in a given motoneurone. As Fig. 19*a* shows, a VR-EPSP that was very prominent during orthodromic stimulation could be completely blocked in the occasional presentations when the same orthodromic stimulus resulted in an action potential. The period of occlusion lasts 20–50 msec (Fig. 19*b*). Similarly, when a given motoneurone was fired antidromically, the spike sometimes blocked or depressed a VR-EPSP for up to 50 msec, although in other cases there was no occlusion, perhaps because of insufficient invasion of dendrites. If the antidromic impulse did not invade the soma, there was no apparent occlusion.

As Kubota & Brookhart (1963) found, the VR-EPSP had no effect on the amplitude of an invading antidromic spike. It did facilitate invasion, however, just as in Kubota & Brookhart's experiments it increased excitability to intracellularly passed currents. In both cases the change in excitability follows the time course of the VR-EPSP and reflects the

size of the VR-EPSP. VR-EPSPs had no apparent effect on an orthodromic EPSP, although they often summed to produce a spike.

The VR-DRP, on the other hand, has a very potent inhibitory effect on the DR-VRP. This feedback interaction works by depolarization of dorsal root afferents in a manner presumably analogous to the presynaptic inhibition of the mammalian spinal cord. The time course of inhibition closely follows that of the VR–DRP (not that of the VR-VRP). Figure 20*a* shows such an inhibitory curve. Interestingly, this inhibitory effect appears to act selectively on the polysynaptic components of the DR-VRP, leaving the monosynaptic potential intact even when it was timed to occur at the height of the VR-DRP, as in the example of Fig. 20*b*. When the VR-DRP is blocked by tubocurarine or an equivalent drug, there is no inhibition of the orthodromic pathway.

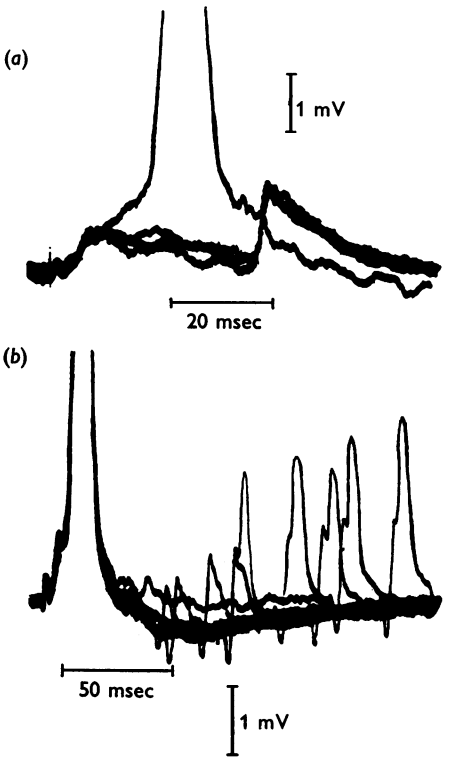

Fig. 19. *a*. Superimposed records from a VR 8 motoneurone showing the blocking effect of an orthodromically elicited spike on the VR 9-EPSP. All records were made with the same DR stimulus intensity, but the orthodromic EPSP led to a spike in only one of the presentations shown. *b*. Record from a VR 8 motoneurone showing the time course of occlusion of a VR 8-EPSP by an orthodromically elicited spike. The partial spikes riding on the largest VR-EPSPs may be signs of activity arising at the initial segment or first node of the motoneurone axon.

