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DELAYED DEPOLARIZATION AND THE REPETITIVE RESPONSE TO INTRACELLULAR STIMULATION OF MAMMALIAN MOTONEURONES

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The present work was undertaken in order to investigate quantitative and qualitative aspects of tonic firing of motoneurones by the intracellular technique, being in this respect a sequence to studies of maintained unit discharges in ventral roots pursued over some years (e.g. recently by Granit & Rutledge, 1960, and Granit & Renkin, 1961). On the basis of the experiments by Araki & Otani (1955), Coombs, Curtis & Eccles (1957*a*, *b*) and Fuortes, Frank & Becker (1957), the axon hillock (A- or IS-zone) is nowadays visualized as a specific firing zone of low electrical threshold. The latter makes it, as it were, so sensitive to depolarization that its spike activates the rest of the motoneurone antidromically. If this also be true for tonic discharges, one is curious to know why the soma-dendrite region (B- or SD-zone) is invaded at all to deliver an ionic display, or, at least, what this region thereby might contribute to the sum total of events, because—on the theory—synaptic knobs would be expected to occur preferentially on the axon hillock, where, in reality, they are known to be absent or scarce. Quite consistently Coombs *et al.* (1957*b*) have also concluded that remote synapses are functionally ineffective. It is not a particularly attractive notion that demonstrable structures because of geometrical reasons would be lacking functional significance and this *a priori* standpoint has recently been strengthened by the work of Hild & Tasaki (1962) on tissue cultures of cerebellar neurones. By stimulating a visible dendrite they found impulses started 100 μ from the soma to be capable of travelling and eliciting a soma spike.

Most of our experiments were performed on rats, though specific points were checked with cat motoneurones. The feasibility of using rats has recently been demonstrated by Bradley & Somjen (1961). They observed that after-hyperpolarization as a sequence to the spike was smaller there

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than in cat motoneurones. This, we find, is partly due to delayed depolarization, as seen with responses to single antidromic shocks. In tonic firing to inside stimulation the slow depolarization is replaced by after-hyperpolarization. The transition between these two forms of activity will be investigated below and the nature of delayed depolarization analysed. The work has led to the conclusions that delayed depolarization is an invasion of the dendrites and that a repetitive discharge to inside stimulation is a mode of operation quite different from the one seen in response to antidromic stimulation. The next paper (Granit, Kernell & Shortess, 1963) will be devoted to other aspects of rhythmic firing in response to inside, orthodromic and antidromic stimulation.

METHODS

A full description of how to operate upon and set up an anaesthetized rat for intracellular investigation of motoneurones has been given in the *Journal* by Bradley & Somjen (1961) and this was found very helpful. We depart from their technique merely in giving the rat pre-operatively a dose of 60–80 μg isodrine (paredrinol) in order better to maintain blood pressure, and in using an oxygen mixture containing 1% instead of 5% CO_2. Use of isodrine was a relatively late addition to our technique and none of our results were fundamentally influenced by it. Often air alone was supplied by the pump. We have found our best preparations to survive from 5 to 12 hr. When they last so long it will occasionally be necessary to add a little pentobarbitone to the original dose of 55 mg/kg, sometimes also to add D-tubocurarine.

The rat is 'somewhat poikilothermous' (Donaldson, 1924). When ready for experimentation it generally had a rectal temperature of between 36 and 37° C. When, in spite of the heating pad below the suspended animal, its temperature gradually fell to 32° C, this heralded the end of the experiment. A temperature of 34° C is not abnormal in rats.

The majority of our preparations have been both de-efferented and de-afferented from L3 down into the cauda equina. Segment L4 has been used (cf. below). In some cases we have stimulated the sciatic nerve of de-afferented rats. In the rat the lumbar cord is supplied with blood from a main artery whose branches enter in the thoracic region (Sugar & Gerard, 1940). For this reason the rat is a better preparation than the cat for experiments involving complete lumbar root section.

The circuit for stimulating through the intracellular electrode, like similar circuits used by many workers, was modelled on that of Araki & Otani (1955). It has been described and discussed by Frank (1959). Since the main problems were concerned with spike counting and with the slow phenomena subsequent to the spike, we have taken no steps to compensate for capacitative attenuation of spike height (cf. discussion by Eccles, 1957; Frank, 1959). There is probably no more sensitive criterion for the quality of a penetration than that the motoneurone can be made to respond to intracellular stimulation in a maintained fashion over some length of time. All our results have been checked on such motoneurones, the problem of repetitive firing having been in the centre of our interest. Membrane potentials have been measured from time to time but not regularly. We are generally in this paper concerned with spikes from 50 to 80 mV with overshoots between 10 and 20 mV. The record time for maintained repetitive firing to inside tests in one neurone has been 3 hr. The measurements on rat motoneurones given by Bradley & Somjen (1961) fully cover relevant points.

The stimulator for producing rectangular currents has been of the standard type in this laboratory and designed by Frankenhaeuser. Standard KCl micro-electrodes (5–10 MΩ)

were generally used, but a number of experiments were carried out with potassium citrate electrodes. In any one preparation one may succeed in penetrating from 15 to 30 motor cells, but most of the cells do not respond with spikes of constant size for long. Cells giving merely post-synaptic potentials have been rejected as they have been of little interest from our points of view.

A number of cats, de-afferented and de-efferented from L6 to S1, were studied for comparison. The anaesthesia used was either a mixture of pentobarbitone 20 mg with chloralose 20 mg/kg or else pentobarbitone 40 mg/kg alone. Some cats were decerebrated.

RESULTS

Size of motoneurones

It is of some interest in view of comparisons with cat motoneurones to know the number and average size of the large motoneurones in the rat. The main supply to the sciatic nerve is from segment L4, but L5 and L6 also contribute. In Fig. 1 a histogram is presented of cells from L4 and

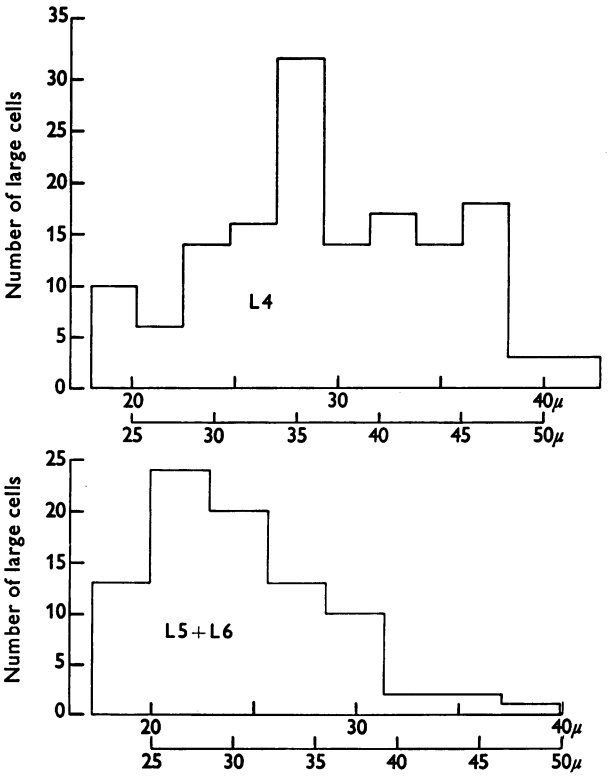

Fig. 1. Histograms of diameters of large cells (see text) in segments L4 and L5+L6 in rat ventral horn. Line below abscissa is a re-scaling of the data on the assumption of a 20% shrinkage.

from the two segments below it, based on measurements of two diameters at right angles. The average values so obtained are plotted against cell frequency. Below each histogram is found another scale based on the assumption of a 20 % shrinkage of the cells.

