RESPONSES OF MOTONEURONS UNDERGOING CHROMATOLYSIS

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ABSTRACT

The delayed and asynchronous firing of chromatolytic motoneurons in response to group I afferent volleys is shown to be evoked monosynaptically, there being an abnormally long and variable delay between onset of monosynaptic action and generation of impulse discharge. Intensity of monosynaptic excitatory action is reduced, and considerable variability in the form of successively evoked postsynaptic potentials is often observed. No evidence has been found for the development of excitatory group I polysynaptic pathways.

Reduction in responsiveness of finer dendrites is indicated by the feeble "*d*" response evoked by an antidromic volley in a chromatolytic motor nucleus. Antidromic impulses appear to invade the cell bodies and coarse dendrites, but die out at points short of the normal extent of dendritic invasion. Vigorous firing of Renshaw cells can be elicited by antidromic volleys.

Chromatolytic motoneurons appear to maintain reasonably normal resting membrane potentials, but are more susceptible to damage than are normal cells. Action potentials are large and usually overshoot the resting potential level. Post spike potentials are similar to those of normal cells except for a less prominent, or absent, early phase of depolarisation.

In contrast with the reduced responsiveness of peripheral dendrites, there is a lowered threshold for antidromic and segmental reflex synaptic activation of the more central regions, probably the cell bodies and nearby coarse dendrites, of motoneurons undergoing chromatolysis.

INTRODUCTION

It is now well known that characteristic changes take place in the synaptic responses of motoneurons undergoing chromatolysis after interruption of their axons. In particular, disappearance of the normal short latency reflex response to muscle afferent volleys is observed, together with a striking development of asynchronous delayed discharges, resembling the flexor reflex pattern, in response to such volleys (10, 12, 13). This paper describes an attempt to analyse further the nature of the functional changes taking place in single units and in pools of motoneurons undergoing chromatolysis, with particular reference to the suggestion (13) that the reflex delay might represent the development of

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new polysynaptic pathways between group I afferent fibres and the affected motoneurons. As reported earlier in a brief account the present work (4), it has been found that the longer response latency simply represents abnormal delay in the operation of the monosynaptic reflex transmission mechanism. The same conclusion has been reached in a recent paper based on intracellular microelectrode studies by Eccles, Libet, and Young (17). In the present paper, some observations on antidromic invasion of chromatolytic motoneuron pools are also presented.

Methods

In a series of thirty adult cats, operative section of a selected ventral root (usually L7 or S1) was carried out extradurally under barbiturate anesthesia and with aseptic precautions, as described in earlier papers from this laboratory (13, 18). In some instances, a nerve in the hindlimb was severed in place of a ventral root. 2 to 6 weeks after the initial operations, responses of the affected motoneurons were examined oscillographically and compared with those of normal motoneurons in the corresponding segment on the opposite side, or in an adjacent ipsilateral segment. In some experiments, barbiturate anesthesia (pentobarbital) was employed throughout, while in others the animals were used in the acutely decapitate state, ether being used initially to permit interruption of the cranial arterial supply and section of the spinal cord *via* the atlanto-occipital membrane. After laminectomy and dissection of appropriate spinal roots and hindlimb nerves, motoneuron responses to antidromic or orthodromic stimulation were recorded from ventral roots or from the cord itself. In a few experiments, potential fields within the cord were explored with steel microelectrodes of tip diameter approximating 20 μ; in others, 0.5 to 1.0 μ Ling-Gerard glass capillary micropipettes filled with 3 M KCl were used to obtain intracellular records from individual motoneurons. Details of the experimental procedures employed have been described in previous publications from this laboratory (5, 13).

RESULTS

1. Reflex Discharge of Chromatolytic Motoneurons

Response to Group I Afferent Volleys.—As reported elsewhere (13), volleys restricted to large (group I) afferent fibres of muscle nerves readily evoke reflex discharge of motoneurons undergoing chromatolysis, but the discharge is delayed and asynchronous in comparison with that of a normal motoneuron pool. Fig. 1 gives examples of such responses in two different animals, in each of which the first sacral ventral root (S1 VR) had been severed aseptically some weeks previously. Displayed in each case are the simultaneously recorded responses evoked by a muscle afferent volley in two adjacent ventral roots, the upper trace showing a monosynaptic discharge in the normal seventh lumbar ventral root (L7 VR), and the lower beam the reflex output along the operatively severed ventral root. The nerves stimulated were those supplying the triceps surae in A, and the combined

triceps surae and plantaris nerves in B. Compared with the normal monosynaptic discharge, the response of a chromatolytic ventral root begins later, lasts longer, and is more irregular in contour. That the responses are set up by action in the largest (group I) muscle afferent fibres is readily shown by reducing the stimulus strength. Even very small volleys evoke some discharge from chromatolytic motoneurons, reflex threshold commonly being slightly lower in the chromatolytic than in the normal part of a homonymous motoneuron pool when tested by the same afferent volley. The graph of Fig. 2 plots the growth of mon-

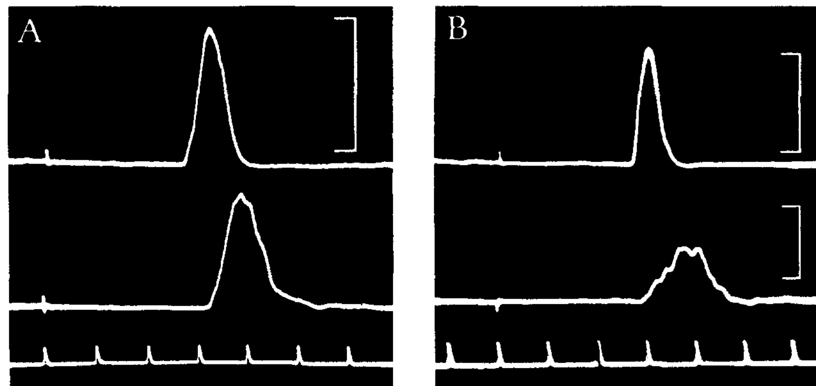

FIG. 1. Reflex responses evoked by group I afferent volleys in normal and chromatolytic motoneurons. In A and B, the upper beam shows the response of the normal L7 VR, and the lower, the simultaneously recorded response of the S1 VR severed 35 days previously in A, 24 days in B. Afferent volley in triceps surae nerve in A, and in the combined plantaris and triceps surae nerves in B. Calibrations, 1 mv.; same amplification for upper and lower beam in A, different amplifications in B. Time marks 1 msec. Two different decapitate preparations.

osynaptic discharge in the L7 VR (filled circles) and the chromatolytic response of the S1 VR (open circles) as the group I muscle afferent volley is increased from zero to maximum (same preparation as Fig. 1 B). The fact that the curve for the S1 reflex actually lies slightly to the left of the monosynaptic L7 reflex not only confirms earlier findings (13) that group I fibres are responsible for the chromatolytic discharge, but also indicates a slightly lower reflex threshold in the motoneurons undergoing chromatolysis.

Cutaneous Afferent Volleys.—Impulses in myelinated cutaneous afferent fibres (groups II and III) evoke an irregular, asynchronous discharge in a chromatolytic as in a normal ventral root, the earliest discharge appearing at about the same latency on both roots (see Figs. 3 and 4). In a normal ventral root, such a response is certainly polysynaptic and represents the flexor reflex (24), so there is good reason to regard the chromatolytic discharge as

a manifestation of the flexor reflex too. The only difference observed has been quantitative, with the discharge consistently more prominent on a chromatolytic than on a normal ventral root, an observation also reported previously

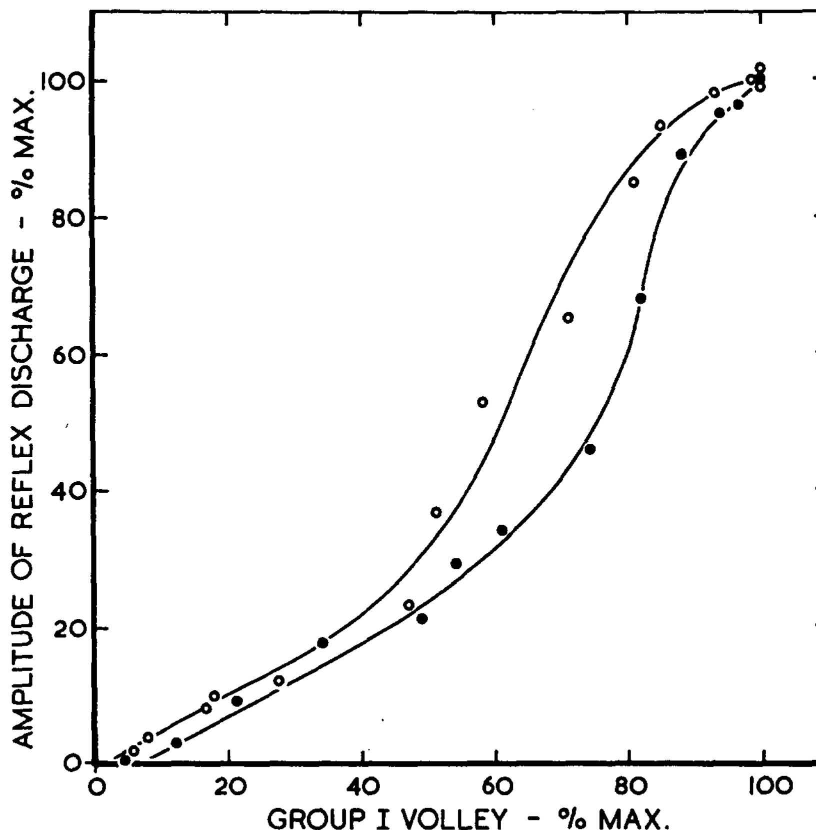

FIG. 2. Input-output curves for reflex responses evoked by group I afferent volleys in combined plantaris and triceps surae nerves. Filled circles, amplitude of monosynaptic discharge on normal (L7) ventral root; open circles, amplitude of response on chromatolytic (S1) ventral root (24 days' chromatolysis). Abscissa, size of group I volley; ordinate, size of reflex response, both expressed as per cent of maximum. Decapitate preparation.