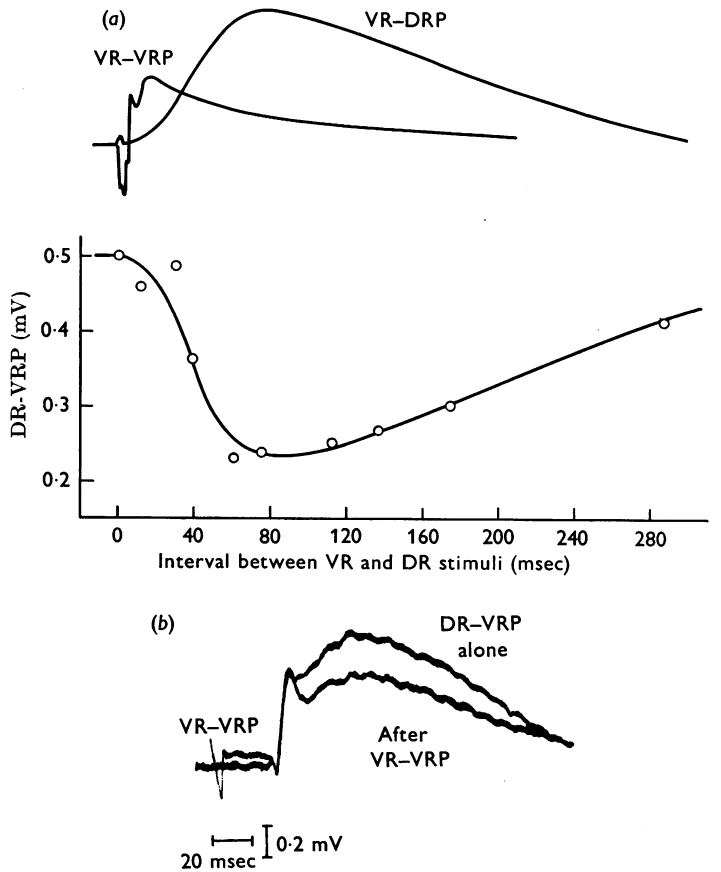

Fig. 20. Inhibition of the DR-VRP by the VR-DRP. *a*. Magnitude and time course of inhibition compared with the time course of the VR-VRP and VR-DRP. The orthodromic stimulus was applied at about 3 cm distance from the cord, where no VR-DRP could be detected. *b*. Example showing that inhibition affects primarily the polysynaptic components of the DR-VRP.

DISCUSSION

Washizu's findings (1960) of asymmetry of motoneurone response to stimulation of two different ventral roots provided good evidence that the response having the greater latency was not the result of antidromic stimulation of an axon collateral of the cell or of a second axon arising independently from the cell soma. The demonstration that the VR-EPSP is graded in nature (Kubota & Brookhart, 1962, 1963; Katz & Miledi, 1963) and facilitated by orthodromic stimulation in proportion to the size of the DR-VRP rather than to the size, polarity, or time course of intra-

cellular potential changes, eliminates even the possibility that antidromic stimulation of a second 'axon' arising from some point on the dendritic tree could be responsible for the VR-EPSP and the VR-VRP. It seems clear that two populations of cells are involved.

Intracellular recordings demonstrate that motoneurones constitute the post-synaptic side of the VR-VR interaction. The presynaptic elements also appear to be motoneurones, since sympathetic fibres have been found to be unessential, and aberrant sensory afferents are presumably present in numbers too small to explain the graded nature of the interaction. What populations of motoneurones are involved, and in what proportions they contribute to the interaction, remains to be learned.

The pharmacological evidence assembled above recommends the hypothesis that the interaction is electrical in nature. None of a large variety of known synaptic blocking agents are effective in reducing it. Likewise, Mg^{2+} and Ca^{2+} both reduce the potential, in contrast to their antagonistic effect at other known chemical synapses. This by no means proves that it is not a chemically mediated synapse, but would require the postulation of quite an atypical one.

The findings of Kubota & Brookhart (1963) that large shifts in membrane potential did not affect the size of the VR-EPSP, and that the VR-EPSP did not affect the size or shape of antidromic action potentials recorded in the motoneurone soma, have been confirmed in the present investigation and are convincing evidence that the synapse, if chemical, is not located near the cell soma. The principal reasons that led Kubota and Brookhart eventually to favour a chemical synapse were (1) the results of preliminary experiments in which curare, succinylcholine, and decamethonium affected the interaction, (2) the anatomical evidence for axon collaterals, and (3) their belief that action potentials do not invade the entire dendritic tree.