The largest motoneurones in the cat are stated to be 50×70 μ in diameter (Rexed, 1952). The rat motoneurones are definitely smaller and probably, too, there is a smaller number of large cells in the rat ventral horn L4 than in the corresponding sciatic projections of the cat (L6–S1). Our results suggest that it would be valuable to possess similar histograms for cat motoneurones in the various segments.

In comparing motoneurones of rats and cats fibre diameter and conduction velocity can be adduced as additional clues to size of cells. Leksell (1945) in his work on gamma motoneurones made a large number of estimates of alpha conduction velocities (knee to root entry) and found wave fronts conducted at between 90 and 115 m/sec. In the rat our fastest fibres group themselves around 85 m/sec but with stronger shocks one may see a massive secondary hump at rates between 50 and 60 m/sec. According to work in course of preparation by A. Mellström and S. Skoglund (personal communication) the largest efferent fibres in the rat sciatic nerve are of the order of 12–14 μ in diameter. Applying a conversion factor of 6 to these figures (Gasser & Grundfest, 1939) leads to reasonably good agreement between anatomical and physiological data.

The intracellular response

In Fig. 2 the responses are antidromic, with the exception of 3*b* and *c*; all calibrations are to 20 mV but highly amplified spikes exceed the proportionality range of the oscillograph. Such sensitivities are used to display the slow changes succeeding the spikes. There the most conspicuous feature is the delayed depolarization which sometimes is preceded by a slight notch at the foot of the completed spike. Delayed depolarizations of this type seem to be common also with amphibian motoneurones (Araki, Otani & Furukawa, 1953; Fadiga & Brookhart, 1960). After-hyperpolarization is small, as has already been pointed out by Bradley & Somjen (1961). With the exception of spike 6, which specifically is meant to illustrate a small spike (47 mV), the others range from 60 to 80 mV. Spike 6, possibly from a depolarized cell, also has the largest after-hyperpolarization of the lot and looks like a replica of the cat motoneurone illustrated in Fig. 7 (p. 445) of the paper by Brock, Coombs & Eccles (1952). The others can be said to represent typical rat motoneurones in segment L4.

Eccles (1957) discusses delayed depolarization as an after-depolarization,

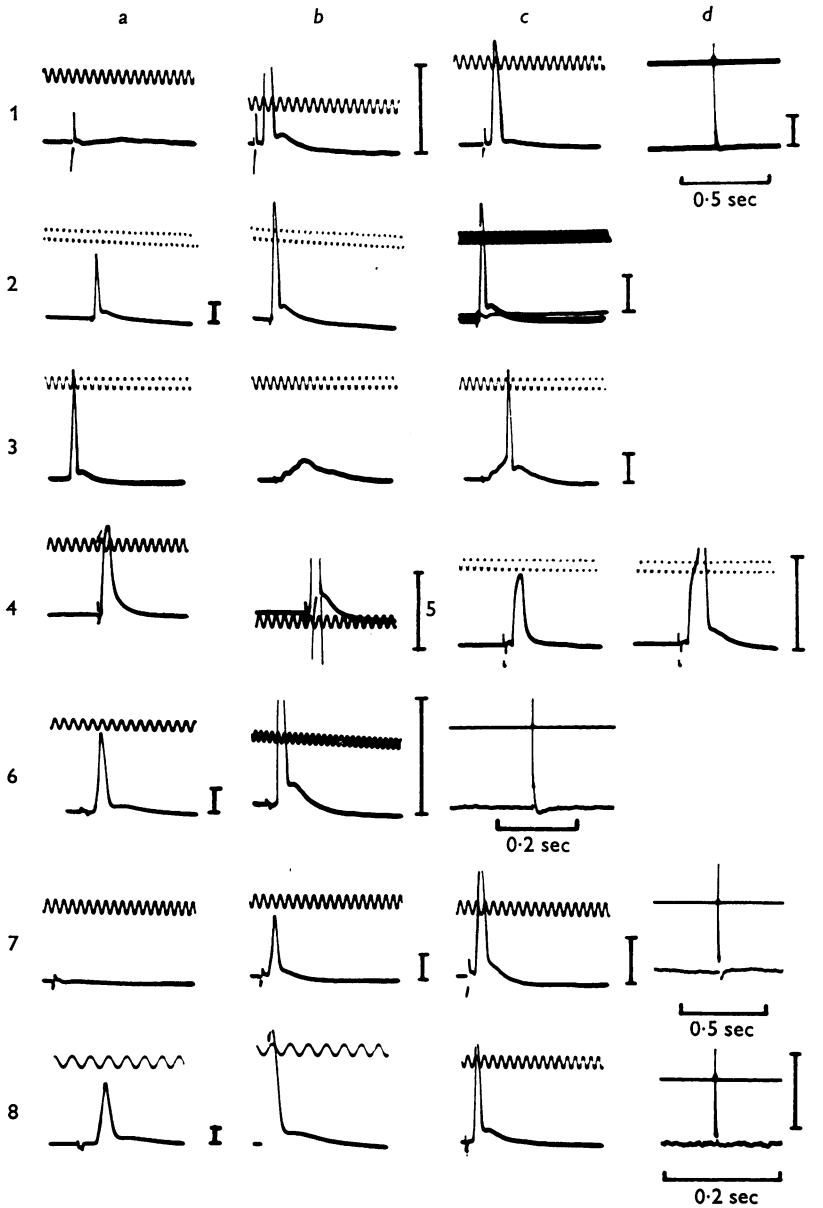

Fig. 2. Sampling intracellular records from eight ventral horn cells in rat segment L4, all being antidromic responses except 3*b* and *c*, which are orthodromic ones around threshold. Calibrations to 20 mV and time in msec unless otherwise marked. 1*a*, subthreshold, *b*, *c* and *d* at threshold; 2, threshold responses, note threshold play in 2*c* at standstill of film; 4*a*, IS spike alternating with *b*, full-size spike; 5*c* and *d*, similar pair but another motoneurone; 6, antidromic response elicited from sciatic nerve; 7*a*, subthreshold, *b*, *c*, *d* threshold responses; 8, the largest spike shown (81 mV); after-hyperpolarization invisible in spite of considerable amplification (cf. also 1*d*, 2*c*, 6*c* and 7*d*).

but in the same paragraph also states (p. 83): 'it is not possible to offer any experimental evidence which leads to an explanation of the brief phase (about 2 msec to 6 msec) of depolarization that lies normally between the end of the spike and the onset of the after-hyperpolarization'. In rats it tends to be visible up to 5 msec from the initiation of the spike and to reach a maximum whose magnitude depends on the membrane current (see below). The maximum value observed in this work during inside hyperpolarization was 10 mV (Fig. 6*B*). Measured from the foot of the rising spike its duration was then 8–9 msec.

