

## THE BEHAVIOUR OF MAMMALIAN MOTONEURONES DURING LONG-LASTING ORTHODROMIC, ANTIDROMIC AND TRANS-MEMBRANE STIMULATION

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The general problem of how well long-lasting discharges can be maintained in motoneurones stimulated in the ways enumerated in the title of this paper forms its subject. We refer to our previous paper (1963) for evidence in support of the facts, first, that the relation between impulse frequency and trans-membrane current strength, measured at a fixed time after beginning of stimulation, is linear for mammalian motoneurones (rat, cat), and secondly, that for any one motoneurone the proportionality constant of such linear curves rapidly diminishes during maintained stimulation, and soon acquires a stationary 'adapted' value; in cats, after a few spikes have been fired in response to the intracellular stimulus. The direct proportionality between discharge frequency ( $f$ ) and current strength ( $I$ ) will be briefly referred to below as the linear  $f$ - $I$  relation. It is now imperative to treat all exceptions from this relation as limiting conditions defining its range of validity. One concern here is with the limiting conditions of the linear  $f$ - $I$  relation under long-lasting stimulation. How stationary is, for instance, the value of the 'adapted' proportionality constant?

An interesting version of this problem was adumbrated in an old paper by Liddell & Sherrington (1925) describing myographic work with the crossed extensor reflex in which a 'stimulation-plateau' was distinguished from an 'after-discharge-plateau' both delivering the same amount of contraction in grams isometric tension. The relevant point is that a brief intercurrent ipsilateral inhibition had a much greater effect on the after-discharge-plateau than on the stimulation-plateau. This seems to be an exception from the linear  $f$ - $I$  relation. A related problem was formulated quantitatively by Granit & Rutledge (1960) while studying unitary discharges of extensor motoneurones activated in decerebrate animals by constant stretch. Although the cell then might fire at a constant rate over

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a considerable time (suggesting a constant depolarizing current), nevertheless, testing the cell with a constant recurrent inhibitory stimulus (= repolarizing current) revealed that this inhibition increased in potency as a function of duration of firing. It should be noted that recurrent inhibition has been shown to be of the repolarizing variety (Eccles, Fatt & Koketsu, 1954) and so this result would also be an exception from the linear  $f-I$  relation. This increase of inhibition could be counteracted by supporting the constant rate of firing by increased stretch and quite generally it was found that, in order to maintain good resistance to repolarizing inhibition over some time, a certain amount of 'excitatory surplus' was necessary. If this precaution was observed, recurrent inhibition operated on the linear  $f-I$  relation (Granit & Renkin, 1961). The present paper will throw light upon the general problem of why in such experiments surplus excitation is necessary when the fixed level of depolarization is assessed by a measured amount of repolarization. It will do so by showing the margin of error by which in tonically discharging motoneurons rate of firing measures net depolarization.

Our technique being intracellular it has been possible to replace tests employing recurrent inhibition by actual membrane repolarization through the electrode tip while the cell has been forced to discharge in response to trans-membrane, or synaptic or antidromic stimulation. Synaptic contributions to the events studied could thus be separated from membrane properties as such.

#### METHODS

Rats anaesthetized with Nembutal (pentobarbitone, 55 mg/kg; Abbott Laboratories) 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 present work makes use of cells from our previous studies (Granit, Kernell & Shortess 1963; Granit, Kernell & Smith, 1963) selected among those which during long-lasting stimulation proved particularly stable. The circuit for intracellular stimulation through the tip of the micro-electrode was similar to that introduced by Araki & Otani (1955).

