

QUANTITATIVE ASPECTS OF REPETITIVE FIRING OF  
MAMMALIAN MOTONEURONES, CAUSED  
BY INJECTED CURRENTS

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Though several authors must have seen that motoneurones may fire repetitively to maintained intracellular stimulation and Frank & Fuortes (personal communication) have found the relation between impulse frequency and current strength to be linear, confirming Barron & Matthews (1938) depolarizing through the ventral root, the quantitative aspects of this relation do not seem to have been closely investigated. One would, for instance, need to know for what duration steady states of firing can be maintained at different current strengths, how well neurones follow the linear law, how 'adaptation' modifies it, etc. Some papers on the role of accommodation early in the discharge have been published (Araki & Otani, 1959; Frank & Fuortes, 1960; Bradley & Somjen, 1961; Sasaki & Otani, 1961) and will be discussed below. Long durations of stimulation do not seem to have been used or, at least, relevant data have not been published. This suggests that, when the experiments include variations of current strength, both technique and interpretation of the results are beset with difficulties and this, we find, is true. On the other hand, it is important to make an attempt to overcome such difficulties because the properties of the cell membrane itself, devoid of synaptic support, is the basic datum required for an understanding of firing also under synaptic bombardment (see Discussion).

In the mean time an attempt had been made by Granit & Renkin (1961) to apply the linear law relating impulse frequency to current strength to an analysis of algebraical summation of 'natural' excitation and inhibition. Their preparation was the cat motoneurone firing tonically to stimulation of muscular afferents and inhibited by the recurrent route. The results were encouraging, inasmuch as they demonstrated the validity of the linear law in a physiological context and thus showed that such problems are accessible to quantitative study. For this reason their work served to underline the need for an investigation of the kind to be presented below.

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## METHODS

Rats anaesthetized with pentobarbitone 55 mg/kg and some cats similarly anaesthetized with a dose of 40 mg/kg have been employed. The procedure for operating on rats to be used in intracellular work on the spinal cord has been described in detail by Bradley & Somjen (1961) and, as applied in this laboratory, by Granit, Kernell & Smith (1963).

The technique for intracellular stimulation is the one introduced by Araki & Otani (1955). The present work, being devoted to a quantitative analysis of the effects of trans-membrane currents of extended duration, is based on experiments which differ only in this respect from those published by Granit *et al.* (1963) in which brief current pulses were used.

## RESULTS

*Typical rat motoneurone*

Repetitive firing to intracellular stimulation can be obtained in a large number of rat motoneurones but most of them give brief phasic bursts and others may not be stable for a long enough time to allow more than a few trials to show that they do respond tonically. These cannot add much to the quantitative aspects of our problem. The results to be presented refer to the final selection of 10 motoneurones which displayed maintained firing.

Figure 1 illustrates sample records of a motoneurone firing to different current strengths and Fig. 2 contains cuttings from a discharge of that motoneurone maintained for 10 sec. Later in the experiment this period was extended to 25 sec without much reduction in firing rate. Sample records on an expanded time base emphasize the finding by Granit *et al.* (1963) that early in the stimulation period the after-hyperpolarization gradually increases (compare Figs. 1 and 2) to the extent of almost or wholly replacing the delayed depolarization characteristic of antidromic SD spikes and of the initial responses to inside stimulation. Since after-hyperpolarization depresses excitability of the motoneurone (Eccles, 1957), augmentation of it reduces firing rate, and therefore this transformation of type of response from one with little to one with much after-hyperpolarization contributes to 'adaptation' of discharge frequency, a term to be used in the descriptive sense rather than 'accommodation', which is beginning to acquire a definite meaning in terms of mechanisms (see below). The establishing of the mode of firing that is characterized by fully developed after-hyperpolarization occupies an interval that varies from cell to cell and also with stimulus strength.

When current strength is much increased, rate of adaptation also greatly increases and the spikes grow smaller, suggesting that firing level is altered (see Fig. 1); finally the generative process encounters difficulties, firing becomes irregular and ultimately full inactivation occurs. This sequence is in every respect similar to what happens when the crustacean stretch receptor is overstretched (Eyzaguirre & Kuffler, 1955). We shall be

interested only in the range within which steady-state conditions can be obtained, except when mentioning a few examples of phasic cells.

For the motoneurone shown in Figs. 1 and 2 there is plotted in Fig. 3 the relation between impulse frequency ( $F$ ) and current strength ( $I$ ) in units of  $10^{-9}$  A (or nA). Curve 1 is the regression line for counts from the

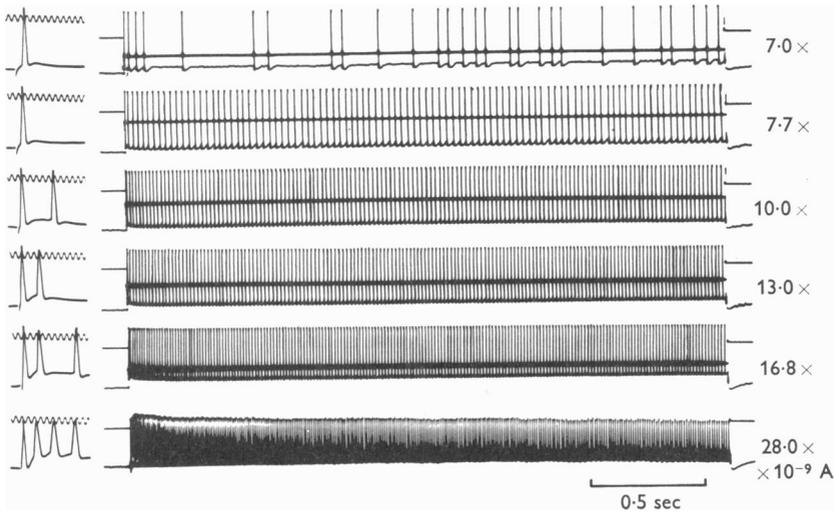


Fig. 1. Intracellular stimulation of rat motoneurone (spike size 81 mV) by currents of different strength. On the left initial spikes recorded on fast sweep circuit with time in msec. Note change of sensitivity of current-recording beam of oscillograph between 7.0 and  $7.7 \times 10^{-9}$  A. For the strongest current spike size has diminished (cf. Fig. 3 for plot).