Delayed depolarization (DD), a neutral term to be preferred since after-depolarization suggests definite events, is elicited by the soma-dendrite spike or by whatever causes the latter to rise. In Fig. 2, 1*a* and 1*b* as well as 7*a* and 7*b* illustrate just subthreshold and threshold responses, while 2*c* shows threshold play during temporary standstill of the film for a few sweeps. These records serve to exclude the possibility of recurrent facilitation (Renshaw, 1941, 1946; Wilson, 1959) as an explanation of DD. Records 4*a* and 5*c* show the so-called IS or A spike, in each case followed by full size soma-dendrite or B spike. Only the latter possess the large component of DD. This always reaches its full magnitude with threshold antidromic shocks.

In 3*a* and 3*c* antidromic and orthodromic threshold responses are compared, while 3*b* illustrates the post-synaptic potential that just failed to elicit the spike. The records have been inserted to demonstrate the fact that orthodromic spikes have not been found wholly to eliminate the post-synaptic potential. If threshold conditions are strictly adhered to, the residuum of slow changes may largely consist of DD which slowly is replaced by a small after-hyperpolarization. The threshold response of the cat motoneurone, on the contrary, is held to destroy the post-synaptic potential and end up in an after-hyperpolarization (Eccles, 1957). Such motoneurones are found also in the rat, especially among those with small spike potentials likely to have been injured by the penetration.

The largest motoneurone in Fig. 2 is no. 8 (81 mV), which is practically without after-hyperpolarization but has a definite DD. This is one that served for $2\frac{1}{2}$ hr in the experiment and so cannot well be called abnormal. Of considerable interest from this point of view is the antidromic response of the *cat* motoneurone in Fig. 3 which in the beginning (1*a*) responded with a spike of 48 mV, magnified in 1*b* and 1*c* to demonstrate the typical after-hyperpolarization. It grew while being observed to the maximum in 2*a* of 80 mV, and was then recorded at the higher sensitivity in 2*b* and 2*c* to show that it now had a good delayed depolarization not followed by much after-hyperpolarization. In fact, it now looked exactly like the majority of rat spikes. This cat was a decerebrate preparation.

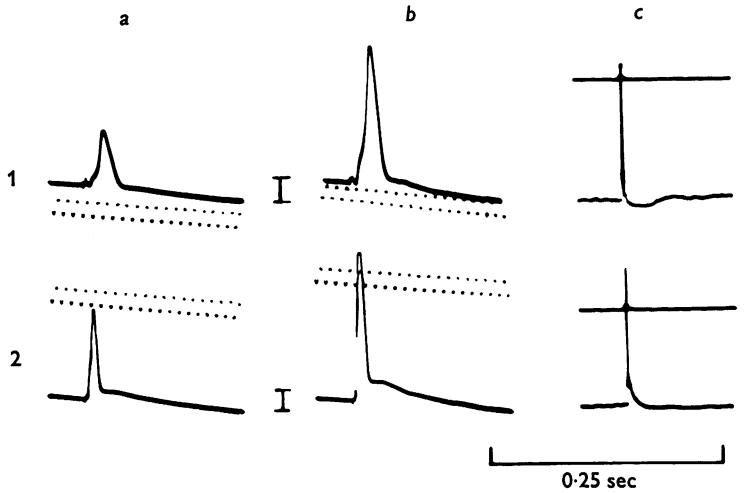

Fig. 3. Motoneurone in decerebrate cat stimulated from efferent root L 7. Calibration to 20 mV of 1*a* and 2*a*, time in msec unless marked. Soon after penetration (records 1) this motoneurone gave a response of 48 mV, later increasing (records 2) to 80 mV. Records *b* and *c*, at 2·4 times increased amplification, inserted to demonstrate sequence after spike. The increase in spike size from 1 to 2 was accompanied by diminution of after-hyperpolarization (records *c*) and increase of delayed depolarization (records *b*).

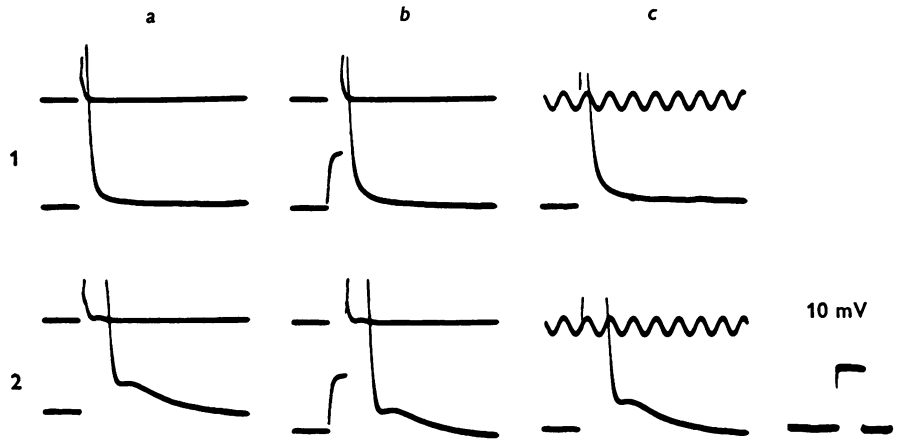

Fig. 4. Rat motoneurone. Threshold responses to inside depolarization lasting (*a*) 0·5 msec, (*b* and *c*) 0·12 msec, the latter (*c*) with time (msec) replacing current recorder. Records 1 are just below, records 2 just above, threshold. The full-size spike was at 66 mV.

In Fig. 4, records 2, DD is produced by a brief inside shock through the electrode. Records 1 are just subthreshold for the spike. We never succeeded in producing DD from the inside except as a sequence to full-size soma-dendrite spikes.

To sum up, delayed depolarization behaves as an all-or-none event elicited by the soma-dendrite spike or whatever process is responsible for the latter. Perhaps it merely reflects the constant spike height.

