

## Maintained Firing of Motoneurons during Transmembrane Stimulation

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When the confluence zones of subsynaptic currents stimulate motoneurons to discharge repetitively, they will do so at a frequency which is some function of current strength. If rate of discharge varies but little with current strength, the motoneurone has little power of amplification, if, on the other hand, slight changes of current strength greatly influence firing rate the motoneurone will serve as an efficient amplifier of input. Motoneurons have been studied a great deal but this function has curiously been neglected. Yet knowledge of it is fundamental for integrative work. A fair imitation of this property of motoneurons can be obtained by stimulating them from the inside by the tip of the micro-capillary in the manner introduced by Araki and Otani (1955). The present paper deals with this problem and is based on recent work in our laboratory by a team consisting of Granit, Kernell, Shortess and Smith (see references). Special references will have to be looked up in the original papers.

The work was begun at a time when there was a temporary shortage of cats in the laboratory and so it was decided to use the rat as our standard preparation. The findings were in the end also verified on a number of cats. Bradley and Somjen (1961) have recently shown that for many purposes the rat is quite serviceable. Especially we have found this animal useful when both dorsal and ventral roots for the hind limb segments are cut because these segments in the rat are supplied by blood vessels from the thoracic end and not by the roots, as are the corresponding segments in the cat. Our preparations were anaesthetized with pentobarbitone, 55 mg/kg, and curarized. They were maintained on artificial respiration by a mixture of oxygen with 1% carbon dioxide. Technical details are given in our first paper (Granit *et al.*, 1963c) and in the work of Bradley and Somjen. The stimulating circuit was that of Araki and Otani (1955) and KCl micro-capillaries were used.

The rat as a preparation proved to be of interest also because most intracellularly recorded spikes, elicited by the antidromic route, were practically without after-hyperpolarization (Fig. 1d) differing in this respect from the majority of cat motoneurons, so well known from the work of Eccles and his colleagues (see *e.g.*, Eccles, 1957). Partly this was due to the prominent delayed depolarization which followed the action potential both when it was fired antidromically (Fig. 1 a-d) and when it was elicited by an intracellular shock (Fig. 1 e-g). We did not succeed in eliciting

delayed depolarization separately. It merely occurred in sequence to a full-size spike. The IS-spike was never followed by delayed depolarization. A considerable number of experiments in our first paper (Granit *et al.*, 1963c) were devoted to a study of delayed depolarization. The outcome of this work was ultimately summarized in the hypothesis that delayed depolarization represents activation of the dendrites. I shall not review these experiments because Kernell has since tried to investigate this hypothesis, and in the discussion he will concentrate on this aspect of our findings and present his own later work.

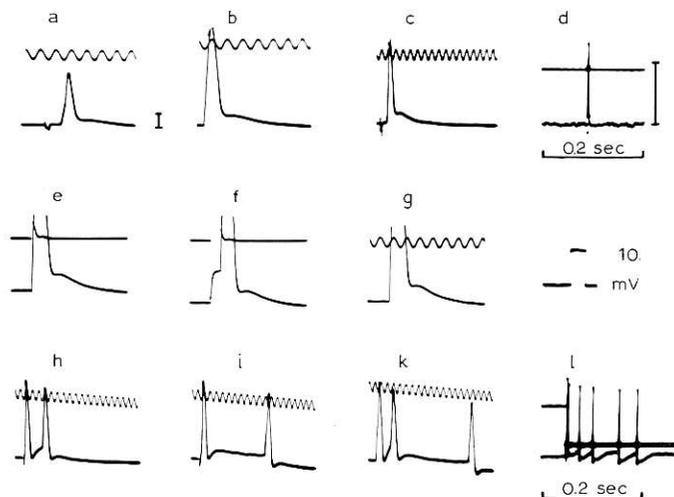


Fig. 1. Intracellular records from rat motoneurons. a-d, antidromic discharge of spike of 81 mV at different amplifications. Times in msec for a-c, calibrations 20 mV; note, that spikes b-d go beyond range of oscillograph deflexion. e-g, another spike stimulated from the inside by shock of 0.5 msec (e) and 0.12 msec (f), the former repeated in g with time (msec) replacing current recorder. h-l, beginning of near-threshold repetitive discharge as in l, of which h-k show variations of initial frequency (time in msec) in different trials. Note development of after-hyperpolarization (Granit *et al.*, 1963c.)