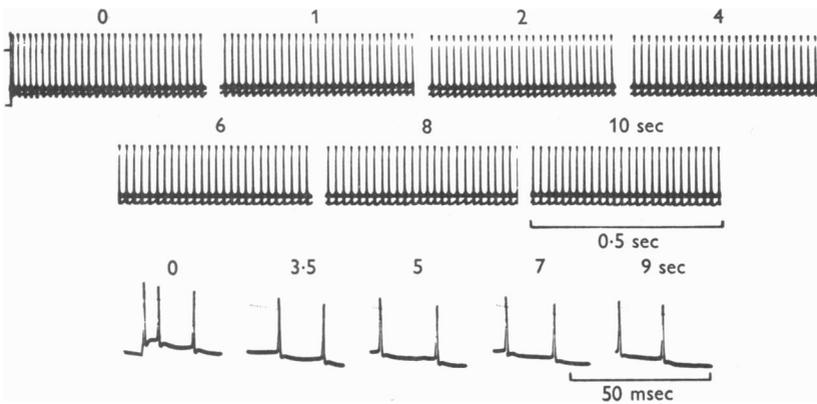


Fig. 2. Same motoneurones as in Fig. 1 stimulated for 10 sec, samples of which have been cut out at the moments marked above the records. Current strength maintained at 13.8 nA. Below, samples on fast sweep circuit demonstrating after-hyperpolarization.

second interval during  $\frac{1}{3}$  sec (frequency of firing =  $F = 5.85I - 6.2$ ). Curve 2 refers to moment 1.3 sec and curve 3 to 2.6 sec. The regression line for the linear portion is  $F = 4.1I + 5.9$ . The region in which the curves begin to flatten out corresponds to that of the strong current in Fig. 1.

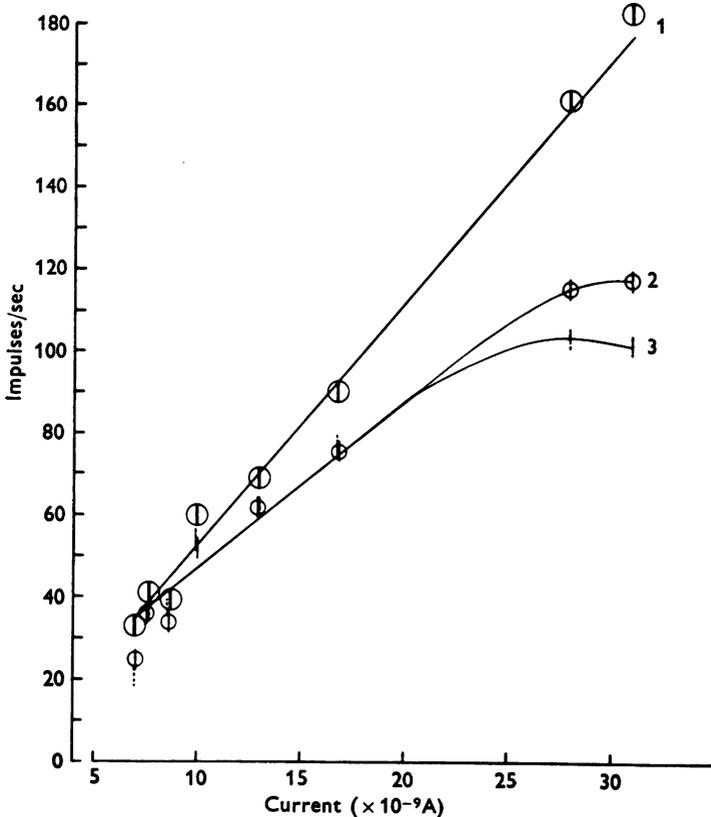


Fig. 3. Frequency of discharge plotted against current strength for motoneurone of Fig. 1. Curve 1, slope constant 5.9 imp/sec/nA derives from  $\frac{1}{3}$  sec following first interval; curve 2, same after 1.3 sec; curve 3, same after 2.6 sec, measuring time extended to  $\frac{1}{3}$  sec. Slope constant of 2 and 3 is 4.1 imp/sec/nA. The rectilinear portion of these curves was calculated by the method of least squares in this experiment but not in those to follow.

Greater accuracy could have been obtained, had we not used stimuli of very long duration. Actually adaptation is fast enough in most of those motoneurones that are prepared ultimately to fire in a maintained fashion, to make 0.5 sec seem sufficient for determining the final or *adapted* value for the linear relation between current strength and impulse frequency. In this case the slope constant varied but little with duration of depolarization, from about 6 to 4 imp/sec/nA, but it is characteristic of all

motoneurones (1) that the slope constants diminish during adaptation and (2) that, when this happens, the range of validity of the linear relation also shrinks, the curves flattening or even bending down for strong current intensities.

#### *Adaptation*

With strong currents it is impossible to sort out the factors which contribute to adaptation, but by investigating a range of current strengths the existence of a true adaptive process in the membrane can be demonstrated in most cells capable of firing at a steady rate for some time. Thus the motoneurone of Fig. 4 exhibited truly tonic behaviour in the medium range of current strengths but adapted quickly at both extremes as is

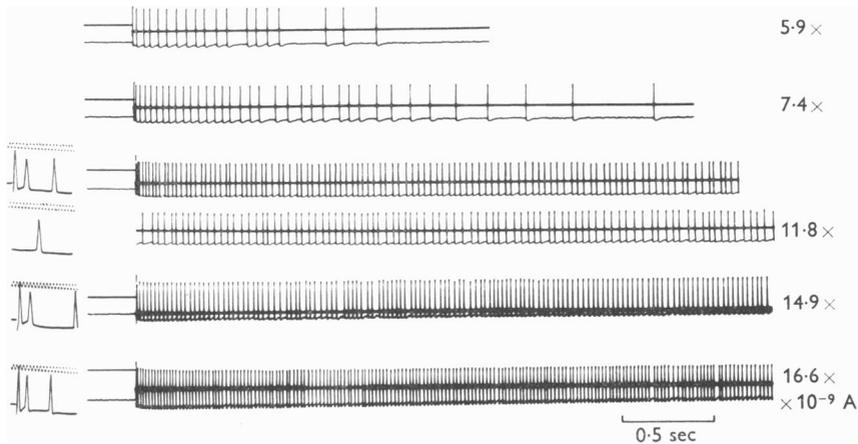


Fig. 4. Rat motoneurone, spike size 75 mV, stimulated intracellularly as in Fig. 1. At 11.8 nA lower record is directly continued from upper record to show how well discharge can be maintained.

illustrated also in Fig. 5 where the discharge frequency is plotted against running time of stimulation. At the highest intensity (27.7 nA) inactivation began to occur. While the combination of cell membrane and micro-electrode may prove detrimental to both when chloride is transported into the cell at excessive rates by very strong currents, clearly it must be properties of the membrane itself that are revealed when, in Fig. 4, there is much adaptation *below* a strength of current to which the firing mechanism can adapt itself perfectly. Hence the cell is responsible for a counter process other than augmentation of after-hyperpolarization, which is a very fast initial event (compare Figs. 1 and 2).