The first of these reasons depends on evidence that is contradicted by the present findings. The explanation for this discrepancy is not clear. The existence of axon collaterals is reassuring in view of their probable function as the first (ACh-releasing) step in the pathway to depolarization of the dorsal root afferents. There is no reason why the interaction with adjacent motoneurones should originate in the same way, however. In fact, it seems clear that the somas of the antidromically stimulated motoneurones must be invaded if the interaction is to be detected. There seems even to be evidence that the dendrites of the invaded motoneurones form the presynaptic side of the VR-VR 'synapse' and probably dendrites of adjacent motoneurones the post-synaptic side.

The VR-VRP is usually associated in root recordings with a negative field potential peak occurring later than the maximal soma discharge

of the invaded motoneurones. This field potential and the VR-VRP can be greatly facilitated by orthodromic depolarization in cases when invasion of the motoneurone somas seems to be relatively little affected. Refractoriness to successive VR-VRPs appears to be longer than that for axon or soma spikes, and the fatigue following sustained VR-VRP tetanus lasts longer than fatigue of motoneurone soma invasion.

Lloyd's (1959) finding that a reduction in temperature of the dendrites of cat lateral plantar motoneurones markedly enhanced their antidromic activation (as did orthodromic stimulation) suggests an explanation for the temperature effect on the VR-VRP, and also implicates the dendrites in this interaction. Two possible mechanisms whereby cooling might enhance the interaction are: an increase in soma invasion due to increased input resistance, perhaps due to a decrease in resting potassium conductance; and an increase in duration of activity, which could enhance soma and dendrite invasion and improve electrical coupling between adjacent membranes by increasing the time available for capacitative charge transfer.

Perhaps the observations most critical to our understanding of the interaction are those of occlusion and facilitation between successive VR-VRPs arising from stimulation of different ventral roots. This is a presumed electrical synapse that shows signs both of convergence and occlusion. The explanations proposed for both effects are remarkably similar. Occlusion results from dendritic depolarization, and so does facilitation! This requires the assumption that conduction in, or invasion of, dendrites is reduced by large depolarizations, enhanced by smaller depolarizations. Yet this assumption seems justified on the basis of the effects of orthodromic depolarization and refractoriness following antidromic invasion already mentioned.

A very important question is whether there is, in fact, a synapse at all, i.e. whether this interaction could arise simply as a result of field effects. This appears unlikely in view of the large size (6–7 mV) of some of the VR-EPSPs, which would require a very large extracellular field around the motoneurone dendrites. No such field with the correct polarity has yet been observed in the many micro-electrode penetrations through the cord.

It is clearly necessary to know more about the anatomy of the frog cord, particularly the relations between different motoneurone dendrites. Earlier studies (Sala y Pons, 1892; Silver, 1942) have revealed that the motoneurone dendrites may extend 1 mm or more and intertwine along much of their length. There is so far no information available on the fine structure of these cells, but if the motoneurone interaction is electrical one might expect to find regions of very close apposition or fusion of the surface membranes of adjacent dendrites, such as have been observed in

known or presumed electrical synapses, e.g. the annelid giant fibres (Hama, 1959) and the crayfish giant motor synapse (Robertson, 1955; de Lorenzo, 1959); chick ciliary ganglion (Martin & Pilar, 1963*a*, *b*; de Lorenzo, 1960) and the club endings on Mauthner cells (Furshpan, 1964). It is of particular interest that Bennett, Aljure, Nakajima & Pappas (1963) find fusion of the surface membranes of dendrites of spinal electromotor neurones of Mormyrid fishes over long distances without accumulation of vesicles, and that Hama (cf. Bullock, 1964) is reported to have observed similar 'tight junctions' in the rat C.N.S. It should be noted, however, that none of the electrophysiological data obtained from study of this interaction can differentiate between close apposition of membranes and the existence of membrane fusion or of fine dendritic bridges.