Delayed depolarization is taken up in this connexion because it has the interesting property of disappearing when repetitive firing is elicited by maintained intracellular stimulation. This is shown in Fig. 1 h-l. Repetitive firing is here elicited at stimulus strengths near threshold so that the initial frequency of discharge varies a little from case to case. The initial spikes were separately recorded on the sweep circuit in records h to k, and on the expanded time scale it is seen that during transmembrane stimulation delayed depolarization is gradually replaced by after-hyperpolarization. In fact, the rat spike, fired repetitively from the inside by transmembrane currents, now behaves like (according to the Canberra group) most cat spikes seem to do howsoever fired, *i.e.* they now show good after-hyperpolarization. This made us look at the cat motoneurons with renewed interest and, actually, among them, too, a considerable number failed to give after-hyperpolarization when fired antidromically. Kernell has since made further observations dealing with the relative frequency and properties of cat motoneurons that discharge antidromic spikes succeeded by delayed depolarization.

It is clear that the employment of the firing mechanism must be different when the motoneurone discharges repetitively as compared with how it operates in response to an antidromic invasion. The simplest explanation seems to be that the rhythmically discharging motoneurone employs a very much enlarged firing area and that it, only when it operates in this manner, can deliver (in rats) the phase of after-hyperpolarization which develops early during transmembrane stimulation. The reason why many, if not most, cat motoneurones are in this state before rhythmic firing has begun, remains obscure.

When the transmembrane current is allowed to act for several seconds a number

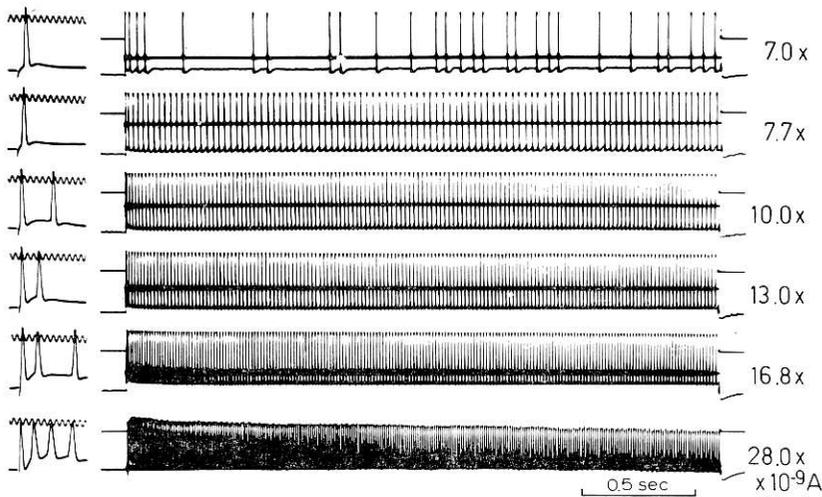


Fig. 2. Intracellular stimulation of rat motoneurone to discharge repetitively by current strengths marked on the right. Note change of sensitivity of current recording beam of oscillograph at 7.7 nA. For the strongest current spike has diminished. On the left, initial spikes on sweep and time in msec (Granit *et al.*, 1963b).

of motoneurones respond repetitively throughout the period of stimulation. The majority merely responds phasically and when current strength is increased the rates of discharge in the burst merely increases without the latter expanding much in duration. The discharge of a tonically firing motoneurone is shown in Fig. 2. The initial spikes have also been recorded on the fast sweep circuit. These are shown on the left in the figure, and the increase of the after-hyperpolarization early in transmembrane stimulation is conspicuous also in these records.