#### RESULTS

*Trans-membrane stimulation.* To this form of stimulation tonic motoneurons adapt quickly; in rats within something of the order of half a second, meaning that the proportionality constant by which firing rate is related to current strength then has assumed a fixed value (Granit, Kernell & Smith, 1963). In the experiments on surplus excitation referred to, and in tests of the linear  $f-I$  relation by recurrent inhibition (Granit & Renkin, 1961) motoneurons were often kept firing tonically for minutes. In terms of trans-membrane stimulation this means that, as one switches from stronger to weaker currents or vice versa, the proportionality con-

stant must remain stationary even if firing rate were to suffer a reduction in absolute terms. With two of our best motoneurones this proposition was scrutinized. One of them hardly altered its firing rates at all during minutes of stimulation at different current strengths. The results with the other one are shown in Fig. 1.

In Fig. 1 displacement of the current beam upwards indicates repolarizing current (inhibition), downwards, depolarizing current (excitation). The experiment was carried out twice with a brief pause between the tests which lasted between 5 and 6 min. The stimulator was connected to the delay circuit of the sweep which introduced a pause of around 1 sec every

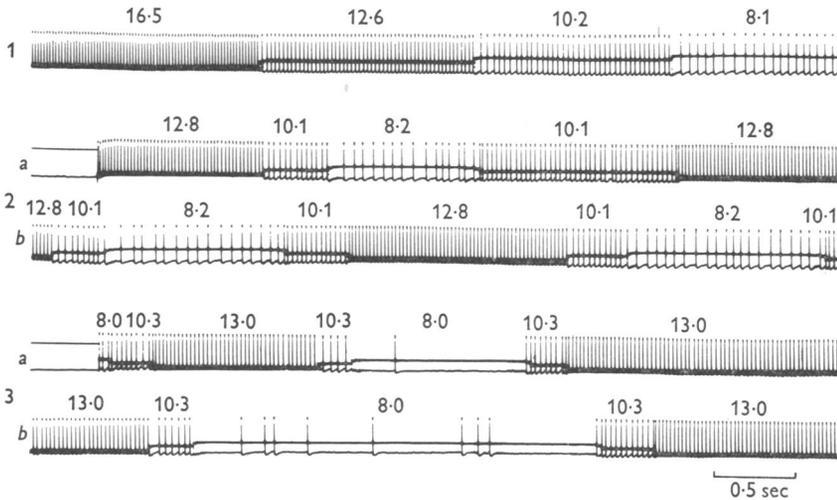


Fig. 1. Rat motoneurone of 81 mV, maintained under intracellular stimulation for about 200 sec with brief interruptions around 1 sec every 10th sec. Current strength in nA above each record, as read with an estimated accuracy of the order of 0.2 nA from recorder displayed in the figure. 1 from 27 to 32 sec; 2, from 99 to 109 sec, record *b* directly continued from *a*; 3, between 179 and 189 sec.

10th second. For obvious reasons very strong currents cannot be used in such a test. Figure 1 shows that there was a slow rise of threshold, best visible with the weak current of around 8 nA, whose effect gradually became subthreshold if by the (rhythmic) threshold we mean the current required to obtain minimum frequency of regular firing. It is further seen that when the change is from 'excitation' to 'inhibition' the discharge commences at a lower rate than when it takes place the other way round.

An experiment with the same motoneurone is analysed in detail in Fig. 2. The uppermost straight line refers to the first experiment soon after penetration and is taken from Fig. 3 of Granit, Kernell & Shortess (1963), the others were begun about half an hour later. Clearly the propor-

tionality constant (of the linear  $f-I$  relation) remained at its fixed 'adapted' value but the threshold rose slowly. The curve as a whole therefore shifted downwards during extended stimulation and this automatically involved the rhythmic threshold which in this case was at a high rate of discharge.