If the interaction does in fact require invasion of the dendrites along much of their length, and particularly if the post-synaptic elements are also dendrites, the conduction time necessary is probably a sufficient explanation of the 2 msec or more of 'synaptic delay' observed at 5–10° C.

Finally, it must be noted that no attempt has yet been made to detect specific connexions between motoneurones, which one might expect to find if the interaction is functionally important to the animal. On the other hand, in view of the probable tendency of the motoneurones of a given muscle nerve to be clustered together in the cord, it may be that the random formation of 'synapses' between contiguous dendrites could function nearly as efficiently. Then perhaps their most likely purpose would be the synchronization of activity of the motoneurones of any given region of the cord.

It is conceivable that similar interaction might exist widely throughout the central nervous system, providing synchronizing depolarization perhaps largely restricted to dendrites. Its discovery in the frog spinal cord may simply be the fortuitous result of an anatomical arrangement allowing massive but selective stimulation and recording from mixed populations of neurones.

It is a pleasure to express my gratitude to Dr R. Miledi and Professor B. Katz for their consistently rewarding interest and advice throughout the course of this research, and to Dr S. Hagiwara, Dr T. H. Bullock, and Dr E. J. Furshpan for critically reading the manuscript. Unfailing technical assistance was afforded by Mr J. L. Parkinson of University College London and by Mr F. W. Bloodworth of the University of California, Los Angeles. Much of this research was done while the author was the holder of a U.S. National Science Foundation Post-doctoral Fellowship.

REFERENCES

ANDEN, N. E., LUNDBERG, A., ROSENGREN, E. & VYKLICKÝ, L. (1963). The effect of DOPA on spinal reflexes from the FRA (flexor reflex afferents). *Experientia*, **19**, 654–655.

BARRON, D. A. & MATTHEWS, B. H. C. (1938). The interpretation of potential changes in the spinal cord. *J. Physiol.* **92**, 276–321.

BENNETT, M. V. L., ALJURE, E., NAKAJIMA, Y. & PAPPAS, G. D. (1963). Electrotonic junctions between teleost spinal neurones: electrophysiology and ultrastructure. *Science, N.Y.*, **141**, 262–264.

BROOKHART, J. M. & FADIGA, E. (1960). Potential fields initiated during monosynaptic activation of frog motoneurones. *J. Physiol.* **150**, 633–655.

BULLOCK, T. H. (1964). Neurophysiology: United States–Japan Joint Symposium. *Science, N.Y.*, **144**, 1361–1364.

CURTIS, D. R. & ECCLES, R. M. (1958). The effect of diffusional barriers upon the pharmacology of cells within the central nervous system. *J. Physiol.* **141**, 446–463.

DEL CASTILLO, J. & ENGBAEK, L. (1954). The nature of the neuromuscular block produced by magnesium. *J. Physiol.* **124**, 370–384.

DEL CASTILLO, J. & KATZ, B. (1957). A study of curare action with an electrical micromethod. *Proc. R. Soc.* B, **146**, 339–356.

DE LORENZO, A. J. (1959). The fine structure of synapses. *Biol. Bull. mar. biol. Lab., Woods Hole*, **117**, 390.

DE LORENZO, A. J. (1960). The fine structure of synapses in the ciliary ganglion of the chick. *J. biophys. biochem. Cytol.* **7**, 31–36.

DUNN, ELIZABETH, H. (1914). The presence of medullated nerve fibers passing from the spinal ganglion to the ventral root of the frog, *Rana pipiens*. *J. comp. Neurol.* **24**, 429–436.

ECCLES, J. C. (1947). ACh and synaptic transmission in the spinal cord. *J. Neurophysiol.* **10**, 197–204.

ECCLES, J. C. & MALCOLM, J. L. (1946). Dorsal root potentials of the spinal cord. *J. Neurophysiol.* **9**, 139–160.