Two graphs of adaptation in terms of impulse frequency against duration of stimulation for different current strengths are plotted in Figs. 5 and 6, of which the former, as stated, refers to the cell and spike illustrated

in Fig. 4. As long as frequency of firing changes rapidly slope constants may not be very accurate, but they can be approximately determined with an accuracy that is quite sufficient to support one of our fundamental conclusions; namely, that the drop in the value of these constants is one

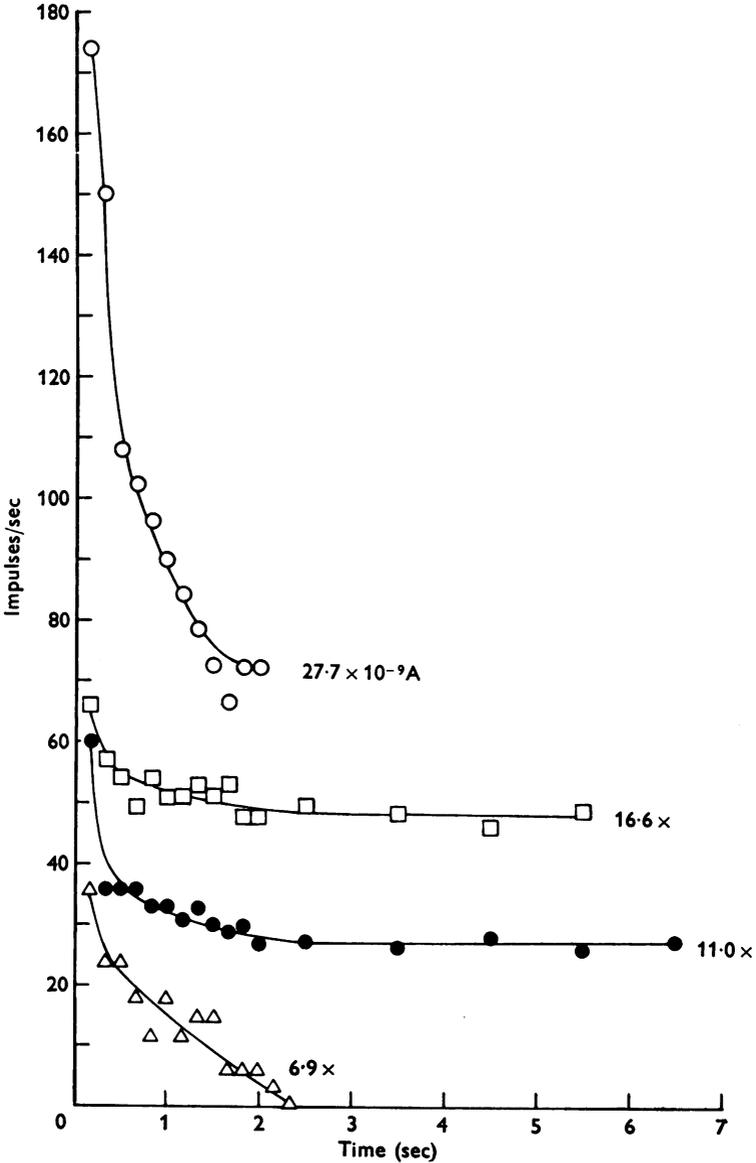


Fig. 5. Adaptation of rat motoneurone of Fig. 4 plotted in terms of rate of firing against time of current maintained at strengths marked in the figure.

of the main characteristics of adaptation. The cell in Fig. 6 adapted very rapidly. Variations in adaptation rate such as those illustrated in Figs. 5 and 6 are reminiscent of well known similar phenomena in receptors. Our findings may also be compared with those of Hodgkin (1948) stimulating

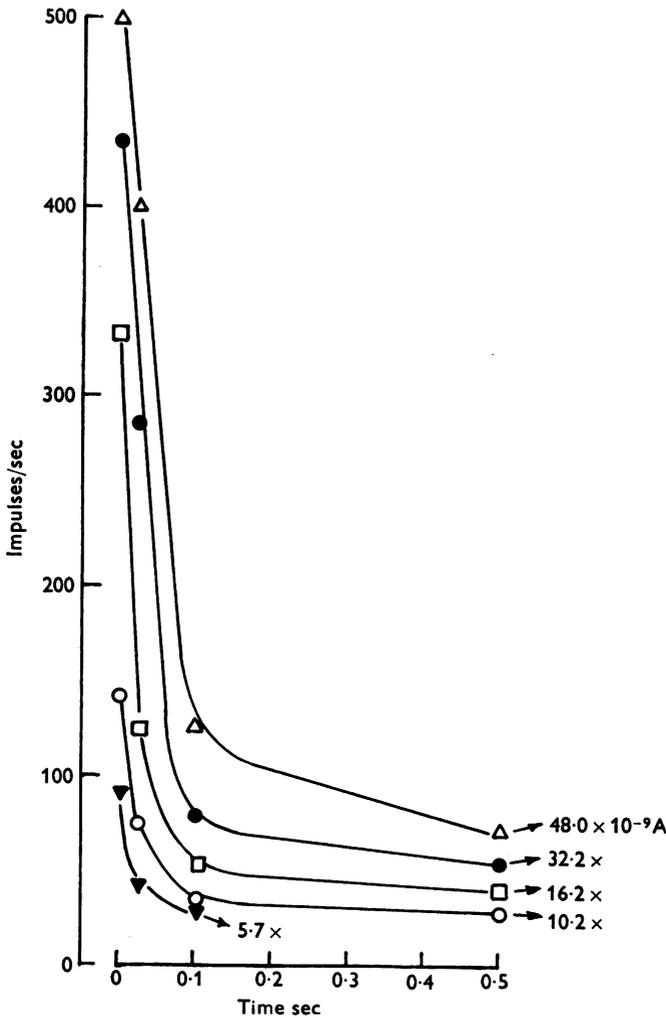


Fig. 6. As Fig. 5 but for another motoneurone; spike size 67 mV.

single fibres of *Carcinus*. Our Fig. 1 corresponds, for instance, to his Plate 2, our Fig. 4 to his Plate 1 (see also Fessard, 1936; Arvanitaki, 1938), though we have never seen any 'warming up' or frequency increase during stimulation.