Influence of current strength and polarity

If an antidromic spike was generated during simultaneous stimulation from the inside, DD increased with inward currents (inside −) and decreased with outward currents (inside +). Figures 5 and 6 show two different types of response, one with a negative notch (6*B*), the other without a conspicuous negative notch (5*B*) in the records marked Cont(rol). Looking first at the curves (Figs. 5*A* and 6*A*) relating magnitude of DD to current strength, clearly it has been possible to draw reasonably straight lines through the values for the range of currents within which changes in size occur. Similar results have been obtained by Araki (1960) with toad motoneurones. Another matter is, then, whether this result really means that the curves approach an equilibrium on the right in the Figures. With large hyperpolarizations, at the limit of blocking the antidromic spike (Coombs *et al.* 1957*a*; Fuortes *et al.* 1957), DDs in Figs. 5*B* and 6*B* are large and start without a preceding dip. With increasing depolarization the DDs are preceded by a dip increasing in size to a maximum, better visible in Fig. 6*B*, while delayed depolarization no more is seen above the base line, but may well be contained within the rapid rise towards it. This is the 'negative rebound' described by Araki *et al.* (1953) in slightly depolarized toad motoneurones, the nature of which they regard as unknown. In particular, it proved impossible in Fig. 6*B* to assign zero level to a definite current strength because DD merely took on another aspect and did not change with further increase in current strength from 3 to 5 of Fig. 6*B*. Stronger currents (record 5) would induce repetitive firing (see below) or offset amplifier balance in a disturbing manner.

Influence of shock interval on DD

Depending upon its magnitude and duration, delayed depolarization (which may vary from neurone to neurone) will mask after-hyperpolarization more or less effectively. This and the question of additivity are studied in Fig. 7. Record 1 is the control spike to an antidromic shock, 2 is an inside depolarization and 3 the response to the same current strength but of inverted direction (inside −). The currents were symmetrical and so, in 2, there may be a small local response superimposed on the charging of

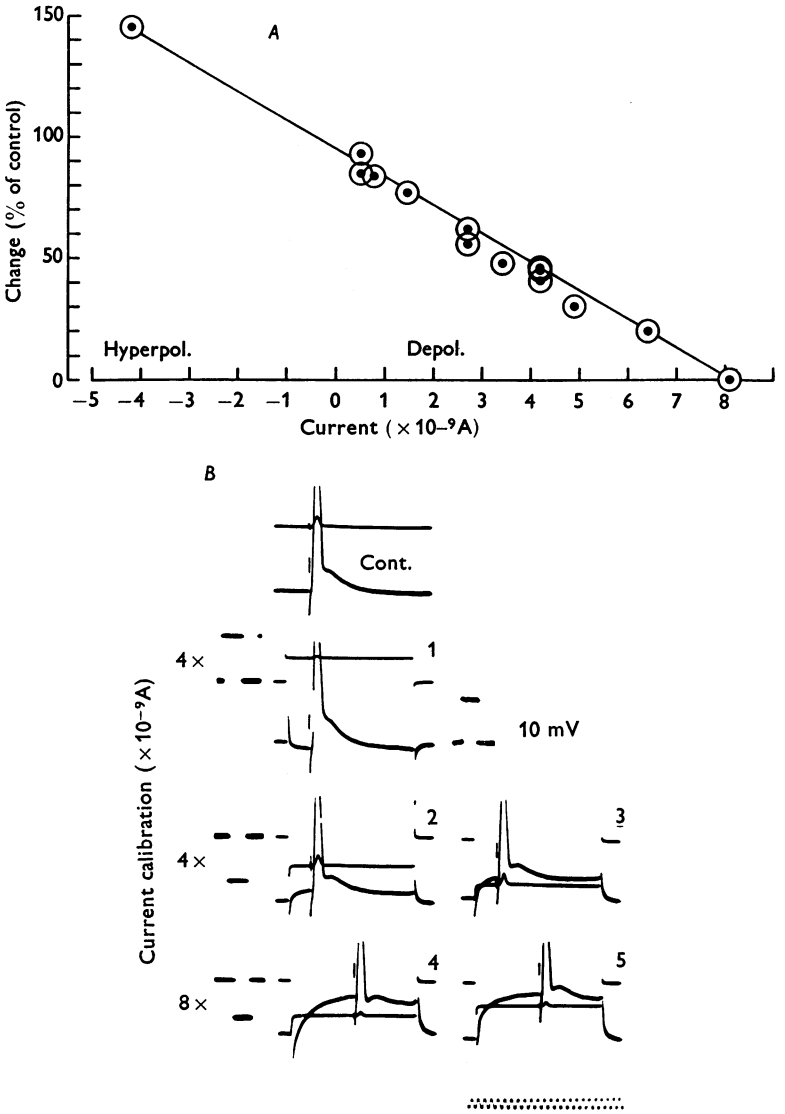

Fig. 5. *A*, plot of full experiment, samples of which are given in *B*. Spikes antidromic. Curve shows magnitude of delayed depolarization in percentage of control as function of current intensity and direction. *B*, sample records (note calibrations of current and voltage) of control (Cont.), hyperpolarization (1) and depolarization (2–5); 3 and 5 represent much the same current strength but location of antidromic spike was shifted. Size of spike 56 mV, time msec. Note that current recorder also records the passage of current during spike response.