ECCLES, R. M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol.* **157**, 484–503.

ECKER, A. & WIEDERSHEIM, R. (1899). *Anatomie des Frosches*, pt. II, p. 548. Braunschweig: Vieweg.

FURSHPAN, E. J. (1964). *Electrical Transmission* at an excitatory synapse in a vertebrate brain. *Science, N.Y.*, **144**, 878–880.

HAMA, K. (1959). Some observations of the fine structure of the giant nerve fibers of the earth-worm, *Eisenia foetida*. *J. biophys. biochem. Cytol.* **6**, 61–66.

HUTTER, O. & KOSTIAL, K. (1954). Effect of magnesium and calcium ions on the release of acetylcholine. *J. Physiol.* **124**, 234–241.

KATZ, B. & MILEDI, R. (1963). A study of spontaneous miniature potentials in spinal motoneurones. *J. Physiol.* **168**, 389–422.

KIRALY, J. D. & PHILLIS, J. W. (1961). Action of some drugs on the dorsal root potentials of the isolated frog spinal cord. *Brit. J. Pharmac. Chemother.* **17**, 224–231.

KOKETSU, K. (1956). Intracellular slow potential of dorsal root fibers. *Amer. J. Physiol.* **184**, 338–344.

KUBOTA, K. & BROOKHART, J. M. (1962). Recurrent facilitation of frog motor neurons. *Physiologist*, **5**, 170.

KUBOTA, K. & BROOKHART, J. M. (1963). Recurrent facilitation of frog motoneurons. *J. Neurophysiol.* **26**, 877–893.

KUSANO, K. & HAGIWARA, S. (1961). On the integrative synaptic potentials of *Onchidium* nerve cell. *Jap. J. Physiol.* **11**, 96–101.

LLOYD, D. P. C. (1951). Electrical signs of impulse conduction in spinal motoneurons. *J. gen. Physiol.* **35**, 255–288.

LLOYD, D. P. C. (1959). Temperature and dendritic response of spinal motoneurons. *Proc. natn. Acad. Sci. U.S.A.* **45**, 589–592.

MARTIN, A. R. & PILAR, G. (1963*a*). Dual mode of synaptic transmission in the avian ciliary ganglion. *J. Physiol.* **168**, 443–463.

MARTIN, A. R. & PILAR, G. (1963*b*). Transmission through the ciliary ganglion of the chick. *J. Physiol.* **168**, 464–475.

MILEDI, R. (1961). From nerve to muscle. *Discovery*, October, 1961.

NELSON, P. G. & FRANK, K. (1964). Orthodromically produced changes in motoneuronal extracellular fields. *J. Neurophysiol.* **27**, 928–941.

RENSHAW, B. (1942). Reflex discharge in branches of the crural nerve. *J. Neurophysiol.* **5**, 487–498.

ROBERTSON, J. D. (1955). Recent electron microscope observations on the ultrastructure of the crayfish median-to-motor giant synapse. *Expl Cell Res.* **8**, 226–229.

SALA Y PONS, C. (1892). Estructura de la medula espinal de los batracios. *Trab. Lab. Invest. biol. Univ. Barcelona*, 3–22.

SCHMIDT, R. F. (1963). Pharmacological studies on the primary afferent depolarization of the toad spinal cord. *Pflüger's Arch. ges. Physiol.* **277**, 325–346.

SILVER, M. S. (1942). Motoneurons of the spinal cord of the frog. *J. comp. Neurol.* **77**, 1–39.

TAKEUCHI, A. & TAKEUCHI, N. (1962). Electrical changes in pre- and postsynaptic axons of the giant synapse of *Loligo*. *J. gen. Physiol.* **45**, 1181–1193.

WASHIZU, Y. (1960). Single spinal motoneurons excitable from two different antidromic pathways. *Jap. J. Physiol.* **10**, 121–131.