(13). This is a further indication of a lowered reflex threshold in motoneurons undergoing chromatolysis.

The Increase in Group I Reflex Latency.—Failure of monosynaptic connections together with a development of new, polysynaptic pathways between group I afferent fibres and chromatolytic motoneurons (13) could account for the greater latency of reflex discharge; however, simple slowing of the

monosynaptic excitatory process would explain the longer delay equally well. That the simpler explanation is correct is suggested by the observation that in preparations free from the depressant action of narcotic the reflex delay may amount to no more than about 0.3 msec., as in Fig. 1 B. Further

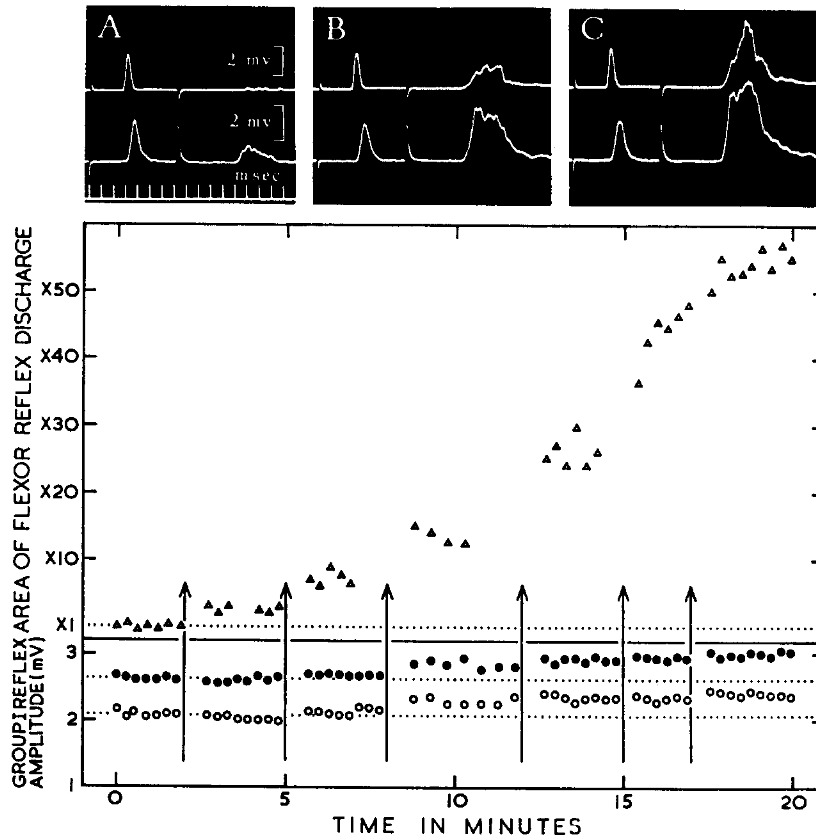

FIG. 3. Above, records showing effects of strychnine on reflex responses to group I (muscle) and group II (cutaneous) afferent volleys. Upper trace of each pair shows responses of normal, L7 VR; lower trace, those of chromatolytic S1 VR (20 days) as in Fig. 1. First stimulus to plantaris and triceps surae nerves, second to sural nerve. A, control, B after 0.04 mg./kg. of strychnine sulfate, C after a further 0.07 mg./kg. of strychnine. Plotted below are the effects of further strychnine administration some hours later. Triangles show area of L7 VR flexor reflex discharge to sural volley, expressed as multiples of average control area. Amplitudes of responses to muscle afferent volley are also shown, filled circles being those of the normal L7 VR, open circles those of the chromatolytic S1 VR. Arrows give the times of strychnine administration, the total dose being 0.2 mg./kg. during the period shown in the graph. All strychnine doses given intravenously. Decapitate preparation.

analysis supports the view that a change in timing but not in pathway of group I reflex action is a sufficient explanation of the phenomenon.

One way of examining the nature of a reflex path yielding responses at a latency consistent with the presence of one or more internuncials is to submit it to the action of agents which in suitable doses exaggerate or depress

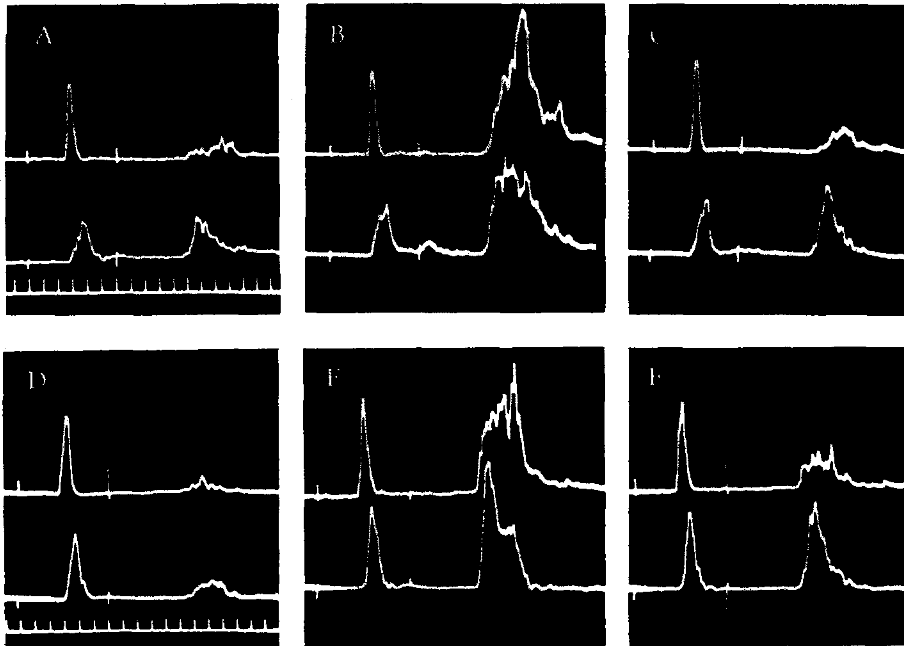

FIG. 4. Effects of strychnine and myanesin in two different decapitate preparations, A, B, and C 35 days, D, E, and F 21 days after severing the S1 VR. Each pair of sweeps simultaneously recorded, the upper from the ipsilateral L7 VR (normal) and the lower from the chromatolytic S1 VR. Each sweep shows responses to successive stimulation of a muscle nerve and a cutaneous nerve, as in Fig. 3. A and D are controls before drug administration; B and E show the effects of strychnine (0.08 mg./kg. in B, 0.1 mg./kg. in E). C and F, action of myanesin upon strychnine-enhanced responses (30 mg./kg. in C, 40 mg./kg. in F). Drugs administered intravenously. Time marks, milliseconds.

transmission in known polysynaptic paths (such as the one mediating the flexor reflex) while having minimal action on the monosynaptic stretch reflex arc. Suitable substances are strychnine and myanesin, the first of which dramatically enhances polysynaptic reflexes while the latter depresses transmission in such pathways (3, 8, 22, 23, 30, 32). Fig. 3 presents the results of an experiment with strychnine. Above are three records each consisting of two simultaneously recorded responses, the upper being derived from the

L7 VR (normal) and the lower from the S1 VR which had been operatively severed 20 days previously. Two stimuli were applied during each sweep, the first to the combined triceps surae and plantaris muscle nerves, the second to the sural nerve. The muscle nerve volley evoked typical responses as in Fig. 1, the one in the normal root certainly being monosynaptic; the cutaneous nerve volley elicited the expected asynchronous flexor reflex response in both roots. A shows typical control responses before administration of any drug; B shows the responses to the same volley after intravenous administration of 0.04 mg./kg. of strychnine sulfate, and C the additional change brought about by a further dose of 0.07 mg./kg. of strychnine. The drug brings about striking potentiation of the polysynaptic responses elicited by the sural nerve volley, but little change is apparent in the response in either ventral root to the muscle nerve volley. Plotted below in Fig. 3 are the results of further doses of strychnine administered several hours later to the same preparation. The vertical arrows show the times of giving successive doses of the drug, the total amount being 0.2 mg./kg. Triangles show the areas (potential-time integrals) of polysynaptic flexor reflex responses to the sural nerve volley in multiples of the average control response area; below are plotted amplitudes of the responses to the muscle afferent volley, filled circles showing the normal monosynaptic response and open circles the delayed discharge of chromatolytic motoneurons. It can be seen that the very great potentiation by the drug of the flexor reflex discharge is not matched by comparable increase in the responses to muscle nerve stimulation, which are scarcely affected until after the third dose and are only moderately increased by the full amount. The graph shows clearly our consistent finding that any strychnine-induced changes in the amount of chromatolytic reflex discharge in response to group I afferent volleys follow closely those occurring simultaneously in a known group I monosynaptic reflex.