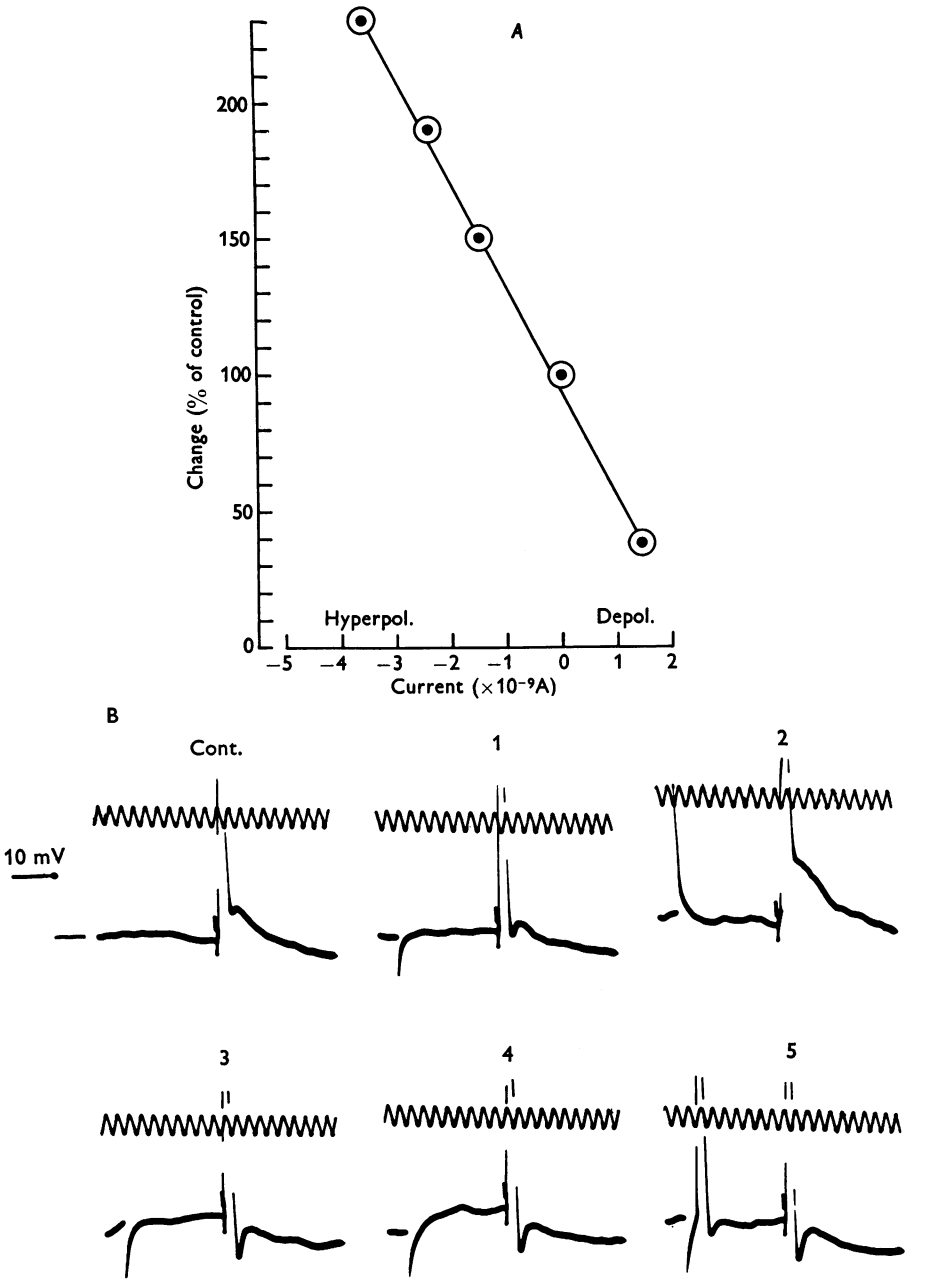

Fig. 6. *A*, diagram, as in Fig. 5*A*, but with another spike, samples of which are shown in 6*B*. Spike size 75 mV. *B*, samples from experiment plotted in 6*A*. 1, current strengths for the records in $\times 10^{-9}$ A are: (Cont.), zero; (1), 1·47; (2), −3·54; (3), 3·84; (4), 5·90; (5), 6·50. Note that current in (5) is strong enough to elicit spike. Time in msec.

the membrane capacity. In 4 the response to depolarization (2) is displayed with current recorder replacing time. In 5 current strength was slightly increased so as to elicit a threshold inside response generating a spike.

Record 6 (next vertical row) shows the response to a brief but stronger shock. Spike and DD are practically identical with those of the antidromic response (1). In both cases after-hyperpolarization was lacking (at the amplification used). When, in 7, duration of current was extended to the end of the sweep, the base line stayed up, but (records 8–10) reached its original level soon after current was interrupted.

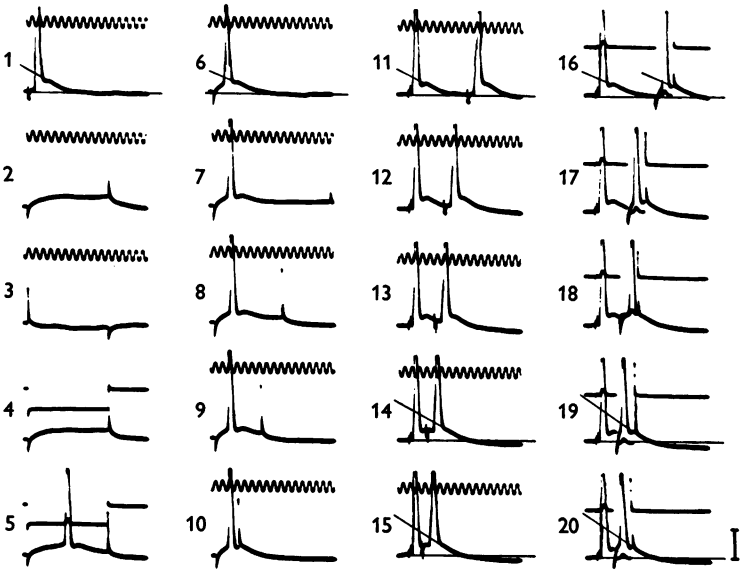

Fig. 7. Rat motoneurone, spike size 81 mV. 1, antidromic control; 2, subthreshold depolarization; 3, response to same current inverted; 4, same as 2 but with current recorder measuring $4{\cdot}4 \times 10^{-9}$ A; 5, spike elicited when current increased to $4{\cdot}6 \times 10^{-9}$ A; 6, response to very brief intracellular depolarization; 7–10, variations in duration of stimulating current as shown by artifacts; 11–15, double antidromic shocks at decreasing intervals; 16–20, antidromic spike succeeded by spike elicited by inside depolarization as in 10 and following antidromic shock at decreasing intervals. All times in msec. Calibration to 20 mV. The lines accentuating rate of decay of delayed depolarization and after-hyperpolarizations below base line were inserted into magnified copy of this figure.

In the series 11–15 a second antidromic shock follows a conditioning shock from another stimulator. The important point brought out is that the delayed depolarizations never summed: in fact refractoriness prevailed and the second shock now elicited a spike that was followed by after-hyperpolarization. The same is seen in records 16–20 in which an antidromic shock is followed by an inside shock to the motoneurone, both

eliciting spikes. Not only were the after-hyperpolarizations increased in magnitude at brief shock intervals but their rate of fall after the foot of the second spike likewise became accelerated.

Double antidromic shocks were used also in the experiment of Fig. 8 with a motoneurone of 40 mV. As the second shock approached the first, the after-hyperpolarization increased and the DD diminished just as in the previous motoneurone of twice larger potential. When in 2*d* the second spike was replaced by an IS-spike there was no increase of after-hyperpolarization.