The question of why all of the motoneurones could not be forced to respond tonically is difficult to answer. Intracellular recording is beset with pitfalls and it is easy to ascribe failure of lasting repetitive firing to technical faults. On the other hand, we had a number of penetrations which to all appearance were excellent in the sense that the spikes were large and well-maintained. When such motoneurones refuse to respond tonically to long-lasting intracellular stimulation we hesitate to ascribe this to a faulty technique. It seems more reasonable to us to accept the best findings at

their face value and to conclude that there actually are motoneurons which 'adapt' so quickly that maintained firing is quickly suppressed.

This brings us to the general problem of 'adaptation' of the motoneurone membrane. A good introduction to this question is the detailed analysis of the records of Fig. 2,

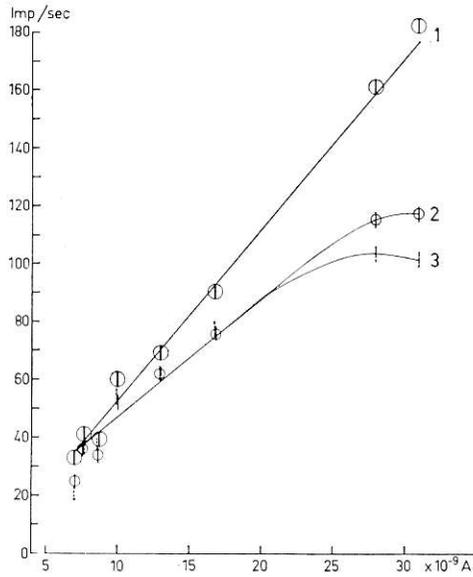


Fig. 3. Frequency of discharge plotted against current strength for motoneurone of Fig. 2. Curve 1, slope constant 5.9 imp/sec/nA, derives from 1/3 sec following first interval; curve 2, same after 1.3 sec; curve 3, same after 2.6 sec but measuring time extended to 1/2 sec. Slope constant of 2 and 3 is 4.1 imp/sec/nA (Granit *et al.*, 1963b).

presented in Fig. 3. Impulse frequency is here plotted against current strength and the three curves refer to the moments 1/3, 1.3 and 2.6 sec after initiation of transmembrane stimulation. Leaving out the first pair of spikes, all of our experiments show that the relation between current strength and impulse frequency is linear over a range which exceeds the normal firing rate of the motoneurons. When such curves are plotted for different moments during the rhythmic discharge, adaptation presents itself as a decrease in the slope of the curves, as in Fig. 3. In most cases the adaptive process is completed within half a second. Occasionally it is a little slower. When the slope decreases, the range within which the linear relation between current strength and firing is maintained also tends to decrease (Fig. 3).

It has become customary to speak of 'accommodation' in a rather loose manner and so, perhaps, it is best to emphasize at the outset that adaptation by no means can be correctly referred to as 'accommodation'. The motoneurons which fire tonically are non-accommodative, or they would not fire in this manner. A general discussion of accommodation and adaptation has been given in our second paper (Granit *et al.*, 1963a).

The adaptive process is very prominent in the phasic motoneurons. Fig. 4 shows the response of such a motoneurone analyzed as in Fig. 3. It never acquired a steady

state of maintained firing. The slopes, however, are linear there, too, and rapidly diminish as a function of duration of stimulation.

The initial slopes of many motoneurones are very high and spread over a considerable range of values. The 'adapted' slopes, however, do not vary very much. Selecting our 16 best tonically firing motoneurones, 6 of which were from cats, the average adapted slope constants were of the order of 2.5 imp/sec/nA with a range of from 4.1 to 1.3 imp/sec/nA. Since phasic motoneurones only utilize the initial discharge, their slope constants will always be high and vary from case to case.

Tonic firing thus takes place in a manner which makes the motoneurones relatively independent of membrane current, provided the rhythmic threshold has been reached. Some of them were stimulated for very long times (Granit *et al.*, 1963b) and the adapted