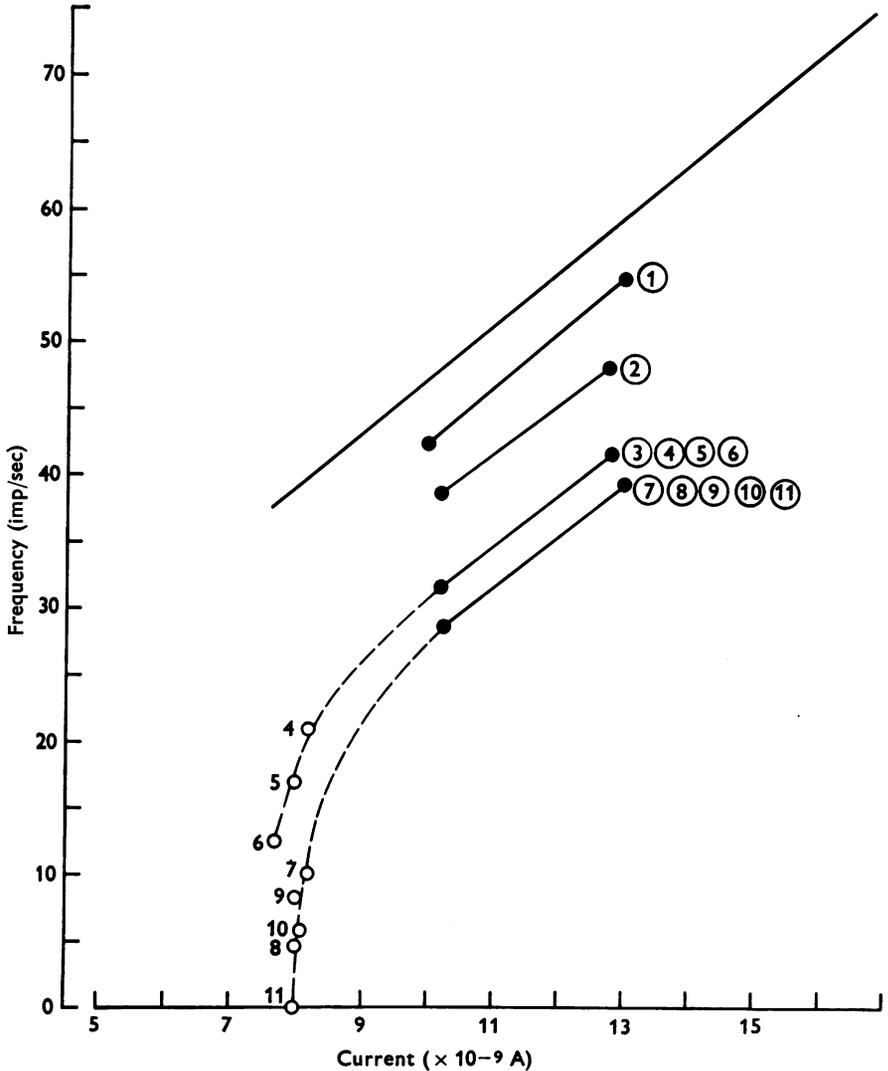


Fig. 2. Same spike as in Fig. 1. Uppermost line refers to moment 2.6 sec after initiation of trans-membrane stimulation and is taken from Fig. 3 of Granit, Kernell & Shortess (1963). The other curves join readings obtained during 110 sec of stimulation as in Fig. 1. 1, time 0 to 4.5 sec; 2, 4.5 to 10.5 sec; 3, 11.6 to 21.6 sec; 4, 22.7 to 32.7 sec; 5, 33.8 to 43.8 sec; 6, 44.9 to 54.9 sec; 7, 56.0 to 66.0 sec; 8, 67.1 to 77.1 sec; 9, 78.2 to 88.2 sec; 10, 89.3 to 99.3 sec; 11, 100.4 to 110.0 sec.

Thus current strength gradually grew insufficient for maintaining the rate at which the cell just could fire rhythmically, as is likewise shown by original records in Fig. 1.