Fig. 4 demonstrates that similar considerations hold for the action of myanesin, a powerful depressant of internuncially mediated reflex discharge (8, 22, 23, 32). The three upper records, A, B and C are from one preparation, 5 weeks after ventral root section; D, E, and F are from another animal, 24 days after operation, the first sacral root having been severed in both cases. As in Figs. 1 and 3, the upper trace in each instance shows discharge of the normal (ipsilateral seventh lumbar) ventral root, while the lower is from the operatively severed first sacral root. The first stimulus engages muscle afferent fibres and the second evokes a cutaneous afferent volley. A and D are control responses before drug administration, B and E responses to the same volleys after strychnine administration. C and F were recorded within a few minutes of giving myanesin (30 to 40 mg./kg.) by intravenous injection. In both preparations, the strychnine-potentiated flexor reflex discharges are seen to be greatly reduced by the myanesin, though little change is apparent in the responses along either root to the muscle afferent volley.

Thus both with strychnine and myanesin, the delayed responses of chroma-

tolytic motoneurons to a group I afferent volley behave in the same way as a known monosynaptic reflex, and not in the manner characteristic of polysynaptic segmental reflex discharges. The pharmacological experiments, therefore, provide strong evidence that the chromatolytic responses to group I volleys are mediated monosynaptically despite the increased central delay.

Reflex Responses of Individual Motoneurons.—Intracellular recording from single motoneurons of a pool undergoing chromatolysis can provide further information about the nature and timing of the synaptic events determining reflex discharge, including changes subthreshold for motoneuron firing. Of the seventy-two motoneurons successfully impaled with Ling-Gerard type micropipettes during the course of this study, thirty-one could be examined for their response to volleys in homonymous afferent fibres, homonymous at least in the sense that in each case they were presumably derived from receptors in the muscle (or a synergist) pertaining to the motoneuron under investigation. It was sometimes possible to compare, in the course of the same experiment, responses of chromatolytic cells to a given volley with those of normal motoneurons in an adjacent segment (for example, A and B in Fig. 5). The disadvantages of intracellular recording, such as the inevitable damage inflicted on the impaled neuron and the uncertainty that a representative population of units from a pool can be selected, are to a considerable extent outweighed by the directness with which answers can be given to problems such as the sequence of postsynaptic events leading to reflex discharge.

Under the experimental conditions required for intracellular recording "normal" motoneurons fired by homonymous group I afferent volleys appear to discharge as a result of postsynaptic depolarisation beginning after a latency so short that it must be monosynaptically evoked. Fig. 5 displays a series of synaptically evoked potentials showing that depolarisation of chromatolytic motoneurons by homonymous group I volleys begins as early as it does in normal cells, thus demonstrating the presence of functional monosynaptic connections with the usual latency of action. Postsynaptic potentials of normal cells ordinarily reach a peak in less than 1.5 msec. (5, 15), and are of reasonably constant configuration (Fig. 5 A). Those of chromatolytic cells, however, do not usually rise as steeply as those of normal motoneurons, but may take from 1.5 to 3.0 msec. or even more to reach an ill-defined, rounded summit (Fig. 5 B and C). Furthermore, in some units there is much more variation in the shape of successively evoked postsynaptic potentials than there is in normal cells. The superimposed tracings of Fig. 6 illustrate this variability, which may be striking, with irregular, random increments of depolarisation appearing as steps or humps (Fig. 6 A). However, the variability may be quite small, as in the responses of the other motoneuron (B) shown in Fig. 6. The postsynaptic potentials may subside smoothly, as shown in the upper record of Fig. 5 B, with a time-course similar to that seen in normal cells (half-decay in 3 to 5 msec.),

but in motoneurons giving the more irregular and variable type of response estimation of their time-course is not possible.

The most striking feature, however, about the responses of chromatolytic motoneurons is the long and variable latency for the synaptic generation of spike action potentials by group I volleys. Typical examples of this are shown in Fig. 5 B and C, in which the superimposed sweeps of C show the phenomenon most clearly. The delay between onset of depolarisation and

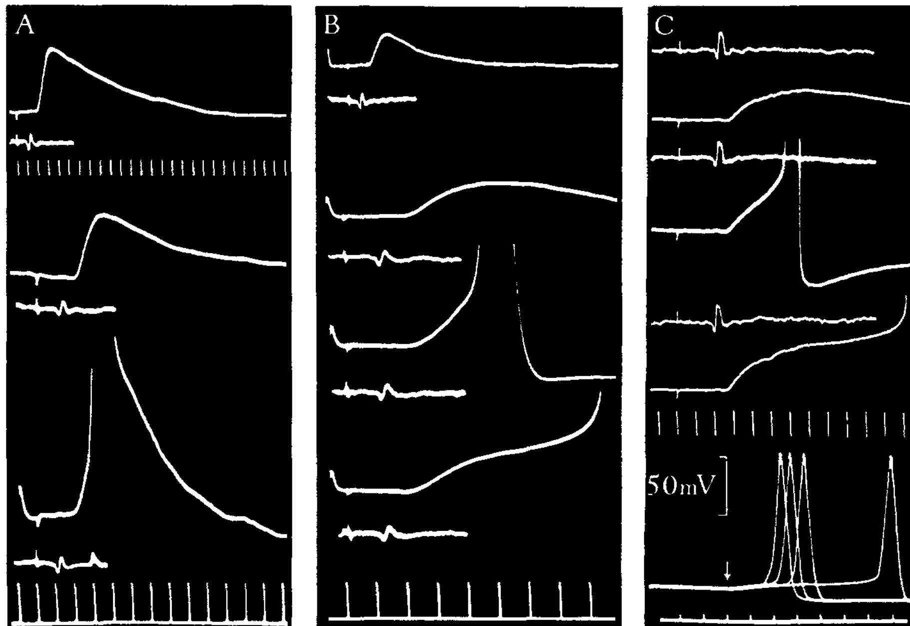

FIG. 5. Intracellular responses of three different motoneurons to group I afferent volleys. Column A, normal flexor hamstring motoneuron showing action of group I afferent volleys in combined biceps and semitendinosus nerves. Upper two sweeps, postsynaptic potential only, at slow and faster speeds; lower record shows motoneuron firing (most of spike off screen). Afferent volley shown below each sweep. B, a chromatolytic hamstring motoneuron (32 days), in same preparation as A. Upper two sweeps, postsynaptic potential evoked by same afferent volley as in A, at two sweep speeds. Lower two sweeps show the same neuron firing to the afferent volley, which is displayed below each sweep. C, chromatolytic flexor longus digitorum (F.L.D.) motoneuron (34 days). From above downwards, postsynaptic potential alone, and two examples of the cell firing to the F.L.D. afferent volley, shown above in each case. Lowest record, superimposed sweeps at low amplification showing variable latency of orthodromic firing of same motoneuron. Calibration applies only to this record; arrow marks onset of postsynaptic potential. All time marks show milliseconds, and apply to the records above them, except for the upper sweep in B which is at the same speed as the top record in A. Barbiturate anesthesia.

generation of an impulse may vary in individual trials from as little as 1.0 to more than 5.0 msec. The longest delay observed in this study was 9.0 msec. During this long latency, the postsynaptic potential usually remains smooth in contour, or presents only minor irregularities, showing that the late discharge of the cell is not brought about by additional internuncially relayed

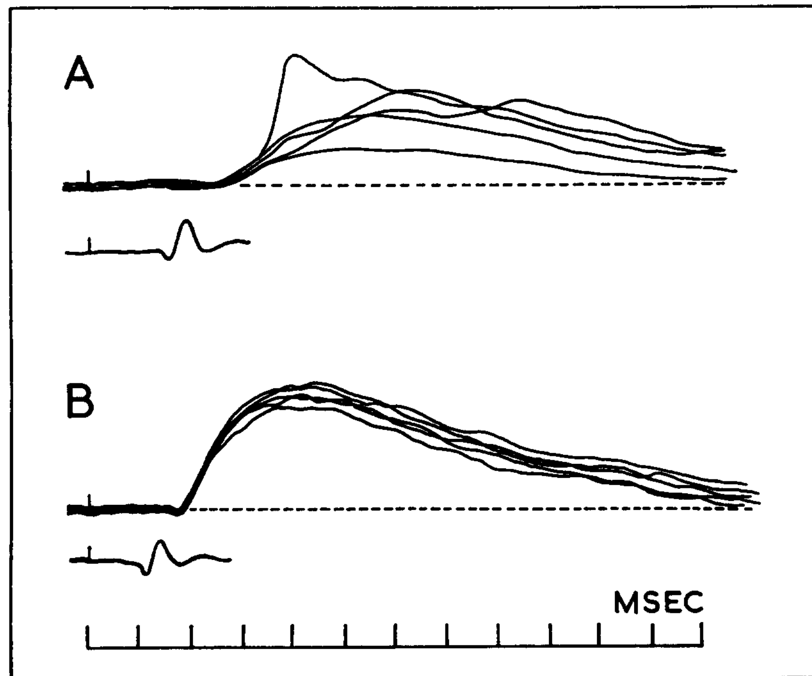

FIG. 6. Superimposed tracings showing, for two chromatolytic motoneurons, the range of variability of the postsynaptic potentials evoked by group I afferent volleys. A, a gastrocnemius motoneuron, 42 days after axotomy; B, a flexor hamstring motoneuron, 20 days after ventral root section. In each case five records have been selected from a series of successively evoked responses to show the range of variability. Afferent volley at entry to cord traced below.