In Fig. 7 a phasic cell is introduced for comparison. It never acquired a steady-state value, although it fired for a couple of seconds. Nevertheless, the slopes of the curves characteristically diminish as a function of time of stimulation. The first interval is not included in this plot. It is,

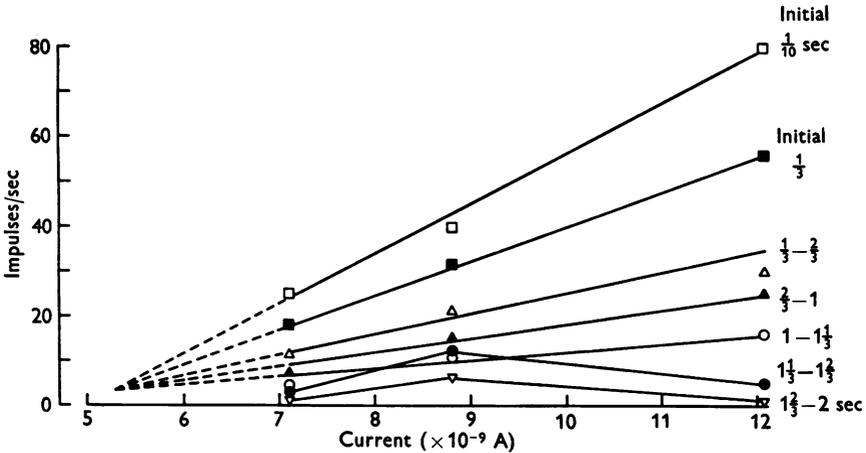


Fig. 7. Adaptation of phasic rat motoneurone, spike size 75 mV, in terms of curves relating frequency of discharge to current strength for the moments marked in the figure. First interval not included in initial count.

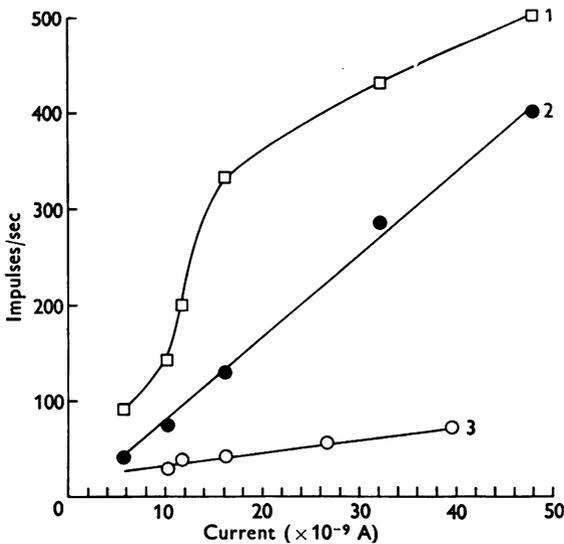


Fig. 8. Frequency of discharge plotted against stimulus strength for rat motoneurone of 67 mV. Curve 1, inverse value of first interval; 2, same for interval between second and third spike; 3, adapted value for 0.1 sec between 0.5 and 0.6 sec of stimulation.

however, plotted for another motoneurone as curve 1 in Fig. 8. The form of this curve varied a great deal from cell to cell; sometimes it, too, was linear but it is seen here that linearity is quite good when for the determination of  $F$  the inverse value of the interval between the second and third spike was averaged (curve 2). The slope constant then was 8.6 imp/sec/nA and the corresponding value was 1.9 imp/sec/nA (curve 3) in the fully adapted cell. Most phasic cells responded with brief bursts of a few impulses only. Increase of current strength merely unbalanced the compensating device in the stimulating circuit. Yet some of these neurones had large spikes.

Finally, it should be emphasized that when motoneurones cease to fire in response to trans-membrane currents they never exhibit firing with so-called A or IS spikes. To this mode of stimulation the cell either discharges a full B or SD spike or is silent. With excessive current strengths a variety of stunted spikes is seen, but there is no point in trying to assign such pathological events to any particular zone.

The range of variation of slope constants in the adapted state and of the validity of the linear laws relating spike frequency to current strength is further exemplified in Figs. 9 and 10. Curves 1 and 2 of Fig. 9 are from a cell which rapidly reached steady-state conditions; the counts for curve 1 are averages from the initial  $\frac{1}{3}$  sec, those of curve 2 from the final  $\frac{1}{3}$  sec of a stimulation lasting some 0.7 sec. The small change in the value of the slope constant, from 4.3 in curve 1 to 3.4 imp/sec/nA in curve 2 again emphasizes that typically the cells that are going to respond tonically very rapidly reach steady-state conditions. At the end of the experiment this cell was kept discharging for 10 sec at current strength 10.0 nA without change of discharge frequency. Curve 3 is the corresponding curve of Fig. 8 put in for comparison on the same scale.

In these figures (see also Fig. 3) it will be noted that for weak currents near threshold the values often tend to lie below the curve. This exemplifies the difficulty in determining the rhythmic threshold accurately. It is a fluctuating quantity. A good approximate value is obtained by plotting curves such as those of Fig. 3 and by taking the current strength from the region of intersection as the rhythmic threshold. The absolute threshold is lower. On an average the rhythmic threshold was found to be 1.4 times the absolute threshold.

Curve 1 of Fig. 10 is interesting because it refers to a motoneurone that quickly set up a steady state, but nevertheless at current strengths from the arrow upwards ceased to discharge in a maintained fashion to inside stimulation lasting  $\frac{2}{3}$  sec. In that range of current the cell delivered a short burst. It is not included among the ten truly tonic motoneurones whose slope constants after adaptation will be considered separately below.

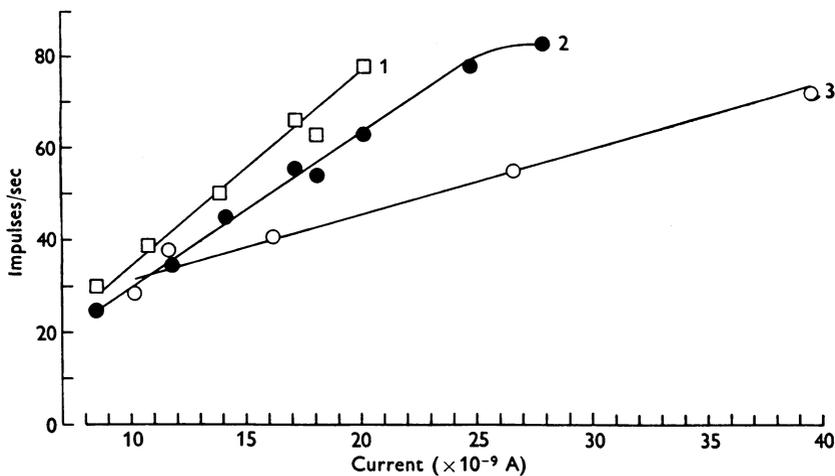


Fig. 9. As Fig. 8. Curves 1 and 2 refer to rat motoneurone of 62 mV spike size. 1, counts for initial  $\frac{1}{3}$  sec; 2, counts for last  $\frac{1}{3}$  at moment  $\frac{2}{3}$  sec. Curve 3 is the similarly numbered curve of Fig. 8, plotted on an expanded scale.