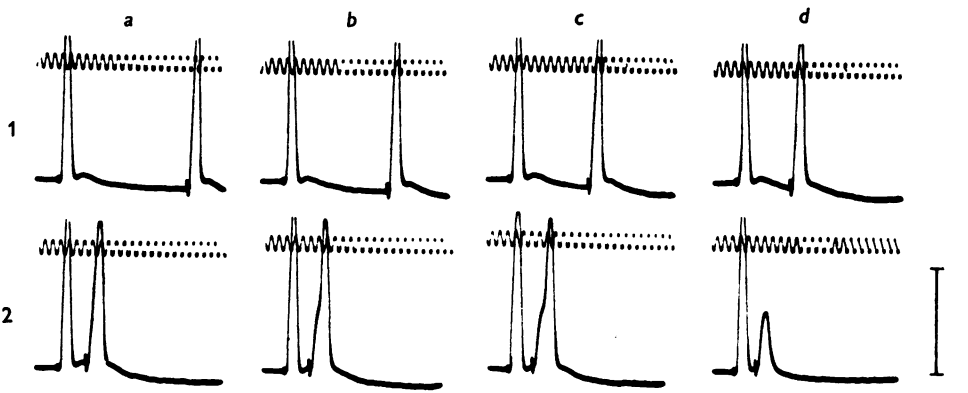

Fig. 8. Rat motoneurone of 40 mV stimulated by two antidromic shocks at intervals increasingly shorter from 1*a* to 2*d*. Calibration to 20 mV, time in msec. Note diminution of delayed depolarization at short intervals and increase of after-hyperpolarization until in 2*d* second spike fails to invade soma-dendrite zone. This spike has then neither DD nor after-hyperpolarization.

It is concluded, therefore, that delayed depolarization, even if it were non-homogeneous and consisted of several components, has a wholly dominating component that behaves as if it were an all-or-none slow cell response possessing refractory period. According to Coombs *et al.* (1957*a*, *b*) the small after-depolarizations following IS-spikes are summative. The large after-depolarizations in hippocampal pyramids are also cumulative (Kandel & Spencer, 1961). The absence or decrease of DD at brief shock intervals may suffice to explain the increase in after-hyperpolarization at short intervals. It would merely be unmasked by the disappearance of DD. Under the circumstances there should be no increase of after-hyperpolarization (Coombs, Eccles & Fatt, 1955), but, if present here, it may have reduced DD.

The rhythmic response

In this paper we restrict ourselves to the question of whether the two slow potentials undergo a change of relative emphasis in tonic firing to

inside stimulation. Figure 9 presents the reply. The rhythmic response of 1*d* to inside stimulation was repeatedly elicited. There was then some variation in the initial response frequency, as seen from the sweeps 1*a*, 1*b* and 1*c*. The feature to be noted there is that the late responses have begun to display prominent after-hyperpolarization and that quite regularly

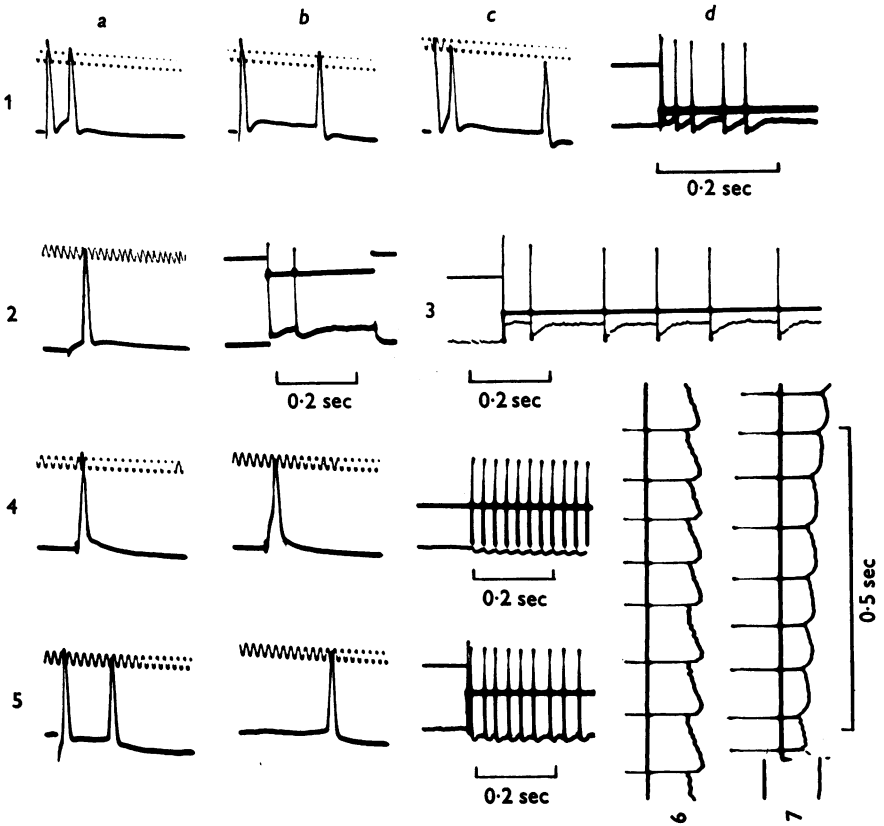

Fig. 9. Rat motoneurones (1–6) and cat motoneurone (7). Time in msec unless otherwise marked. Intracellular stimulations through micro-electrode. 1*a–c*, sweep records of initial phase of repeated depolarizations (current $19{\cdot}2 \times 10^{-9}$ A), one of which shown in 1*d*. Spike, 72 mV. 2, *a* and *b*, similar records of another spike (50 mV) responding to threshold current $3{\cdot}2 \times 10^{-9}$ A. Note large after-hyperpolarization in second spike (2*b*) as compared with first spike (on sweep in 2*a*). 3, spike (68 mV) responding to current intensity $15{\cdot}3 \times 10^{-9}$ A. 4 and 5, same spike (75 mV) stimulated antidromically in 4*c* at rate 60/sec and by inside current of $11{\cdot}4 \times 10^{-9}$ A in 5*c*; 4*a* and 5*a* are the initial spikes in the two cases, 4*b* and 5*b* later spikes. In 4*b* cell then responded at rate 30/sec, in 5*b* at 36/sec. 6, rat motoneurone (92 mV) firing irregularly over some time to threshold current of $2{\cdot}0 \times 10^{-9}$ A, to be compared with 7, cat motoneurone (54 mV) discharging to near-threshold current of $8{\cdot}4 \times 10^{-9}$ A, inserted at onset of record to show development of after-hyperpolarization.

delayed depolarization has greatly diminished or even disappeared. Records 2 and 3 again emphasize this for threshold repetitive responses, and extensive studies of tonic firing confirm our finding (Granit *et al.* 1963). Records 4 and 5 show the same spike fired antidromically (4) and from the inside (5). The first spike of the discharge in 4*c* to repetitive stimulation at 60/sec is illustrated in 4*a*. When after a while the cell merely could respond at a rate of 30/sec (cf. Brock, Coombs & Eccles, 1953), the response still presented the same general appearance. It is displayed in 4*b* and consists of spike and DD. Quite generally, whatever the rate of antidromic stimulation, as long as full-size spikes occurred, they were followed by DD and after-hyperpolarization was then small. By contrast, repetitive firing to inside stimulation, as in records 5, always led to extreme diminution or disappearance of DD which was supplanted by after-hyperpolarization. The juxtaposition of repetitive responses to antidromic and inside stimulation in records 4 and 5 of Fig. 9 strikingly emphasizes the two different functional states of the cell, quickly recognized at a glance by the shift from delayed depolarization to after-hyperpolarization. The antidromic responses remain unaltered because the cell cannot, to a sufficient degree, be depolarized antidromically whatever the rate of repetitive stimulation. High rates, of course, soon lead to block.