increments of synaptic depolarisation, as would be the case if abnormal group I polysynaptic connections were responsible. However, the postsynaptic potentials of some of our units did show one or more steps before take-off of the impulse, suggesting polysynaptic action. Nevertheless, the intracellular experiments on the whole lead to the same conclusion as the observations with strychnine and myanesin, namely, that the delay and asynchrony in the firing of chromatolytic cells by group I afferent volleys are simply the result of abnormal slowing and variability of the processes underlying mono-

synaptic reflex transmission, and do not reflect growth of abnormal reflex connections. A similar conclusion has recently been reached by Eccles *et al.* (17).

2. *Antidromic Invasion of Chromatolytic Motoneuron Pools*

Invasion of a motor nucleus by antidromic impulses is signalled by a sequence of electrical deflections recordable from within or from the surface of the spinal cord (25). This process has been examined in pools of motoneurones undergoing chromatolysis, usually those of the S1 or S2 segment because of the favourable anatomical arrangement for surface recording. Attention has been directed particularly towards the response attributable to the dendrites, the surface-negative "*d*" deflection of Lloyd (25). Because of the great susceptibility of this response to depression by anesthetic agents and minor anoxic insult, these experiments have all been performed in decapitate unanesthetised preparations, and in each case the symmetrical response of the normal segment on the opposite side was recorded as a control. In view of the striking temperature sensitivity of dendritic responses (see Fig. 9), special care was taken to keep the cord temperature constant and within normal limits except when purposely changed. Before the comparison was made, careful search was made to find on each side the site yielding maximum "*d*" responses, usually a point just dorsal to the denticulate ligament near midsegmental level.

Fig. 7 shows the results obtained in an experiment involving the S1 segment, the left-hand records being responses, photographed at two sweep speeds, set up by a maximal stimulus to the S1 VR which had been severed 22 days previously. Those on the right are corresponding responses on the control side. The positive deflections signalling approach of the volleys in the ventral root fibres are reasonably symmetrical, but the striking feature is the gross deficiency of surface-negative "*d*" response on the operated side. This comparison has been made in sixteen animals, and in all but one the "*d*" response was smaller on the chromatolysed side. The experiment in which no systematic difference was detectable involved the L7 segment, which is unfavourable for surface recording of motoneuron pool invasion because of the greater thickness of white matter; furthermore, the animal in question was in poor condition and showed feeble "*d*" responses to any antidromic volley. In the other fifteen experiments, the amount of discrepancy varied considerably as shown in Table I.

The results of experiments in which antidromic responses of motoneuron pools were recorded from the ventral and lateral surfaces of the cord, or from within it by means of steel microelectrodes, also support the conclusion that dendritic invasion is defective in chromatolytic motoneurons; furthermore, the presence of Lloyd's "*b*" response suggests that impulses succeed in entering the somata of such cells in the unimpaled state, or at least invade the axon

hillock regions (25, 15, 20, 21). As described below, the responses of single motoneurons recorded intracellularly by microelectrode impalement indicate that antidromic impulses invade the bodies of the majority of such cells and probably the coarse dendrites as well. It is nevertheless clear from the exter-

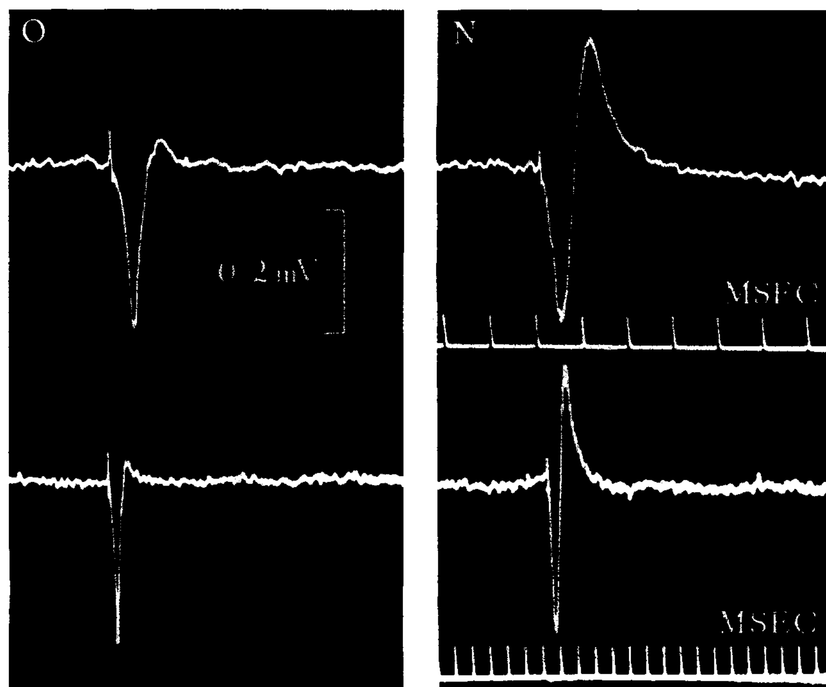

FIG. 7. Potentials set up by an antidromic volley in the whole S1 motoneuron pool on the operated (O) and normal (N) sides of an animal in which the left S1 VR had been severed 22 days previously. Active leads symmetrically disposed on surface of cord at the site on each side yielding the largest "*d*" response, just dorsal to the denticulate ligament at about midsegmental level; indifferent leads on nearby muscle. Response on each side shown at two sweep speeds. Time marks, milliseconds. Calibration, 200 μv. Decapitate preparation.

nally recorded responses that in motoneuron pools undergoing chromatolysis something tends to interfere with the activity responsible for the "*d*" deflection, which presumably represents invasion of the finer dendrites.

Further information can be obtained by examining the ability of a second antidromic volley to set up a "*d*" deflection at various time intervals after the first (25, 26). Fig. 8 illustrates typical findings in an experiment carried out 24 days after section of the left S1 VR. Open circles show amplitudes of the second "*d*" deflection on the operated side, and filled circles those on

the normal side of the same animal, recorded in each case from the dorsolateral surface of the cord. It can be seen that the "*d*" deflection on both sides is depressed for 100 to 120 msec. after a preceding volley, though to a smaller degree on the operated side. Another difference between the two curves is the greater prominence in the chromatolytic nucleus of the phase of relative supernormality. Similarity of the recovery times shows that despite its small size, the chromatolytic "*d*" response does reflect invasion (albeit limited) of structures possessing properties like those of the elements responsible for

TABLE I

Experiment No.	Time after operation	Ventral root or nerve sectioned	Amplitude of chromatolytic "*d*" (as per cent of "*d*" on normal side)
	days		*per cent*
13	20	1st sacral	26.7
14	22	1st sacral	55
16	23	1st sacral	7.4
17	24	1st sacral	28
18	24	1st sacral	20.6
20	28	1st sacral	36
21	23	1st sacral	17.8
22	20	Plantar	23.4
23	23	2nd sacral	48
24	22	1st sacral	10.7
25	26	Plantar	37.5
27	42	1st sacral	7.3
28	24	1st sacral	23.7
29	30	1st sacral	21
30	35	1st sacral	15.8
Average			24.8

normal "*d*" responses, namely dendrites. The smaller amplitude of a chromatolytic "*d*" response could either be the result of blockade somewhere in the dendritic tree of impulses which successfully invade the somata of all or most of the motoneurons, or alternatively it could reflect failure of impulses to negotiate the axon-soma or soma-dendritic junctions of a large proportion of the motoneurons in the pool. Axon-soma blockage does not seem likely in view of the degree of somatic invasion revealed by intracellularly recorded responses, despite the reservations which must be made in view of the procedure involved. It therefore seems probable that antidromic impulses enter the somata and dendritic processes of the majority of chromatolytic cells but die out at some point short of the usual limits of invasion found in normal motoneurons.