Owing to the technical difficulties endangering constancy in long-lasting penetrations, it would be difficult to generalize from a few successful experiments of this kind. Fortunately, we are in possession of the supplementary statistical approach by Granit & Renkin (1961), who studied unit discharge picked up from the ventral roots of decerebrate cats and tested by recurrent inhibition. The linear  $f-I$  relation proved valid in their work. It therefore suffices to have shown here that their result can be repeated by trans-membrane stimulation, even with a cell whose frequency of discharge gradually altered as a function of time. The linear  $f-I$  relation merely requires a fixed proportionality constant for long duration of stimulation. If this condition is fulfilled, then, whatever the firing rate, a constant repolarizing current would always subtract the same number of impulses per second.

*Dorsal root stimulation.* In Fig. 3 are shown results with a cell responding to long-lasting stimulation of the dorsal root L4. Rates of stimulation were changed in the order illustrated and a repolarizing current introduced at regular intervals. Exceptionally, in this record, repolarization is displayed by current recorder shifting *downwards*. This was an excellent penetration because the spike reached 92 mV, our maximum with rats, responded well to inside stimulation (uppermost record) and was kept stimulated anti- and orthodromically from the roots for minutes. The effect of inhibition increased at the higher frequencies when the margin between firing threshold and post-synaptic potential became reduced.

The increasing effectiveness of inhibition with time, shown in Fig. 3, could easily be obtained by constant repolarizing currents and up to a point could also be counteracted by increasing stimulus strength. The moment of onset of the effect was in the expected manner a function of how duration and strength of stimulation were balanced against intensity of the repolarizing current, i.e. of how great the margin of surplus excitation happened to be. It is important in experiments of this type to avoid inactivation (Granit & Phillips, 1956), so easily elicited in rats by stimulating the dorsal roots (Granit, Kernell & Smith, 1963). This state is heralded by a drop in membrane potential and spike size. Dorsal root stimulation is, of course, highly unphysiological as a stimulus.

Figure 4A shows the same increase of the inhibitory effect as a function of duration of stimulation, but this time at a very low rate of repetition. At the higher rate, in records B, the inhibitory effect of repolarization was stronger from the beginning and increased at a faster rate. The records on the fast 'sweep' show that the average rate of rise of post-synaptic

potentials decreased during long-lasting stimulation. Fewer of them reached firing threshold, as also is seen in the continuous records.

Stimulation of dorsal roots brings into the picture both pre- and post-synaptic inhibition (see Discussion) and does not provide a fair comparison with our earlier experiments (Granit & Rutledge, 1960; Granit & Renkin,