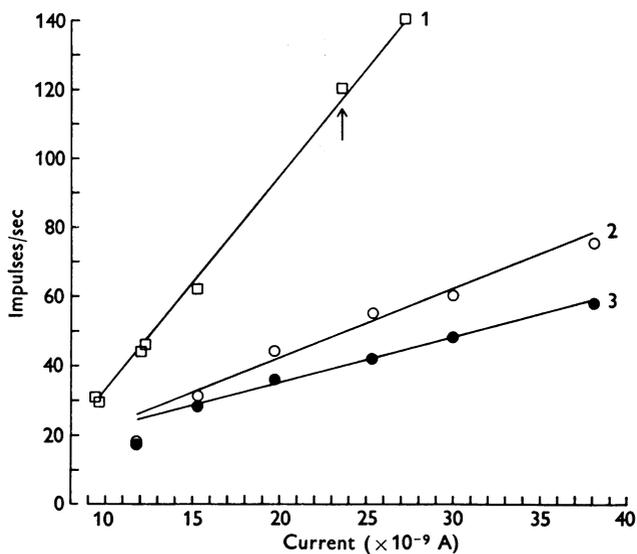


Fig. 10. As Fig. 8. Curve 1 refers to rat motoneurone of 53 mV whose discharge failed to be maintained at strengths of current beyond the arrow. Slope constant 6.2 imp/sec/nA. Curves 2 and 3 are from rat motoneurone with spike of 68 mV. 2, initial  $\frac{1}{10}$  sec excepting first interval. Slope constant around 2 imp/sec/nA; 3, similar plot for  $\frac{1}{3}$  sec. Slope constant 1.3 imp/sec/nA.

Curves 2 and 3 from a tonic motoneurone are inserted in order to give an example of a cell which began by being phasic but later in the experiment became a typical motoneurone which rapidly settled down to steady-state firing. The plot refers to the tonic state. Thus curve 2 with a slope constant around 2 imp/sec/nA is based on counts from the first  $\frac{1}{10}$  sec of stimulation, while the steady-state values of curve 3 gave a constant of 1.3 imp/sec/nA. In the phasic state the slope constant for the first  $\frac{1}{10}$  sec was around 4.5 imp/sec/nA. Since spike size increased when the cell grew tonic it may well have been depolarized early in the experiment while it still was phasic.

#### *Slope constants of adapted cells*

The slope constants of the ten tonic cells were for the adapted state 4.1, 1.3, 2.8, 3.6, 3.4, 1.9, 2.5, 1.4, 2.6 and 2.9 impulses/nA; on an average, 2.7. The rhythmic threshold varied from 15 to 30 impulses/sec, with an average of 22 imp/sec/sec. The linear law relating spike frequency to current strength was valid for an average adapted range of 42 imp/sec/sec, but this value is likely to be a minimum because occasionally technical difficulties were encountered in compensating for the stronger currents. Within the linear range impulse frequency in the steady state is thus, on an average, carried from the threshold up to at least 64 impulses/sec, corresponding to an approximately threefold increase of current strength.

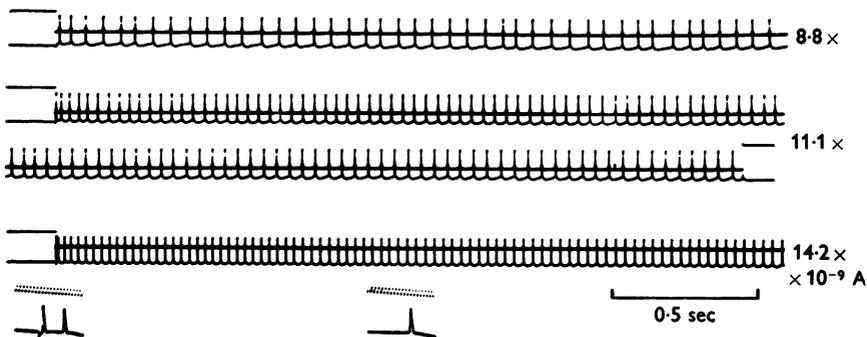


Fig. 11. Cat motoneurone belonging to hamstring nerve; spike size 80 mV. Duration of intracellular stimulation around 6 sec. At 11.1 nA lower record is directly continued from upper record. Samples on sweep (time in msec) below lowermost record (cf. Fig. 13 for plot of data).

#### *Cat motoneurones*

When the work was rounded off with some experiments on cats these on the whole proved to be easier preparations, no doubt due to their larger size and larger motoneurones (cf. anatomical comparison by Granit *et al.* 1963). A tonically firing cat motoneurone is shown in Fig. 11. Compared with Figs. 1 and 4 of rat motoneurones discharge frequency is lower, and

this held good for the six cat motoneurones studied. On the basis of work by Eccles (1957) and his colleagues it was pointed out by Granit *et al.* (1963) that since many, if not most, cat motoneurones have larger after-hyperpolarizations to single shocks than the large majority of rat motoneurones, the transitional stage at the onset of depolarization, during which the rat motoneurone develops after-hyperpolarization, is less in evidence with cats (compare Fig. 11 with Figs. 1 and 2). In them this component of the adaptive process seems practically restricted to the first interval. The lower average frequency of discharge in cats, as has been pointed out by Bradley & Somjen (1961), would follow from the longer duration of after-hyperpolarization, as measured in cats by Eccles, Eccles & Lundberg (1958). Our measurements on rat motoneurones firing at a slow rate to inside depolarization gave values around 50 msec (cf. Bradley & Somjen, 1961).