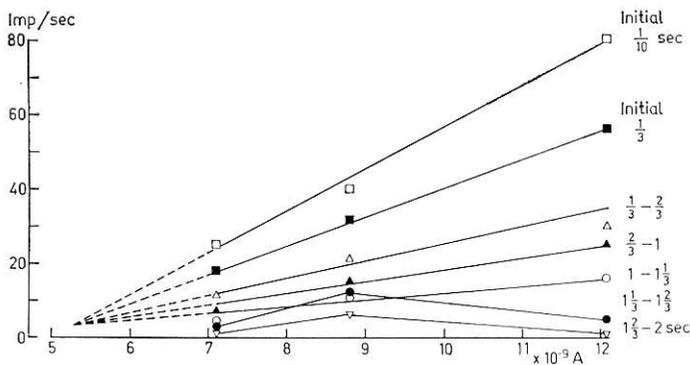


Fig. 4. Adaptation of phasic rat motoneurone, spike size 75 mV, in terms of curves relating frequency of discharge to current strength for the moments marked in the figure. First interval not included in initial count (Granit *et al.*, 1963b).

slope constants then remained constant although the absolute frequency of discharge slowly diminished and the rhythmic threshold rose.

The best known limb reflex in which tonic firing occurs is the stretch reflex. From our results would follow that in this reflex the neurones would have to fire at slow rates and, in the steady state, be relatively independent of the degree of extension. This, of course, is well known to be the case (Denny-Brown, 1929; Adrian and Bronk, 1929; Granit, 1958). It is now clear from the present work that an essential factor in the stabilization of frequency of discharge is the adaptation of the motoneurone membrane itself. To this add themselves the after-hyperpolarization and the recurrent inhibition as further stabilizing influences. Whether the motoneurones that can be fired repetitively over seconds from the inside are the very ones which are tonic with respect to muscular afferents, cannot be decided on the basis of the results presented here. More essential is at the moment that if motoneurones can fire tonically, their membranes are so designed that adapted slope constants are small.

Essentially the motoneurones behave like the sense organs, which start firing at a high rate and adapt quickly. This behaviour is common if they are organs provided with a tonic discharge. Clearly, the properties of the motoneurones are matched to those of the sense organs. Many reflexes also open in (what Sherrington used to

call) *d'emblée* fashion, that is, abruptly with a rapid burst of impulses quickly diminishing in frequency in the manner required by the properties of the cell membranes.

On the other hand, many internuncial cells are known to fire at fast rates for some time. This is generally ascribed to the properties of the transmitter substance (Eccles, 1957) but before the slope constants of such neurones have been measured one should be cautious in ascribing their mode of firing exclusively to the chemical mechanism. It is very likely that such neurones have large slope constants and perhaps also much adaptation. If this were so their function would be to provide amplification of input in addition to what else they might do. This approach to the problems is still an untilled field. We do not yet possess the data that would enable us to distinguish 'amplifying' cells from 'stabilizing' cells on the basis of neural adaptation. But the suggested corollaries to our experiments all represent as many problems capable of an experimental solution. (Granit and Kernell have since confirmed this prediction. Added in proof.)

Of special interest is the strict validity of the linear relation between current strength and impulse frequency. It can be discussed from many points of view. It means, for instance, that in maintained firing the product of current intensity and spike interval is a constant. This constant is the level from which the motoneurone fires and the stronger the current, the sooner it is reached. The linear rule also means that in the steady state postsynaptic inhibition and excitation are added up algebraically, as shown by Granit and Renkin (1962). Inhibition merely implies sliding 'downwards' on the same curve along which excitation slides 'upwards'. The quantitative implications of this finding are extensive.

#### SUMMARY

1. The aim of this work was to study the relation between transmembrane current strength and discharge frequency of rat and cat motoneurons.

2. Comparison of maintained transmembrane stimulation with single antidromic shocks disclosed that the delayed depolarization, seen after the antidromic spike, disappears during intracellular stimulation of a cell to repetitive activity. This transformation of the cell's mode of firing is prominent in most rat cells and in several cat cells (*cf.* p. 52 Kernell in the Discussion).

3. The motoneurons which are capable of firing repetitively to maintained transmembrane stimulation do so along curves relating current strength linearly to firing rate.

4. The slope constants of such curves are initially steep, but after about 0.5 sec they acquire a fixed or 'adapted' value and are then of the order of merely 1.5–4 imp/sec/nA.

5. A process of 'adaptation' is defined by the decline in the firing rate and the decrease of the slope constants.

6. Some implications of these results for integrative work on motor control are discussed.

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