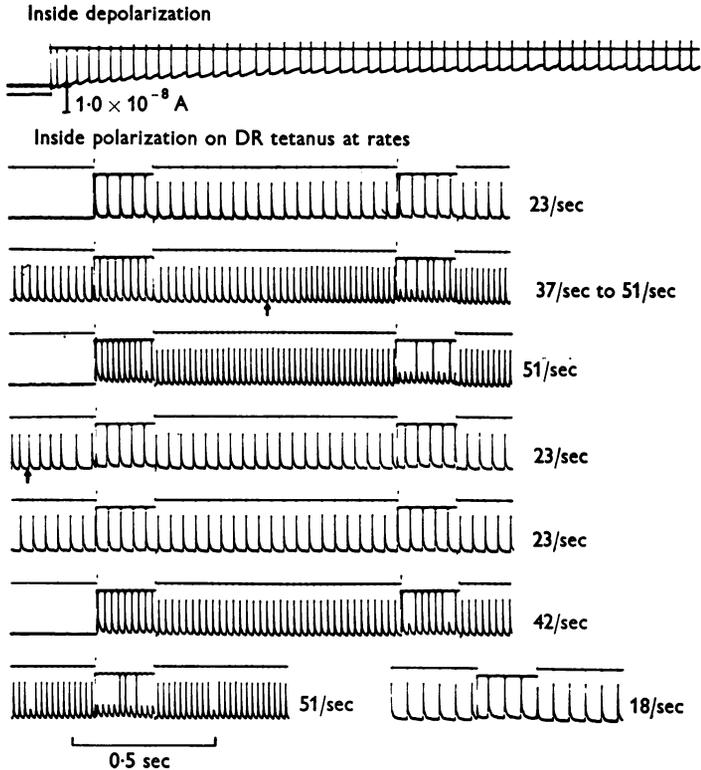


Fig. 3. Rat motoneurone of 92 mV stimulated is marked on the figure. Uppermost record inserted to show how well it responded to trans-membrane current. The others show maintained stimulation of dorsal root L4 interrupted by pauses lasting 0.7 sec every 19th sec. Brief repolarizations inserted in the course of stimulation at different rates. Exceptionally in this experiment the testing trans-membrane currents of strength 2.8 nA (tip of intracellular electrode negative) were displayed *downwards* on current recorder. In all other records repolarizing currents are always illustrated upwards. Arrows mark change of stimulus frequency when introduced in the midst of stimulation. Responses synchronous with stimulation.

1961) on decerebrate cats, in which the spike was studied in root filaments and muscular afferents were used for activation of the motoneurons. But, restricting our comparison to general principles, Figs. 3 and 4 illustrate further sources of error in extracellular work based on measurements of spike frequency. These will be taken up in the Discussion.

Yet even with dorsal root stimulation and intracellular recording it is not impossible to adjust the conditions so that the linear  $f-I$  relation is obeyed. In Fig. 5 is shown an experiment at a faster rate of stimulation

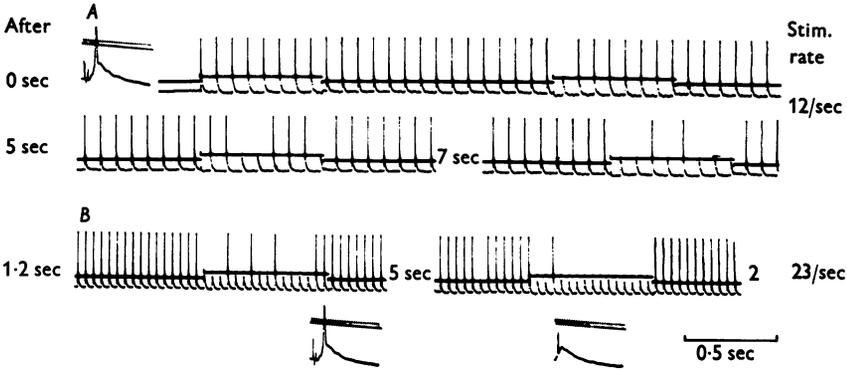


Fig. 4. Rat motoneurone of 67 mV driven from dorsal root L4 at rates marked on the right. *A* begins with fast record on sweep circuit, time 1000 c/s, of first spike in the series. Second row displays moments after 5 and 14 sec of driving, the latter after a pause of 7 sec. *B*, same experiment at faster rate of driving and samples on sweep circuit. Repolarizations of 3.7 nA introduced every 3.7th sec. Responses synchronous with stimulation.

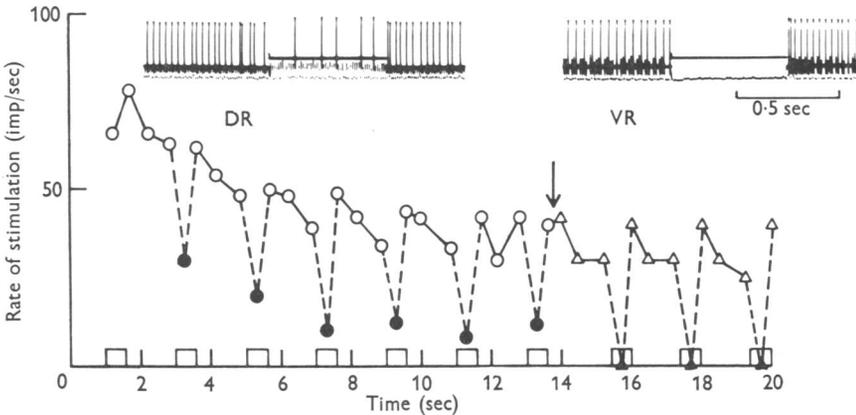


Fig. 5. Rat motoneurone of 72 mV stimulated from dorsal root L4 at a rate of 111/sec for 20 sec as marked on abscissa, on which oblongs represent duration of brief repolarizing currents of 6.8 nA through electrode tip. At arrow dorsal root stimulation is replaced by ventral root stimulation. Inset: samples of records including repolarizations. Responses non-synchronous at this rate of stimulation.