The fact that the second of two successive "*d*" responses in a chromatolytic nucleus is not facilitated (nor for that matter, is the third of three) suggests that the defective invasion is not the result of simple anodal block caused by dendritic hyperpolarisation, for should this be so the first volley would be

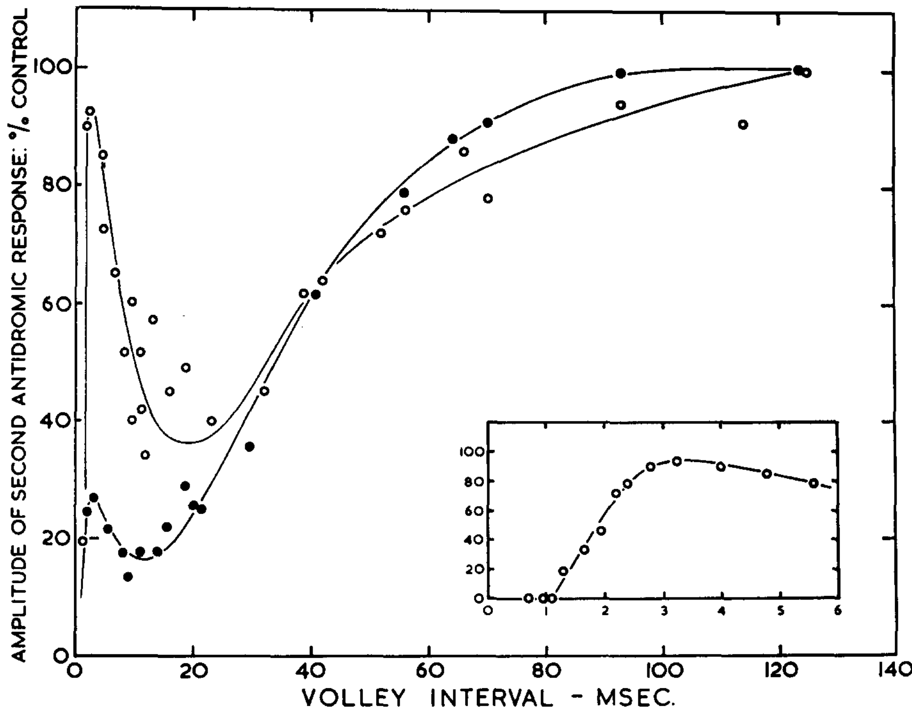

FIG. 8. Recovery of "*d*" response evoked by the second of two antidromic volleys in the S1 VR plotted as per cent of control (ordinate) against volley interval in milliseconds (abscissa), 24 days after section of the left S1 VR. Potentials recorded as in the experiment of Fig. 7. Open circles, operated side; filled circles, normal side. Inset shows early portion of recovery curve for the chromatolytic motor nucleus. Decapitate preparation.

expected, by virtue of electrotonic spread of depolarisation beyond the block, to facilitate invasion by the second volley. On the other hand, acute asphyxia can temporarily relieve the block, as described below, suggesting that hyperpolarisation of dendrites could be one factor contributing towards the reduction of antidromic invasion (26).

The higher peak of relative supernormality and its somewhat longer duration as compared with the curve for normal motoneurons suggest either that the chromatolytic motoneurons are more susceptible to those factors tending to increase excitability during the first 30 msec. or so after an antidromic

volley (7, 25, 26), or that these factors are more powerful (or are less masked by depression) in chromatolytic motoneuron pools. Perhaps the greater relative supernormality simply reflects a difference in the properties and proximity to the somata of the dendritic regions yielding the "*d*" deflection in the two situations. The normal response is probably dominated by action in very large numbers of fine terminal dendrites, whereas the smaller chromatolytic deflection presumably arises from a smaller number of somewhat coarser dendritic elements nearer the center of the motor nucleus. Lloyd (25) has shown that such elements in normal motoneurons are less depressed by a preceding antidromic volley than are the more peripheral dendrites.

Fig. 9 shows the effects of lowering the cord temperature and of asphyxia on the amplitude of "*d*" responses to antidromic volleys. A and C are control responses at 37°C. of the normal and operated side respectively upon stimulating the S1 VR; B and D show the corresponding responses after lowering cord temperature by cooling the paraffin bathing it. Both the operated and normal responses become larger as a result of this procedure, the increase on the normal side being spectacular. Thus it appears that even in a "normal" motoneuron pool many fine dendrites fail under ordinary experimental conditions to be invaded by antidromic impulses, but are able to respond at lower temperatures. The increased response of chromatolytic cells indicates that cooling brings some measure of relief to the much greater degree of block characteristic of the condition, but this relief is no more effective than in a normal nucleus; indeed, it is often less so. In none of our experiments has cooling succeeded in boosting a chromatolytic "*d*" response to a level approaching the amplitude seen in an equivalent normal motoneuron pool. The only maneuvre which has succeeded in temporarily restoring a chromatolytic "*d*" deflection to a size approaching normal has been a period of acute asphyxia, as shown in the lower records of Fig. 9. The large "*d*" deflection seen 100 seconds after ceasing ventilation (Fig. 9 F) shows that there were dendritic elements in this chromatolytic nucleus capable of yielding a response of approximately normal size, and presumably invaded as a result of drastic asphyxial depolarisation; this increase must correspond with the convulsive increment described by Lloyd (27), for at this time the second of two volleys suffered practically no depression. At a later stage of asphyxia, the "*d*" deflection disappeared entirely; upon restoring ventilation, slow recovery took place without any phase of exaggerated response during the period of post anoxic hyperpolarisation. Thus a cathodal type of block is unlikely to be in any way responsible for the impaired dendritic responses of chromatolytic motoneurons, but the evidence is consistent with the hypothesis that an anodal type of block could contribute to the deficiency. It seems likely, however, that factors other than a change in membrane potential are principally responsible for the deficiency of dendritic response.

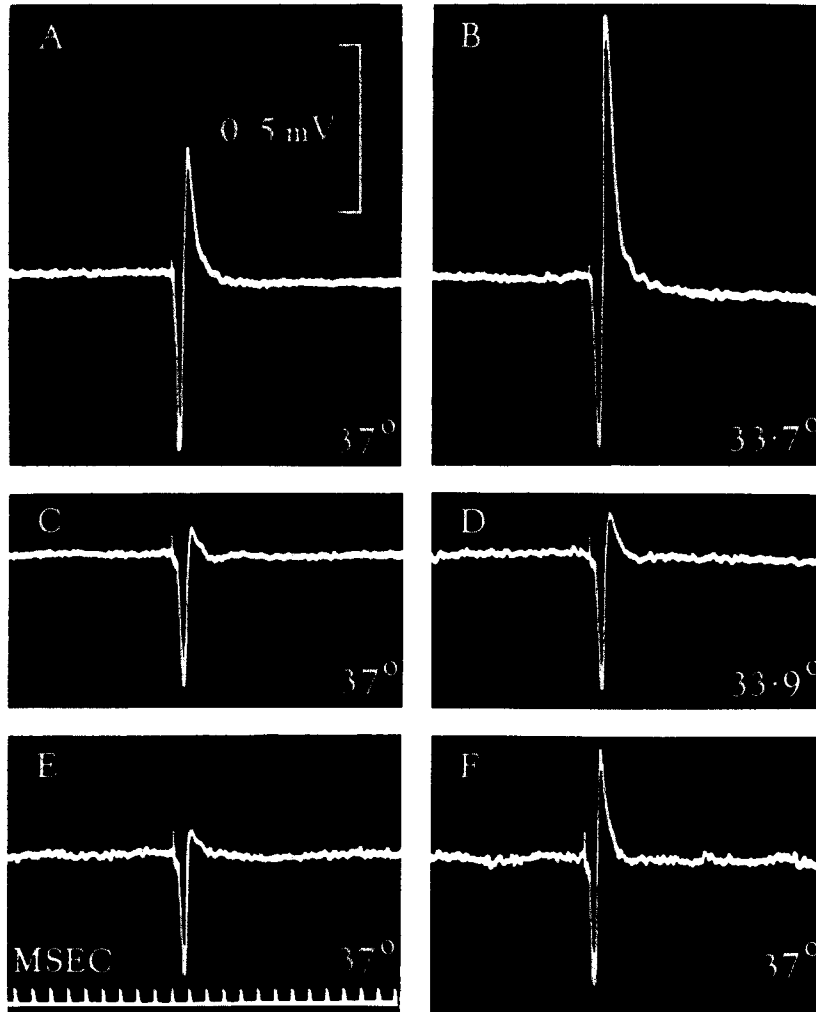

FIG. 9. "*d*" responses recorded from the cord surface as in Fig. 7 to single antidromic volleys in the S1 VR on each side. A and B, normal side at 37 and 33.7°C. respectively; B and C, a similar comparison on the operated side at 37 and 33.9°C. E and F show the effects of asphyxia on the chromatolytic "*d*" response at a later stage in the same experiment, after restoration of temperature to 37°C. E, control, F, 100 seconds after ceasing ventilation. Time marks, milliseconds. Temperatures measured in the paraffin adjacent to the site of recording, not within the cord itself. Decapitate preparation, 24 days after section of the left S1 VR.