Once tonic firing has started, cat and rat spikes behave in the same general way and in our material the only difference between them is that, in the cat, after-potentials on the whole are both larger and possess longer duration. On the other hand, it seems possible to select two records of cat and rat cells respectively from an overlapping zone of properties in which the after-hyperpolarizations approach each other in size though durations always tend to be longer in the cat (which apparently has a more efficient organization for recurrent inhibition). Thus record 6 of Fig. 9 is the threshold rhythmic discharge of a large spike (92 mV) in the rat, and record 7 the corresponding picture of a fairly small spike (54 mV) in the cat which by chance (half a year later) happened to be photographed at amplifications giving much the same spike height. The after-hyperpolarization in the rat spike would be larger here, but in the cat spike it still had a longer duration.

With regard to the relative prominence of delayed depolarization and after-hyperpolarization it therefore looks as if in the cat the motoneurones from the beginning were nearer to the state into which, in the rat, they are brought after some period of depolarization leading to rhythmic activity. In Fig. 9 it can be seen that the change from the one state to the other always requires some time (records 1–3) but analysis of transition time and its significance falls outside the scope of the present paper. After-hyperpolarization generally tends to increase during the first few discharges of the depolarized cell. Along with depolarization, firing as

such seems to be important. It is likely that close study of the process of transition in cat motoneurones would reveal the same sequence of events there, too, once attention now has been drawn to it with the aid of a more favourable preparation (cf. Fig. 9, record 7).

Temperature, anaesthesia. These are not likely wholly to explain the difference between the two species. Cats have been given pentobarbitone 40 mg/kg or else half the dose has been chloralose. Rats have received pentobarbitone 55 mg/kg but would metabolize it at a faster rate than cats. If their temperature as a rule has been lower, this would rather tend to increase excitability (Koizumi, Ushiyama & Brooks, 1960). Delayed depolarization has, however, been seen both in the beginning and at the end of a day. Anaesthesia in the work on cats by others has probably, on the whole, been deeper than in our experiments with rats.

Inactivation

When this process was found by Granit & Phillips (1956) as a normal event in cerebellar Purkinje cells, they concluded from their intracellular records that the membrane temporarily became 'hyperdepolarized' so that the generative process was inactivated (Frankenhaeuser, 1952; Hodgkin & Huxley, 1952). Inactivation by gradual depolarization to excessive degree had, at the time, been seen by Eyzaguirre & Kuffler (1955) in the crayfish's stretch receptor excessively stretched. von Euler & Green (1960*a*, *b*) then found the same process in hippocampal pyramids (extracellular), where they on good evidence concluded that it was responsible for the well-known theta waves of this structure and that it thus could occur in the mammalian central nervous system as a normal regulatory mechanism. (Inactivation has since been seen in several invertebrate structures.) Kandel & Spencer (1961) studied the hippocampal pyramids intracellularly. In these cells after-depolarization occurs and cumulates with repetitive stimulation until the cell is excessively depolarized and by inactivation quenches the burst of impulses which appears to be a characteristic feature of activity in these cells. There is no after-hyperpolarization and so the sequence of bursting, leading to inactivation succeeded by repolarization, can go on as theta waves in the manner demonstrated by von Euler & Green (1960*b*).

In rat motoneurones, stimulated from dorsal roots, the post-synaptic potentials are large and after-hyperpolarization small, unless the neurone is set for rhythmic firing of the kind described above. Thus conditions are favourable for 'inactivation' with strong dorsal-root shocks. Towards the end of the life of an intracellularly recorded spike, inactivations are quite common and records such as those of Fig. 10 show the process gradually developing. In some cases it proved possible to demonstrate that inactivation turned up during base-line drift with spike diminution, indicating fall of membrane potential. As an instance might be mentioned a motoneurone of 54 mV, maintained under antidromic stimulation, so that spike

height could be followed continually. Orthodromic shocks were repeated at regular intervals. When the size of the spike had fallen to 46 mV in compensation for an equivalent drop of membrane potential, the test shocks began to inactivate the cell in a regular manner. The records then became a replica of those shown in Fig. 10. Since there are cat motoneurones very much like the typical rat motoneurones we have not been surprised when occasionally inactivation has been seen also in some cat motoneurones. Repetitive firing to single orthodromic shocks has also been seen with flexor motoneurones in the cat (Wilson & Burgess, 1962; Perl, 1962).

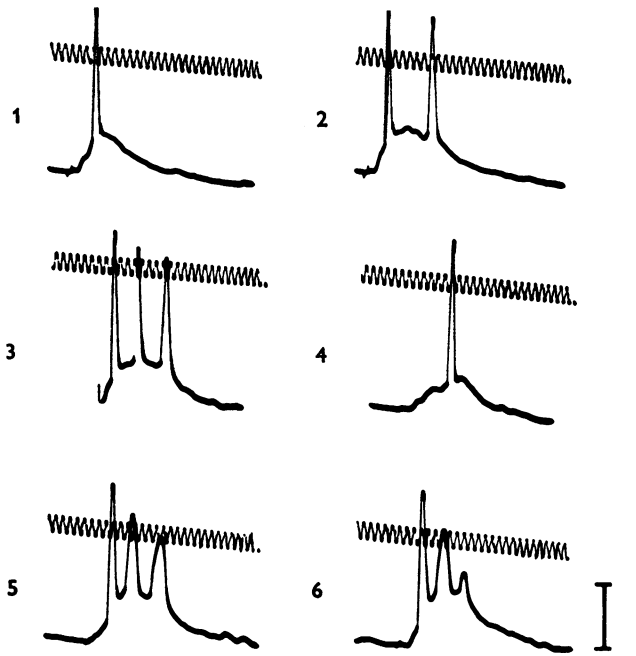

Fig. 10. Rat motoneurone stimulated by dorsal root shock. 1, near-threshold shock of relative strength 1·0; 2, response to same shock after long-lasting dorsal root tetanus; 3, somewhat later relative shock strength 0·8 occasionally excited triple discharges; 4, threshold is now at 0·4 and shock at 0·5 elicits single spikes while (5) original shock strength 1·0 causes inactivation, more marked in 6. Calibration mark, 20 mV.

In depolarized motoneurones stimulation by inside hyperpolarizing currents will often elicit 'post-inhibitory rebound' as an after-effect (cf. Coombs *et al.* 1955; Kuffler & Eyzaguirre, 1955; Ito, 1957; Araki, Ito & Oshima, 1961; Fukami, 1962). To what an extent this may be a normal mechanism has not yet been investigated experimentally by any of the authors cited.