in which as above a constant repolarizing current was repeated at regular intervals during 14 sec of tetanization of the dorsal root L4. It is seen that in spite of a considerable reduction of firing rate artificial inhibition by the trans-membrane repolarizing current removed the same number of

impulses throughout the experiment, as required on the linear  $f$ - $I$  relation. At the arrow stimulation at the same rate was switched to the ventral root L4. There was then complete inhibition to the same repolarizing current and this was followed by rebound.

*Ventral root stimulation.* The rebound shown in Fig. 5 is of some interest because it differs fundamentally from the rebound seen with dorsal root stimulation when the cell has become excessively depolarized, discussed (with references) by Granit, Kernell & Smith (1963). For ventral root

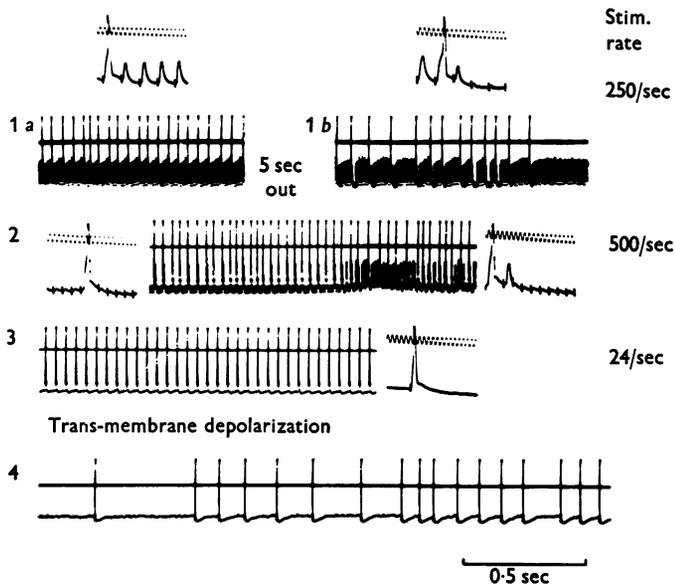


Fig. 6. Rat motoneurone of 75 mV stimulated through ventral root L4 in records 1-3 and through the intracellular electrode at 5.9 nA in record 4 (inside +). Occasional records on the 'sweep' at time 1000 c/s to illustrate type of discharge at the places where they occur. Note that when block has developed at the A- or IS-zone in 1a and 1b, the cell can be made to fire at fast rates by increasing stimulus frequency in record 2 to 500/sec.

rebound the requirement is that there should exist a block between the B- or SD- and A- or IS-spikes, by the intracellular technique first demonstrated by Brock, Coombs & Eccles (1953) and then studied by many authors (e.g. Coombs, Curtis & Eccles, 1957; Fuortes, Frank & Becker, 1957). This block occurs whenever the stimulus rate exceeds the following rate of the motoneurones (see Fig. 6). The moment of rest given the motoneurone during repolarization then allows time for recovery and so the cell immediately afterwards, and for a while, fires at a higher rate.