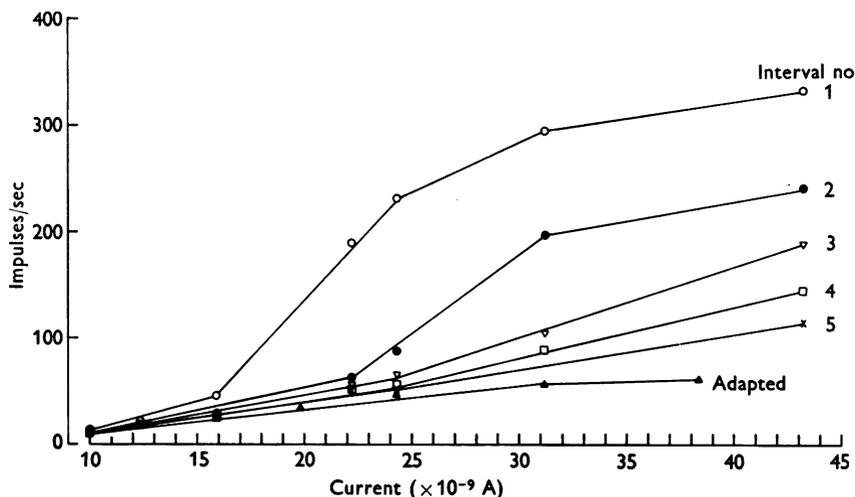


Fig. 12. As Fig. 8. Cat motoneurone of 55 mV. Adaptation illustrated by counts of frequency of discharge based on intervals numbered in graph. Adapted value with slope constant around 2 imp/sec/nA refers to  $\frac{1}{2}$  sec at 2.5 sec and is replotted as curve 3 of Fig. 14 on expanded scale.

Two studies of adaptation are illustrated in Figs. 12 and 13. Reciprocals of early intervals 1–5 plotted in Fig. 12 show that adaptation was practically completed during the discharge of five spikes. In Fig. 13 the uppermost curve (1) refers to the first interval and has a slope constant of 15.4 imp/sec/nA. Counting average frequency of discharge for the first half second gave curve 2, with a slope constant of 4, the second part of the first half second gave curve 3 with a slope constant of 3.5. The range of validity of the linear law was small in the adapted state. After 5–6 sec (curve 4) firing was not maintained for the strong current but a linear

relation between discharge frequency and current strength prevailed for weaker currents with a slope constant of 2.5 imp/sec/nA. This is the motoneurone illustrated in Fig. 11.

Slope constants of two cat motoneurones are shown in Fig. 14 (see legend). The six adapted cat motoneurones had slope constants 2.3, 2.5, 1.9, 3.5, 2.0 and 3.5 imp/sec/nA, the average being 2.6. The average rhythmic threshold was at 10 impulses/sec and the average range of validity of the linear relation between spike frequency and current strength

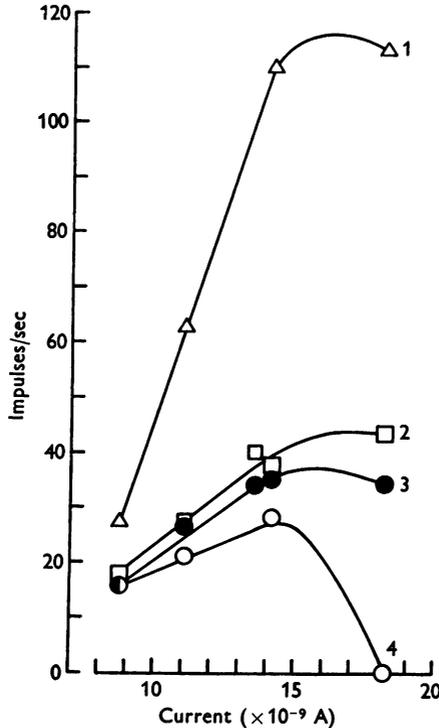


Fig. 13. As Figs. 8 and 12 but for cat motoneurone of 80 mV shown in Fig. 11. Curve 1 refers to first interval, slope constant 15.4 imp/sec/nA; 2, first half second; 3, second half second; 4, between 5 and 6 seconds. The slope constants decrease from 4 to 3.5 to 2.5 imp/sec/nA in curves 2, 3 and 4 respectively.

carried rate of firing up to 37 impulses/sec. The material is too small to show if the smaller range is significant by comparison with rat motoneurones, nor is it possible to conclude that the fair agreement between slope constants, 2.7 with rats, 2.6 with cats, is more than general evidence demonstrating that slope constants in the adapted state on the whole tend to have low values between 1.5 and 4 imp/sec/nA. The average constants may well be slightly different in cats and rats.

*Comparison between rat and cat motoneurones*

We possess information on threshold and maximum firing rates for thirty-three cat motoneurones stimulated to discharge tonically by stimulation of extensor muscular afferents (Granit, Haase & Rutledge, 1960). In their histograms the minimum value of the rhythmic threshold fell on a peak located between 6 and 10 impulses/sec and is thus fully in agreement with the low value of 10 impulses/sec found above for inside stimulation.

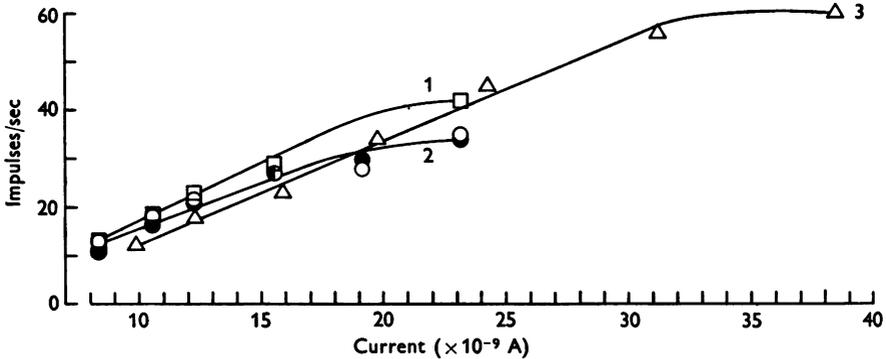


Fig. 14. As Fig. 8. Curves 1 and 2, cat motoneurone of 72 mV. Curve 1, plots from initial  $\frac{1}{2}$  sec; 2, next  $\frac{1}{2}$  sec period. Slope constant for rectilinear portion of 1 is 2.4; of 2, 1.9 imp/sec/nA. Curve 3 is from motoneurone referred to in legend of Fig. 12.

The maxima were spread over a considerable range, with a broad peak between 13 and 25 impulses/sec. Since maximum activation of tonic motoneurones need not necessarily be obtained from muscular afferents, which include inhibitory Golgi tendon organs adding some antagonistic repolarization as well as inhibition by the recurrent route, our value here of some 40 impulses/sec is reasonable and in full accord with what is known about the low steady firing rates of cat motoneurones from a large body of work from Adrian & Bronk (1929) and Denny-Brown (1929) onwards. We feel therefore entirely justified in concluding that within the physiological operative range of tonically discharging cat motoneurones linearity prevails between current strength and impulse frequency, justifying the theoretical approach adopted by Granit & Renkin (1961) in treating their results on algebraical summation of excitation and inhibition.