DISCUSSION

The results obtained have defined some properties of delayed depolarization in rat and cat motoneurones and differentiated between two possible modes of operation of motoneurones in repetitive firing, the one with delayed depolarization and little after-hyperpolarization, the other one with much after-hyperpolarization and little delayed depolarization. Conditions have been favourable for demonstrating these two varieties of repetitive firing but have not excluded intermediates. The results have further emphasized that (from the inside) depolarized motoneurones, after some spikes, exhibit firing of the type interrupted by after-hyperpolarization. There can be little doubt but that normal tonic firing is of the same type, seen also in the Betz cells (Phillips, 1956). Phasic bursts may well be different. We have seen such bursts with little if any after-hyperpolarizations as well as maintained spontaneous firing with large after-hyperpolarizations. Reflex tonic activity can hardly be maintained without after-hyperpolarization. This, on our findings, requires a little time and an amount of depolarization which cannot be produced from the IS-end.

Previous work (cf. Eccles, 1957) has uniformly shown that IS-spikes do not produce a significant amount of after-hyperpolarization (cf. Fig. 2, records 4*a* and 5*c*). In itself this zone can transmit frequencies in excess of what is required in tonic control of muscle. If this property was coupled to a pace-maker function, it is likely that the discharge of motoneurones would be both disorderly as well as highly unsuitable for the driving of muscle. It is clear then that in tonic discharge a much larger or else a different firing zone must be implicated. This mode of firing may well be initiated from any site provided with a sufficient number of synapses. At this point we could be satisfied for the moment and go on to a brief discussion of possibilities for explaining delayed depolarization. Little is to be gained by presenting various alternatives based on the ionic theory. Observation does not carry us that far nor are the conditions ideal for putting such propositions to a test. But for progress in this field it seems necessary, nevertheless, to formulate a theory useful in the planning of further work. The hypothesis preferred assumes that delayed depolarization is connected with the spike transmitted into the dendrites. It is supported by the fact that delayed depolarization appears to be elicited by the spike. The dendritic event must be slow in order to appear the way it does, late in the soma spike. The interference picture obtained does not show the actual moment at which it arises. Since delayed depolarization may rise to 10 mV, it seems far too large for an after-potential.

Indirect support for our explanation of delayed depolarization may perhaps be sought in the work of Terzuolo & Araki (1961) and that of Hild

& Tasaki (1962). The former workers used two parallel micro-electrodes, joined together, but one of them having its tip slightly withdrawn by comparison with the other. The second tip might then be just outside the cell or, sometimes, both tips might be inside the same cell. This electrode was occasionally found to register a slower and delayed deflexion or an expanded spike, suggesting to the authors that it could have recorded a dendritic wave. As stated, Hild & Tasaki have directly recorded dendritic invasion in tissue cultures of cerebellar cells.

The fact that delayed depolarization diminishes or disappears in tonic firing to inside stimulation should now be reconsidered from the standpoint assumed. Clearly one essential factor is the amount of dendritic depolarization that has been achieved by such means. Like the SD spike so also the DD event will diminish when the site where it occurs has become sufficiently depolarized. This state cannot be achieved by firing the cell merely at the IS zone. It is likely that dendritic depolarization serves as a pressure head contributing in a significant manner to the maintenance of somatic depolarization, much as in the lobster cardiac ganglion (Hagiwara & Bullock, 1957) the activity of a firing zone merely is reflected as small fluctuations of potential in a soma which stays depolarized. We need not in this connexion explain why under such circumstances after-hyperpolarization occurs, though it is clear that part of this process is due to unmasking when DD diminishes. After-hyperpolarization is known to increase with increasing depolarization (Coombs *et al.* 1955).

In the rat a considerable number of seemingly normal spikes cannot be fired tonically by inside depolarization. Strong currents elicit brief bursts and excessive strengths change electrode properties so as to unbalance the compensation. One may, of course, attribute phasic discharges to any of several factors connected with the quality of the penetration. Several of those motoneurones, after having been for some time stimulated ortho- and antidromically in routine tests, later turned out to respond with perfect regularity to inside depolarization of long duration. Again it is possible to ascribe this result to technical factors, say, for instance, that in the mean time the membrane spontaneously had sealed up a leak around the penetrating tip. Purely evasive explanations may, on the other hand, make us lose sight of important differences between motoneurones. With many a good penetration it has seemed to us more likely that the intervening period of stimulation had produced dendritic changes of importance for tonic firing. By itself stimulation through the micro-electrode (possibly for geometrical reasons) may not always be sufficient to achieve the desired state without support from the cell itself and so these neurones are phasic from the point of view of our tests. Such neurones seem to be more common in the rat if to our own findings (with cats) we add those reported

by Pascoe (1957) and Frank & Fuortes (1960). These authors assume that all motoneurones can be tonically fired from the inside but duration of the repetitive response was not given. Possibly a more highly developed synaptic and dendritic organization keeps many cat motoneurones in a state which rat motoneurones have to be forced into by some time of orthodromic stimulation.

Finally, our results are of considerable interest as an addition to observations on shifts of firing zones by previous authors, especially by Sasaki & Otani (1961) with cat motoneurones, which they, in accord with Granit, Henatsch & Steg (1956), find divisible into phasic and tonic cells both with regard to intracellular stimulation and cell accommodation. The findings by Homma, Kano & Takano (1962) also fit well into the notion that tonic (soleus) and phasic (tibialis anterior) reflexes in the cat emanate from different firing zones. Bradley & Somjen (1961) have reported that accommodation in rat motoneurones may undergo sudden unexplicable changes, a result reminiscent of the findings quoted in the previous paragraph. They also distinguish two types of motoneurones in the rat. Multiple firing zones seem to be a regular feature of crab and lobster ganglion cells (Bullock & Terzuolo, 1957). The safest course seems to be not to exclude them in mammalian motoneurones where at least two modes of firing can be demonstrated.

SUMMARY

1. Most motoneurones in the rat and some in the cat deliver antidromic intracellular spikes which are succeeded by a phase of delayed depolarization masking the small after-hyperpolarization.

2. Delayed depolarization is found to imitate a process of all-or-none character. It is held to be elicited by the soma spike and is ascribed to dendritic invasion.

3. When motoneurones possessing delayed depolarization are fired repetitively by inside stimulation the delayed depolarization is replaced by after-hyperpolarization. This change does not take place when the motoneurones discharge in response to antidromic tetani.

4. Thus two modes of repetitive firing have been described and it is shown that duration of inside stimulation and the number of spikes fired also are of importance for the transition from discharge with delayed depolarization to discharge with after-hyperpolarization.

5. The discussion of these facts is chiefly concerned with the significance of dendritic depolarization for maintained repetitive firing and possible firing zones.

6. In addition some results on the phenomenon of inactivation in rat

motoneurones and on post-anodal firing have been described. Two histograms of cell diameters in segments L 4 and L 5 + L 6 of the rat ventral horn are included.

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