The important point here is that development of the block is cumulative,

as shown in Fig. 6, records 1*a* and 1*b*, which agrees with findings previously reported by Brock *et al.* (1953). A new finding seems to be that a considerable number of the best motoneurones—though not all of them—in both rats and cats fire at very much higher rates and with little evidence of A- or IS spikes when stimulus rate is greatly increased. This is shown in record 2 of Fig. 6, selected also because the block reappeared spontaneously for a brief while together with IS-spikes and then with a change of base line suggesting some spontaneous depolarization. When this

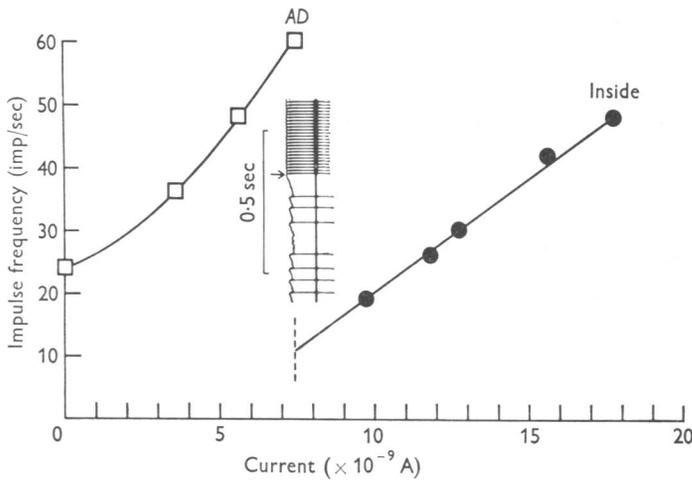


Fig. 7. Rat motoneurone of 75 mV. Stimulated to discharge from the inside it fires in the adapted state along the curve thus marked. Curve *AD* refers to a maintained antidromic stimulation of ventral root L4 at rate 60/sec to which the cell had equilibrated by firing at 24/sec when the experiment began. Curve *AD* shows at what rate antidromic impulses entered during trans-membrane depolarization to the current strengths marked on the abscissa. At the very point when the cell began to fire sporadically to inside stimulation, marked by vertical interrupted line, it responded to antidromic rate 60/sec. This moment is illustrated by inset, in which *AD* stimulation is suddenly interrupted while intracellular stimulation to 7.4 nA is still being maintained.

happened, discharge frequency again was reduced. No doubt, what takes place at high rates of stimulation is that the site of the block is shifted outwards on the axon and so, by summation, a certain number of spikes can traverse the resting IS-zone.

As pointed out previously (Granit, Kernell & Smith, 1963), the antidromically elicited full-size spikes are characterized by delayed depolarization which diminishes or disappears in trans-membrane depolarization (record 4) when after-hyperpolarization greatly increases. Record 3 shows the maximum synchronous firing rate that could be maintained during antidromic stimulation of this cell.

It may finally be of some interest in this connexion to compare the strength of depolarizing current needed to remove an IS-SD block with that required to fire the cell from the inside. In Fig. 7 the motoneurone was stimulated antidromically at 60/sec and soon settled down to a steady rate of discharge at 24/sec. The *AD*-curve of the figure shows rate of antidromic invasion of the cell as a function of trans-membrane current strength while the curve marked 'Inside' illustrates the relation between impulse frequency and trans-membrane current strength (the linear *f-I* relation) in the adapted state of the cell. The interrupted vertical line indicates at what strength (7.4 nA) irregular firing occurred. This was also the current strength at which antidromic impulses followed the stimulus repetition rate (60/sec), as shown by the 'Inset' record. At the arrow in this record antidromic stimulation was stopped.

#### DISCUSSION

Figures 1 and 2 have shown that when the absolute rate of firing diminishes as a function of time of stimulation, this does not influence the slope of the linear *f-I* relation within the medium range of firing rates but at the lower end dramatic changes take place owing to the corresponding and automatic rise of the rhythmic threshold. If a slight repolarizing inhibition was inserted in that region of depolarizing currents, it would immediately silence the cell. This effect could simply be compensated for by taking care to add synaptic excitation (excitatory surplus) on the afferent side, as was done by Granit & Rutledge (1960). It should further be recalled that their experiments, as well as those of Liddell & Sherrington (1925), were carried out with tonic motoneurones, which fire at very low rates. It has been shown that the low rates of firing really signify that tonic motoneurones are being discharged near the rhythmic threshold, when stimulated through their muscular afferents, because they can be discharged at very much higher rates, e.g. in response to the pinna reflex (Granit, Haase & Rutledge, 1960).