Figure 15 is a comparison of slope constants of rat and cat motoneurones based on the material of this work. The frequency of discharge for any given current strength is faster for the rat. Having concluded above that at least for tonic reflexes our results with the cat motoneurones are a fair representation of a range of firing frequencies met with in experimental work, we believe the same must be said about the rat motoneurones, whose

reflexes have not been studied from this point of view. Our diagram suggests that rat muscles also would require faster firing rates and so themselves must be faster. This is easily shown to be the case. Thus the cat gastrocnemius muscle has a fusion frequency of the order of 80 impulses/sec when stimulated from its motor nerve, soleus a fusion frequency around 30 impulses/sec (Cooper & Eccles, 1930). One rat on which a similar experiment was carried out with the medial gastrocnemius and soleus gave fusion frequencies of the order of 180 and 55 respectively.

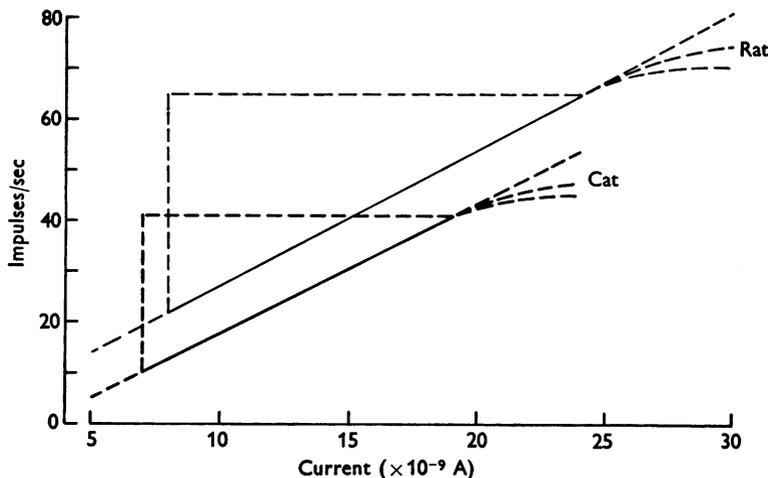


Fig. 15. Schematic. Intracellular depolarization. Comparison of rat and cat motoneurons in plot of impulse frequency against current strength. Lines drawn in full represent averages from ten rat cells and six cat cells discharging repetitively at a steady 'adapted' rate. The average range, as measured in this work, indicated by the triangles.

#### DISCUSSION

##### *The linear relation*

The linear relation of impulse frequency  $F$  to current strength  $I$  is likely to be of general validity, though previously investigated with currents of different strengths only for one motoneurone extracellularly stimulated (Barron & Matthews, 1938), and for some intracellularly stimulated motoneurons (Frank & Fuortes, personal communication). In addition it has been proved to be valid for algebraical summation of excitation and inhibition in tonic motoneurons (Granit & Renkin, 1961). To this should be added that investigations in sense organs of the relation between impulse frequency and generator potential from Katz (1950) onwards support the same generalization (cf. MacNichol, 1956; Fuortes, 1958, 1959; Loewenstein, 1960; Wolbarsht, 1960; Terzuolo & Washizu,

1962). In the present work the whole physiological operative range of tonically active motoneurons was found to be covered by current strengths below those at which the resistance of the stimulating micro-electrode was influenced, and long-lasting tonic discharges were well maintained. The small values of the slope constants of adapted neurons are of considerable interest from the point of view of control of rate of firing.

In the steady state of firing the depression by after-hyperpolarization may be regarded as an 'apparatus constant' characterizing the particular motoneuron studied. Above we have seen after-hyperpolarization rapidly acquire a definite value (cf. also Granit *et al.* 1963) early during transmembrane stimulation. In order to eliminate the constants we define  $f$  as  $F$  minus threshold frequency and  $i$  as  $I$  minus the rhythmic threshold current. The time or duration of the spike interval is then  $t$  ( $f = 1/t$ ). In these terms the linear relation discussed means that the product  $i \times t$  is constant, suggesting that the rate of rise of the generative process can be regarded as being proportional to current strength. The constant value of  $i \times t$  necessary for spike generation is the threshold of the cell. Whenever it is reached, and howsoever, whether slowly or rapidly, the cell fires, and so, for steady-state conditions, there is no accommodation.

There is a striking likeness between the tonically firing motoneuron and the slowly-adapting stretch receptor of the crayfish (Eyzaguirre & Kuffler, 1955), discharging in response to maintained stretch. Each spike in that receptor starts from the same level independently of firing rate until stretch becomes excessive. The level of depolarization then rises and the spikes become smaller until finally inactivation occurs. The spike of the stretch receptor is also followed by after-hyperpolarization just like the spike recorded from the inside of many types of invertebrate nerve fibres (see e.g. Arvanitaki & Chalazonitis, 1956; Frankenhaeuser & Hodgkin, 1956). For *Carcinus* fibres the decisive process setting repetition rate, as above with motoneurons, is the rise of the local generative process (Hodgkin, 1948) operating to overcome after-hyperpolarization which, as stated, can be regarded as something of an 'apparatus constant'. The refractory period of the motoneuron does not enter into the picture.

Amount of after-hyperpolarization for any one motoneuron depends on the level of membrane potential before the spike (Eccles, 1957) and upon the manner or site of firing (Granit *et al.* 1963), but neither factor is of importance under steady-state conditions because within the physiological range of operation the motoneuron always appears to fire from a fixed level of threshold depolarization, and then always will have constant after-hyperpolarization. To judge by the intracellular records of Granit, Phillips, Skoglund & Steg (1957) showing the cat motoneuron firing in response to muscle stretch, tonic firing in reflex activity cannot be distinguished from

the samples of similar activity presented above in response to intracellular stimulation.

#### *Accommodation*

Araki & Otani (1959) initiated systematic work on accommodation, using toad motoneurons. They found generation of an IS spike dependent on rate of rise of current, thus possessing accommodation, while the spike generated directly from a local response, ascribed to the soma, responded to strength of current and was little influenced by its rate of rise. Sasaki & Otani (1961) extended this work to the cat and found two types of motoneurons, (1) one with a higher threshold for slowly rising currents as a sign of accommodation and with a mean duration for after-hyperpolarization of 72 msec, (2) another without accommodation and with a duration of after-hyperpolarization amounting on an average to 102 msec. These were held to be the phasic and tonic motoneurons respectively of Granit, Henatsch & Steg (1956) and of Eccles *et al.* (1958). Our technique has nothing to add to the question of accommodation to slowly rising currents; but it is of interest in the present connexion that in Sasaki & Otani's first type the threshold for slowly rising currents (maintained in our case) rose to very high values, and so those motoneurons could not fire tonically to reasonable strengths of current. This finding suggests that some of our phasic neurones have been truly accommodative or phasic, namely those which, although their spikes were well over 70 mV, were yet incapable of delivering more than a brief burst of spikes while strong currents could not be compensated for. The other type is likely to have been the non-accommodative ones to which above we have devoted attention. Pascoe (1957) and Frank & Fuortes (1960), who did not find any accommodation, may have been studying motoneurons of the second group.