One interesting action of impulses coursing either antidromically or orthodromically in axons of normal spinal motoneurons is the synaptic activation, by way of recurrent collaterals, of the small interneurons in the ventral horn known as Renshaw cells after their discoverer (16, 31). Mark (29) some time ago presented indirect evidence that this system remains active during chromatolysis, and we have been able to confirm this. Fig. 10 displays records

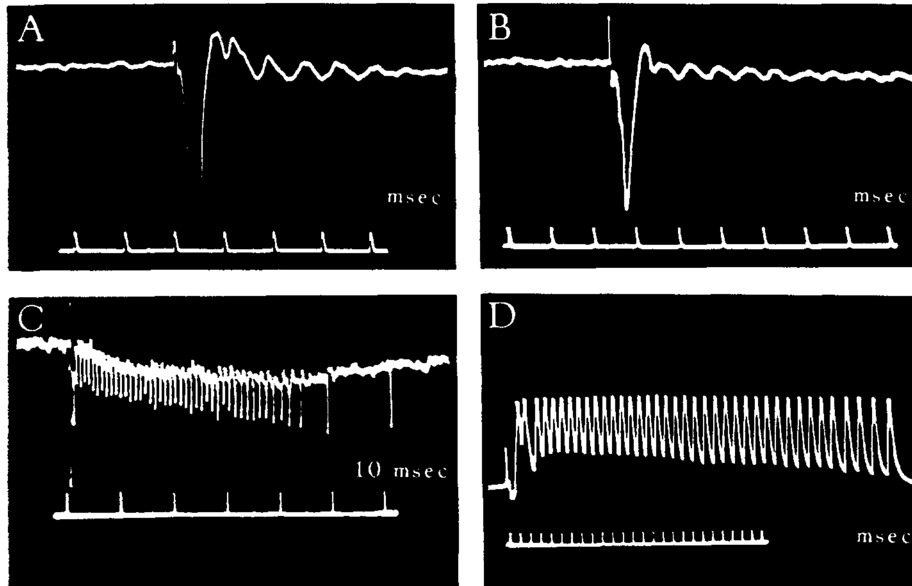

FIG. 10. Responses to antidromic volleys in operatively severed ventral roots. A and B recorded from lateral surface of the S1 segment of the spinal cord in two different preparations; stimulation in each case of the S1 VR which had been severed 24 days before in A, 23 days in B. C and D show the repetitive responses of two Renshaw cells, each evoked by a single antidromic volley in the L7 VR which had been severed 22 days before. Time marks, 1 msec. for A, B, and D; 10 msec. for C.

from which it can be seen that antidromic impulses in the axons of motoneurons undergoing chromatolysis retain the ability to fire Renshaw cells. Records A and B, recorded from the lateral surface of the cord in two different experiments, show the rapid rhythmic waves which signal the firing of many Renshaw cells, in each case set up by antidromic volleys in the S1 VR severed some weeks previously. Records C and D are examples of the spectacular repetitive firing of individual Renshaw cells evoked in another experiment by volleys in the chromatolytic L7 VR. In addition to the intrinsic interest of this finding, showing as it does the apparent full retention of the normal very powerful transmitter action by presynaptic endings derived from

parent neurons undergoing chromatolysis, the observation appears to rule out deficiency of Renshaw inhibition (16) as an explanation for the smaller degree of depression suffered by the second of two antidromic volleys in a chromatolytic motoneuron pool (Fig. 8).

3. Antidromic Responses of Single Motoneurons

Antidromic responses have been recorded from all the chromatolysed motoneurons successfully impaled by microelectrodes, for indeed their identification as motoneurons undergoing chromatolysis depended upon their yielding short latency responses to antidromic volleys set up in the operatively severed ventral root or muscle nerve. All but three of the seventy-two cells so identified generated large spike potentials which overshot the resting membrane potential to a greater or lesser degree (Fig. 11 B). The remaining three yielded smaller non-reversing responses suggesting invasion of the axon hillock region only (6, 15, 21).

Resting Membrane and Spike Potentials.—The difficulty of making reliable estimates of the resting membrane potential of neurons impaled blindly by microelectrodes is familiar to all who have used the method. In addition to errors introduced by the electrodes themselves (2), there must be considerable and uncontrollable variation in the location of, and manner of penetration by, the electrode tip (14). The recorded value in the majority of insertions shows some decline with time, and this decrease may be rapid and substantial; commonly, however, there is a brief initial fall to a plateau which may stay reasonably constant for up to half an hour or more in favourable instances. It has been our impression (and this has also been the experience of Eccles *et al.* (17)), that chromatolytic motoneurons are even more susceptible to microelectrode injury than normal cells, which makes still more difficult the problem of evaluating the true resting membrane potential. This impression is based on the more rapid decline of membrane potential often observed in such motoneurons together with the common appearance of other signs suggesting injury, in particular, spikes of relatively long duration, and abnormality in the sequence of post spike potentials, as described below.

The convention adopted herein has been to assign to each cell the highest membrane potential recorded; in most instances this has been the earliest measurement made after impalement, but occasionally, the highest value has been registered some minutes after insertion of the electrode. The values for resting membrane potential obtained from sixty-nine motoneurons fired by antidromic volleys in chromatolytic ventral roots are shown in the frequency plot of Fig. 12 A; they range from 44 to 83 mv.; the mean value for these cells was 61 mv. The distribution suggests a shift in the direction of lower values than those of normal motoneurons (14); almost certainly, however, the true membrane potentials of many of these cells were higher than the values meas-

ured. The fact that more than half the chromatolytic motoneurons gave values greater than 60 mv., despite their greater vulnerability to injury, makes it seem likely that the true resting membrane potentials of such motoneurons are similar to those of normal cells.

Amplitudes of the antidromic spike potentials recorded from the chromatolytic motoneurons also varied over a wide range. Excluding three cells giving small, non-reversing responses (presumably partial invasion) the spike voltages

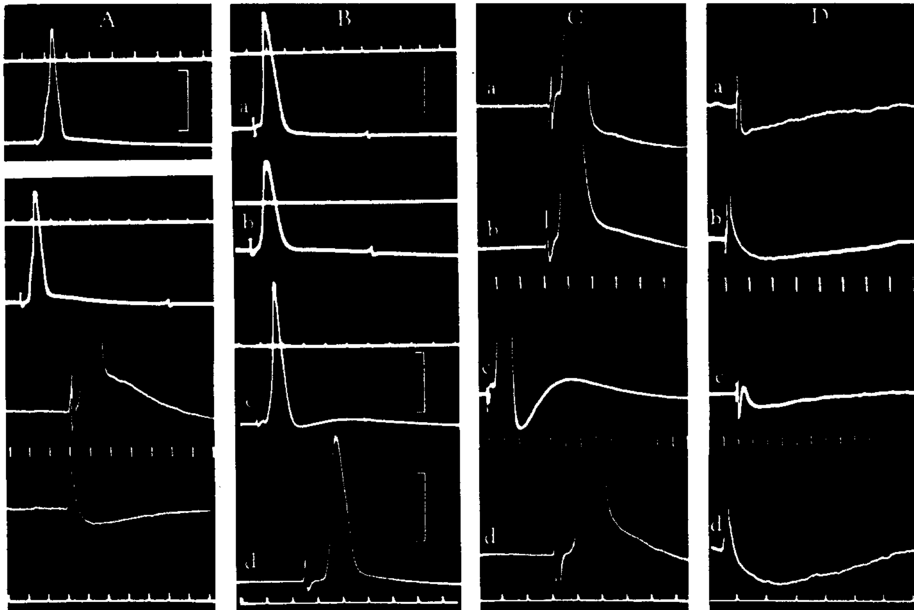

FIG. 11. Intracellular records from antidromically activated individual motoneurons. Those in column A, normal cells; columns B, C, and D display responses of chromatolytic motoneurons. The upper record in A is the response of a flexor hamstring motoneuron; the other three are responses of another hamstring motoneuron in a different experiment, the lower two being taken at high gain and two sweep speeds to show the normal sequence of postspike potentials. Column B, low gain records from four chromatolytic motoneurons; responses of the same motoneurons (designated by the small letters) at high gain and fast sweep speed are shown in C, and at high gain and slower speed in D. a, b flexor hamstring motoneurons in the same experiment, 32 days after operation (in each column, sweep speed the same for a and b); c and d, unidentified motoneurons in different preparations, 34 and 43 days respectively after axotomy. All low gain records except d in column B made with direct coupled amplification; the second beam shows zero potential level. Calibrations, 50 mv., the one in column A applying to the upper two records. Top calibration in B applies to records a and b. Time marks, milliseconds, except for bottom trace in A and all those in D which show 10 msec intervals. Preparations under barbiturate anesthesia.

ranged from 56 to 125 (average 90) mv.; their distribution pattern is shown in Fig. 12 B. It can be seen that the majority exceeded 70 mv. in amplitude, and that three attained 120 mv. or more. Thus at least some motoneurons undergoing chromatolysis can generate spike potentials as large as those of normal cells. As already noted, spike duration tends to be somewhat longer in chro-