It should further be considered that spike counting and constant isometric reflex tension were the sole indicators used in the earlier work referred to. Such indices may well be accurate in cells which possess large proportionality constants on the linear *f-I* relation. But it was shown in our previous paper that the slope constants of the tonically firing motoneurones isolated were small, from 1.5 to 4 imp/sec/nA (Granit, Kernell & Shortess, 1963), and the tonically firing extensor motoneurones which failed to follow the linear *f-I* relation in those earlier tests may have had constants at the lower end of that restricted range. This means that spike frequency alone as a measure of net depolarization is afflicted with a large margin of error. Isometric tension would be less critical still. For this

reason, too, it is held that in the earlier experiments far less stringent criteria were used than those of Granit, Kernell & Shortess (1963), in which it was always known how far above the rhythmic threshold firing was being maintained.

To sum up, in tonic firing elicited from muscular afferents both auto-genetic as well as recurrent inhibition combine forces with the naturally low proportionality constants on the linear  $f-I$  relation to hold down firing rate and make it an insensitive index of net depolarization. Many cells therefore fire near their rhythmic threshold from the beginning and continue to approach it when exposed to adaptation during extended stimulation. They will reach threshold at moments which cannot be predicted from firing rates alone. Apparent deviations from the linear  $f-I$  relation thus occur as 'technical artifacts'. Obviously this effect can be compensated for by adding what in terms of afferent stimulation appears as surplus excitation (Granit & Rutledge, 1960).

The experiments of Figs. 3 and 4 with dorsal root stimulation demonstrate an effect of duration of stimulation which may be partly a similar adaptive rise of threshold which the synchronous beats of post-synaptic potential fail to catch up with. But other factors also have to be considered. Repetition may lead to 'desensitization' (Katz & Thesleff, 1957; Thesleff, 1959), to some exhaustion in the supply of transmitter substance or to depolarization of the afferent terminals (Wall, 1958; Eccles & Krnjević, 1959*a, b*) which will reduce the size of the afferent volley, thereby producing what Eccles and his co-workers since have termed presynaptic inhibition. By such factors the linear  $f-I$  relation would not be invalidated in principle, because it refers to the actual level of membrane depolarization achieved but, again, this method of testing for it is clearly subject to the errors inherent in the index of measuring, as discussed above. High rates of stimulation better imitate physiological conditions, as shown in Fig. 5. The linear  $f-I$  relation can then be proved valid, despite dorsal root stimulation, but only in very successful experiments, because this mode of stimulation is highly unphysiological.

The experiments with long-lasting ventral root stimulation (Figs. 6 and 7) fill out some gaps in our knowledge of this mode of activation of motoneurons. It is perhaps not entirely unexpected to see (in Fig. 7) how little current is needed to remove the IS-SD block by comparison with what is necessary for maintained firing above the rhythmic threshold.

#### SUMMARY

1. Motoneurons stimulated by trans-membrane currents may be excited to yield for minutes a discharge whose frequency is proportional to current strength (the linear  $f-I$  relation), even when a gradual rise of

threshold takes place and shifts the level of such curves in the co-ordinate system.

2. Experiments have been designed to throw light on the question of why analysis of the linear  $f-I$  relation by experiments based on measurements of discharge frequency as its sole criterion presupposes some surplus excitation to become technically possible. This is shown to be a technical limitation in such experiments and not a true deviation from the  $f-I$  relation.

3. Antidromic blocking to repetitive stimulation and its release has been studied in its dependence on stimulus frequency and trans-membrane currents.

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