Sasaki & Otani (1961) found both types discharging from the so-called A or IS zone, though the former seemed to shift their firing site to the soma region when stronger currents were used. On their view cat motoneurons therefore fall into groups possessing more or less accommodative IS zones. Implicit in this conclusion seems to be that, on their data, somehow duration of after-hyperpolarization is connected to the accommodative properties of the IS zone, which is curious because the IS spike itself is lacking an after-hyperpolarization (Eccles, 1957). Transitions also occurred from one type to the other, though whether or not after-hyperpolarizations followed suit is not stated. Inexplicable changes in accommodation between cells with and cells without a minimum current gradient also occurred in the work of Bradley & Somjen (1961) on rat motoneurons, but most cells quickly reached a ceiling, high or low, after which rate of rise of stimulating current lost significance and the threshold or 'ceiling' (their term) became decisive.

#### *Adaptation*

It remains to enumerate the reasons why we have preferred to speak in general terms of adaptation. Work by Hodgkin & Huxley (1952), by Frankenhaeuser (1952) and recently again by Vallbo (personal communication) has shown how the concept 'accommodation' might be given a real meaning in terms of measurable events at the membrane and also how difficult it is to define, even when the preparation is a single fibre with one node operating under a voltage clamp while the adjacent ones are cocaineized. The more protean structure of the motoneurone can clearly fire in different ways (see previous section) and we have found (Granit *et al.* 1963) that, in particular, the rat motoneurons, during trans-membrane depolarization, change their mode of firing from one with delayed depolarization and little after-hyperpolarization to another with much after-

hyperpolarization and little delayed depolarization. This must, to say the least, involve an expansion of the firing zone to engage a much larger fraction of the cell in the well-regulated process of rhythmic activity (cf. Fuortes, Frank & Becker, 1957). The question therefore arises of what the slowly rising current in accommodation experiments really is measuring when motoneurones are concerned.

In the graphs of Bradley & Somjen (1961), who plotted firing threshold against time for currents rising at different rates, the initial part of the curve took a very complicated course, suggesting that alongside accommodative changes something else happened, but when all those rapid changes were over the majority of their motoneurones emerged without accommodation.

The adaptive process that we have traced, both in terms of spike frequency and slope constants, can be described in the statement that, since current is constant, more and more time is needed for the motoneurone before it is prepared to generate the next spike. Thus adaptation slows down spike generation. Araki (1960), when measuring the threshold to electrical excitation during maintained polarization of toad motoneurones, found a rise that was small with depolarizing currents and grew larger when the cells were hyperpolarized by the maintained current. He thus described an adaptive process that depended upon direction of current. This factor is likely to be very important. It underlines the complexity of the situation.

Our results clearly show that an early rapid adaptation by no means excludes development of a steady state of non-accommodative firing, but how the motoneurones achieve this transformation must for the moment be regarded as unknown. We would also pay considerable attention to the fact that in our experience tonically firing motoneurones never turn silent with a final display of IS spikes, supporting the view that tonic firing to depolarization is a form of integrated or joint action on the part of a very large portion of the motoneurone (cf. Fuortes *et al.* 1957).

#### *Relevance of findings for integrative work*

Some comments on this aspect of our work—the one which has inspired it—should be of interest. The properties of the cell membrane itself provide each motoneurone with a characteristic curve relating firing rate to depolarization, along which the synaptic apparatus can operate to throw the cell in or out or set its rate of repetition at any desired level.

It is striking that the rheobase or threshold for one impulse varies but little from cell to cell by comparison with the relative firing rates at the so-called rhythmic threshold. The latter apparently is largely determined by the amount and time course of after-hyperpolarization. Phasic moto-

neurones would therefore have a higher firing rate at the rhythmic threshold than tonic ones, because they have less after-hyperpolarization (Eccles *et al.* 1958; Kuno, 1959). For the same reason rat motoneurones would have higher discharge frequencies at the rhythmic threshold than cat motoneurones, as in Fig. 15 (see also Bradley & Somjen, 1961). The phasic motoneurones of Sasaki & Otani (1961) possessing accommodation also had less after-hyperpolarization than those without accommodation.

In this work also the slope constants distinguish phasic motoneurones and early spikes of many tonic ones from the truly tonically firing motoneurones whose slope constants are small in the adapted state. Phasic activity of motoneurones therefore requires but little depolarization for a great rise in frequency. Tonic neurones will be more dependent on synaptic density in order to be able to operate along the full range of their characteristic curves. This is exemplified by the Ia afferent projections whose density in the cat is some 25 % greater on tonic than on phasic cells (Eccles, Eccles & Lundberg, 1957).

Granit & Jurna (1961), studying in the cat unitary extensor spikes from roots L7 and S1 in response to muscle stretch before and after strychnine, found two categories: (1) soleus motoneurones whose discharge rate was very little influenced by removal of post-synaptic inhibitions (cf. Bradley, Easton & Eccles, 1953), in spite of the fact that especially these motoneurones are subject to heavy recurrent inhibition (Granit, Pascoe & Steg, 1957; Kuno, 1959; Eccles, Eccles, Iggo & Ito, 1961); (2) motoneurones whose frequency of discharge to stretch underwent considerable increase after strychnine. Their conclusion, entirely justified by our results, was that the truly tonic motoneurones have a small range of frequency variation at their disposal because the 'slope constants' are small. These comments will have to suffice to illustrate the integrative significance of the ideas pursued in this paper.

#### SUMMARY

1. In response to long-lasting trans-membrane depolarization by current through the tip of the micro-electrode a number of rat and cat motoneurones set up a steady discharge during stimulation.

2. The frequency of discharge is linearly related to current strength within a range of physiological frequency variation.

3. The steady state of firing is preceded by a brief phase of 'adaptation' during which impulse frequency diminishes at a rate varying from cell to cell and depending on current strength.

4. Early in adaptation, during the delivery of initial spikes, after-hyperpolarization develops and contributes to the adaptive process by diminishing firing rate.

5. A second component of adaptation is a true opposing activity on the part of the membrane leading to an increase of threshold and characterized by a drop in the value of the slope constants of the curves relating firing rate to current strength.

6. In tonic cells the adaptive process is generally over in some tenth of a second. Slope constants are given for the steady state of firing in 10 rat and 6 cat cells. The latter fired at a lower average rate. The discussion deals with the general significance of measurements of slope constants and firing thresholds for the understanding of neural control as well as with some problems of accommodation and adaptation.

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