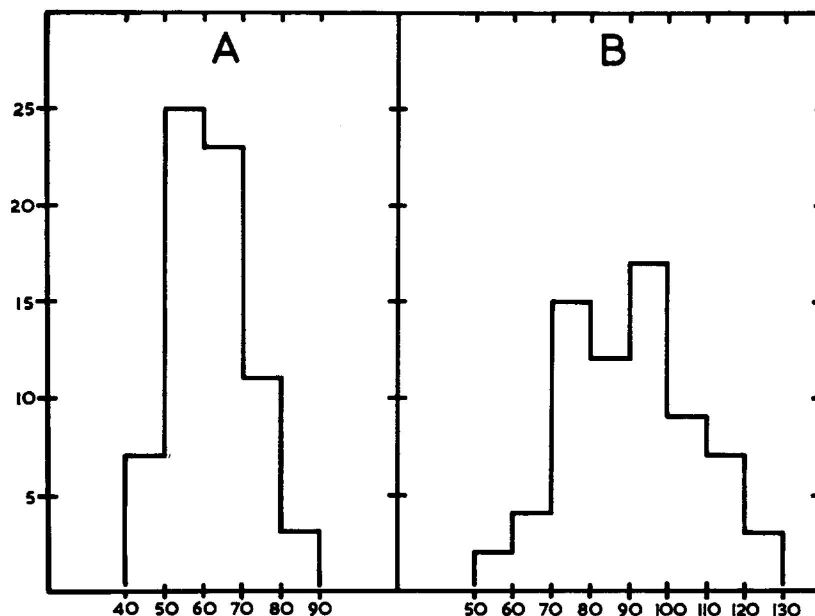

FIG. 12. Frequency plots to show distribution of values for resting potential (A) and action potential (B), the total number of motoneurons being sixty-nine in each case (of the seventy-two studied, resting potential values were not obtained for three cells; another three were omitted from the action potential plot because full sized action potentials did not appear). Ordinates, number of cells giving values within a particular 10 mv. range of potential; abscissa, potential in millivolts.

matolytic cells (1.0 to 1.9 msec.); this may simply reflect susceptibility to injury, since deteriorating "normal" motoneurons also yield spikes of long duration.

Potential Changes after the Spike.—After a full sized action potential, normal motoneurons in good condition show first a phase of slight depolarisation lasting a few milliseconds, then a larger hyperpolarisation which does not subside for about 100 msec. (5, 14, 15). Some chromatolytic motoneurons show the same sequence of potential changes after the spike, but with others, the declining phase of the spike descends directly into hyperpolarisation without an early phase of depolarisation (Fig. 11 C and D). Sometimes there is a

short phase of relative (or even overt) depolarisation after an initial brief trough of hyperpolarisation; thereafter the membrane potential increases once more, returning to the original resting level about 80 to 100 msec. after the spike. All the cells in our series with large, reversing action potentials displayed a prolonged after-hyperpolarisation whether or not an early period of post spike depolarisation was present.

In our earlier experiments, we gained the impression that absence of post spike depolarisation was a characteristic feature of motoneurons undergoing chromatolysis; however, it became apparent later that a large proportion of such cells do show this early phase of depolarisation if their responses are examined at high gain soon enough after impalement. However, it is often small and brief, and tends to disappear within a minute or two, while the onset of hyperpolarisation becomes earlier and steeper.

We have observed that in "normal" impaled cells the post spike depolarisation is extremely labile, being, for example, rapidly abolished by a brief period of asphyxia, and it is well known (5, 14) that its disappearance is an early sign of cell deterioration, with loss of membrane potential, caused by the trauma of microelectrode insertion. Absence or small size of the post spike depolarisation in many chromatolytic cells could, therefore, simply be another indication of a greater vulnerability of such cells to injury. However, this is probably not an adequate explanation, for some of these cells from the moment of impalement yielded responses with no phase of depolarisation after the spike, even when the membrane and action potentials were large. For example, the neuron yielding the upper record of Fig. 11 B had a resting potential of 72 mv. and a spike of 113 mv., yet the latter's descending limb leads straight into hyperpolarisation. One possible interpretation of the post spike depolarisation is that it signals invasion of more distal dendritic branches; should this be so, its deficiency or absence in many chromatolytic motoneurons could be regarded as further evidence of the defective dendritic invasion shown by external recording. However, in preliminary trials we have not observed any augmentation of post spike depolarisation by lowering cord temperature, a maneuvre which increases dendritic invasion (Fig. 9 B and D), and there are other indications that the depolarisation is a manifestation of a true after-potential process (5) whatever other factors such as current flow between different regions of the cell (26) may help to determine its recorded form. It is therefore unlikely that, when present, peculiarities in the intracellularly recorded post spike potentials of chromatolytic motoneurons reflect changes in the properties of distal dendrites. They probably indicate a disorder of the recovery processes in the membrane adjacent to the recording microelectrode, a disorder for which injury by the microelectrode may be partly responsible.

Stages in Antidromic Invasion.—The inflection or step on the rising phase of normal intracellular antidromic action potentials is also visible in the

responses of chromatolytic motoneurons. However, it is less conspicuous, and the potential level at which it occurs is usually some 10 to 30 per cent of the full spike amplitude as compared with the 30 to 40 per cent characteristic of normal cells (Fig. 11 A and B). This lower level of the inflection has also been reported by Eccles *et al.* (17). After the inflection, some of our records show a remarkably steep rising phase (up to 900 volts,sec.), though the falling phase is either within normal limits or else somewhat slower than normal. However, the majority showed no unusual feature except for the low level of the inflection.

The low voltage which initiates the final phase of a chromatolytic motoneuron spike is a clear indication that the region of the cell responsible for this major component of the action potential is more easily fired than normally. Of relevance in this connection is the observation of a low reflex threshold in chromatolytic motoneurons, especially to polysynaptic activation, which makes it seem probable that the region of a motoneuron which generates the second phase of an antidromic spike is also closely concerned with the synaptic generation of impulses.

When two antidromic impulses are set up in the axon of a normal motoneuron, intracellular recording reveals that the second, at a critical interval, usually fails to evoke a full cell spike, the later component failing to appear and instead a smaller, non-reversing spike-like action potential appears (Eccles' "NM" (14), or "IS" (15) response) apparently generated by the part of the cell responsible for the first phase of the full action potential before the inflection. If two successive impulses are backfired into a chromatolytic motoneuron, either the same phenomenon may be seen (Fig. 13 C and D), or more frequently the second impulse fails to set up a response of any size, both principal components of the cell's action potential dropping out at a critical interval leaving only a small deflection (Eccles' "M" response), presumably of axonal origin (Fig. 13 A and B). The response illustrated in the upper record of Fig. 13 D, is remarkable for the long delay at the region of low safety factor before the impulse succeeds in completing its invasion. It recalls the long latency for group I reflex discharge of chromatolytic motoneurons (Fig. 5 B and C).

Different opinions have been expressed as to the anatomical location of the zone of low safety factor for antidromic invasion shown by the inflection on the full action potential and by the partial responses to a second impulse (6, 15, 20, 21). However, in general agreement with Eccles (15) and Fuortes *et al.* (21) it seems to us that the most likely location of this zone is the transitional region between the bouton free axon hillock and the greatly expanded synaptic surface of the motoneuron. On this interpretation, therefore, it must be concluded that the cell body and presumably at least the nearby portions of the coarse dendrites were invaded in the great majority of our impaled chromatolytic motoneurons. Failure of the impulse to invade dendrites at all is a possibility that cannot be excluded by intracellular recordings alone; but pool studies

(25), and Fatt's external recordings of antidromic conduction in single motoneurons (19) provide strong evidence that in normal motoneurons the impulse spreads well up the dendritic tree once the region of low safety factor has been negotiated. Though it must be kept in mind that the degree of antidromic in-

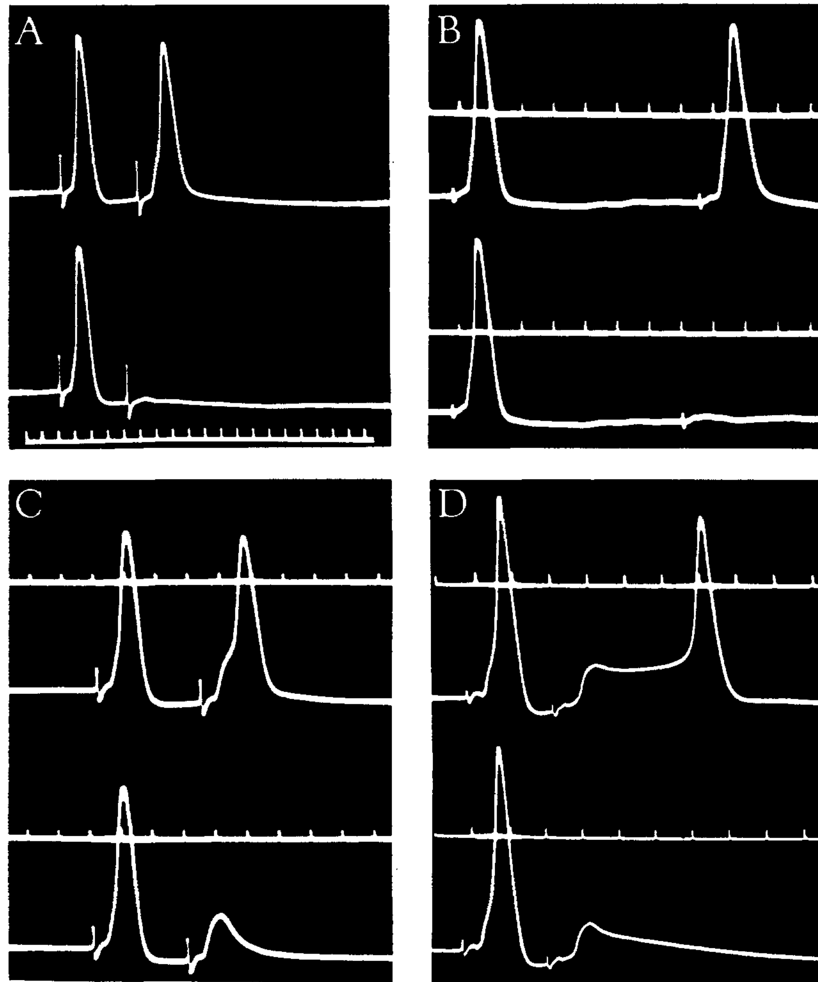

FIG. 13. Intracellular responses of four chromatolytic motoneurons to double antidromic activation, the lower record of each pair at an interval too short to permit a full sized response to the second antidromic impulse. A, B, and D, unidentified motoneurons 43, 26, and 34 days respectively after axon section. C, a flexor hamstring motoneuron 26 days after axotomy. Time marks, milliseconds. Four different preparations, under barbiturate anesthesia.

vasion indicated by intracellular records might in part be a result of the experimental procedure, nevertheless taking the evidence as a whole it is considered likely that spread of antidromic impulses into the soma and coarse proximal dendrites also occurs in most chromatolytic cells even in the intact state. However, the deficiency of externally recorded "*d*" response (Figs. 7 and 9) demonstrates that invasion of the more distal dendrites is usually much less complete than in normal motor nuclei.

Motoneuron Time Constant.—The lower record of Fig. 13 D illustrates an unusual form of response to a second antidromic impulse failing to invade beyond the region of low safety factor. In a number of trials, the declining phase was nearly exponential in form, and from records made at higher amplification the time constant (time to $1/\epsilon$) was estimated to be 2.4 msec., on the assumption of a passive decay of depolarisation beyond the block. The small responses of two of the three cells not fully invaded by a single antidromic impulse also declined in nearly exponential fashion; their time constants were 2.0 and 2.1 msec. These estimates of time constants are also close to the values given by Eccles (15) for normal motoneurons. These observations indicate, in agreement with Eccles *et al.* (17), that the time constant of motoneurons is unchanged during chromatolysis.

DISCUSSION

Reflex Responses of Chromatolytic Motoneurons.—The most clear-cut result of the present work is the demonstration that the delayed, asynchronous reflex firing of chromatolytic motoneurons to group I afferent volleys is generated largely if not entirely by monosynaptic action. The experiments with strychnine and myanesin, together with the intracellular records from orthodromically stimulated motoneurons, show that the increased reflex latency is brought about not by lengthening of the reflex pathway (13) but by slowing of the monosynaptic impulse-generating process. The remarkable fluctuation in the latency for monosynaptic firing of individual motoneurons (Fig. 5 B and C) accounts satisfactorily for the asynchrony and variability in discharge pattern of the responses to group I volleys recorded in a chromatolytic ventral root.

Earlier work in this laboratory (13) provided some evidence that the reflex threshold of motoneurons undergoing chromatolysis becomes lowered, especially to polysynaptic flexor reflex action. The present study has confirmed this impression, flexor reflex responses in a chromatolytic ventral root being consistently more prominent than those in a normal root; and group I reflex threshold is also slightly reduced (Fig. 2). These findings contrast with the results of Campbell, Mark, and Gasteiger (12) who reported an increase in threshold to flexor reflex activation of chromatolytic motoneurons. However, our studies and those of Eccles *et al.* (17) with intracellular microelectrodes have now revealed a reduced threshold for invasion by antidromic impulses

in chromatolytic motoneurons; the latter authors have also shown a reduction of the rheobase in a few such neurons (17). These observations agree well with our findings of a lowered threshold for segmental reflex activation. It is not possible to say whether this lowering is general, applying equally well to synaptic activation from any source, such as the long descending tracts; the deficiency in respiratory discharge of chromatolytic phrenic motoneurons shown by Acheson, Lee, and Morison (1) might suggest an elevation of threshold to some forms of synaptic action. The absence of any striking increase in "spontaneous" firing of chromatolytic motoneurons in our experiments is noteworthy in this context, as it suggests that the lowered threshold to segmental reflex activation might to some extent be compensated by reduced effectiveness of other components of the total synaptic scale.

In one experiment of the present series, the amount of long spinal reflex discharge in the plantar nerves evoked by maximal ipsilateral brachial plexus volleys was compared on the two sides, both nerves on the left side having been operatively severed 20 days previously: there was consistently less discharge on the chromatolytic side. Thus the possibility must be kept in mind that chromatolytic motoneurons might show changes in reflex threshold of opposite character depending upon the source of synaptic stimulation.

Antidromic Invasion of Chromatolytic Motoneurons.—External recording from pools of chromatolytic motoneurons reveals a gross deficiency in the response of the finer dendrites. Campbell (11) in a brief note many years ago pointed out the small size of the surface-negative response yielded by antidromic activation of a motor nucleus in the chromatolytic state, but the significance of the "*d*" response was not known at the time. Intracellular recording, on the other hand, gives no clear indication of defective antidromic invasion, the great majority of chromatolytic motoneurons yielding action potentials as large as those of normal cells.

The only abnormality possibly attributable to a change in dendritic response is the tendency towards deficiency of the phase of depolarisation just after the spike; however, the nature of this deficit of membrane potential in normal motoneurons is not yet understood, and proneness to injury of chromatolytic cells could largely explain our findings.

The failure of intracellular recording to show any obvious abnormality of invasion, despite the deficiency in dendritic response revealed by external recording, emphasises the inability of the intracellular microelectrode method to provide information about the whole motoneuron. Probably the regions in which action can readily be detected by the intracellular method are limited to the cell body proper, the coarse proximal dendrites, the axon hillock, and to some extent the initial segment of the axon.

A Possible Gradient of Responsiveness in Chromatolytic Motoneurons.—The defective antidromic response of finer dendrites stands in contrast with the lowered threshold of chromatolytic cells to reflex and antidromic activa-

tion of the cell body. It would appear that in a large and complex cell such as a motoneuron changes of opposing nature can take place in different regions, in this case the finer dendrites being less responsive while the cell body and probably the nearby coarse dendrites are more easily excited than normally. The simultaneous existence in the same cell of regions with reciprocally altered responsiveness virtually excludes any simple explanation in terms of membrane potential displacements. Unless the core conductor properties of dendrites were abolished, current flow would lead to the collapse of any substantial differences in membrane potential. It would nevertheless be possible to explain the phenomenon by postulating a small over-all reduction in membrane potential together with a large rise in threshold of the peripheral dendrites. However, intracellular records indicate that the enhanced excitability of the cell body region is not a result of depolarisation, but is a true lowering of threshold for the spike-generating mechanism.

If further investigation should confirm the suggestion that reflex threshold to some forms of synaptic excitation is elevated, while to others it is lowered, this could be explained by differences in the spatial arrangements of the respective presynaptic endings together with a gradient of responsiveness in dendrites. For example, the segmental reflex presynaptic endings with enhanced effectiveness might predominate on the cell body and coarse dendrites, the threshold of which appears to be lowered to antidromic and direct electrical excitation; while other endings might have a subnormal reflex effect by virtue of their location on more peripheral dendritic elements of reduced responsiveness. Such speculations, however, should not be taken seriously until more factual information is available.

Monosynaptic Reflex Action.—As described above, monosynaptic excitatory action begins in chromatolytic motoneurons as early as it does in normal cells, but takes longer to reach its maximum effect. Furthermore, there appears to be an over-all deficiency of transmitter action, for should this be normal the lowered motoneuron threshold would lead to a much greater effectiveness of monosynaptic action than is actually observed. It does not seem possible to explain the long delay in monosynaptic reflex firing of chromatolytic motoneurons in terms of a simple change in electrical properties of the postsynaptic membrane, for the motoneuron time constant appears to be unaltered. The slowing and lowered intensity of action must reflect abnormality in the operation of the presynaptic terminals or in the responsiveness of the subsynaptic membrane to the transmitter agent. Presynaptic function could be disturbed by structural or metabolic changes in the closely contiguous neuron or in the surrounding glia cells; for example, the invasion of many fine terminal presynaptic branches might be retarded, asynchronous, and incomplete, or the output of a chemical transmitter agent reduced despite normal invasion of terminals. Although there is little direct evidence bearing on this possibility, the normal "focal synaptic potential" recordable from a chromatolytic motor

nucleus (Fig. 7, reference 13) could be regarded as evidence against the existence of significant change in the time course or synchrony of presynaptic action (28). Furthermore, the general similarity in form of the input-output curves for normal and chromatolytic motoneurons (Fig. 2) is an indication that the group I terminals ending upon the abnormal cells retain normal function.

Alteration in the responsiveness of chromatolytic motoneurons to essentially normal presynaptic action appears to be a more likely explanation for the abnormalities in monosynaptic excitation, and this has been shown to be the case for sympathetic ganglion cells after section of their axons (9). However, there is no direct evidence in the case of chromatolytic spinal motoneurons, and much further work will have to be done before their abnormalities can be adequately explained.

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