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REGULATION OF DISCHARGE RATE BY INHIBITION, ESPECIALLY BY RECURRENT INHIBITION

RAGNAR GRANIT

From the Nobel Institute for Neurophysiology, Karolinska Institutet,
Stockholm 60, Sweden

PROBLEMS of regulation and control in the mammalian preparation present us with the inherent difficulty of how to analyze equilibria. One tries to disturb them—and a chain of events is mobilized to establish a new state of equilibrium. The combinations and permutations involved in such readjustments are not easily disentangled. In the hope of being able to contribute to the understanding of the role of inhibition in the regulation of activity of the motoneurons it was decided to make a test case out of recurrent inhibition whose circuit is relatively simple and has the advantage of being on the efferent side and hence not subject to as many influences as is the afferent side. It is not possible here to discuss the old work on antidromic inhibition and the early attempts from the beginning of this century to assign a function to the recurrent collaterals of Golgi. A great step forward was taken when Renshaw (1946) discovered the high-frequency discharge to antidromic stimulation of ventral roots in the cells which today we call the Renshaw cells, and when Eccles *et al.* (1954) by different types of experiments found that it paralleled the course of repolarization of the ventral horn cells that was to be expected if antidromic inhibition essentially was recurrent in nature. The work of Brooks and Wilson (1959) and of Wilson (1959) supports the view of the Canberra group; as also does all the work they have done at Canberra since 1954. We make it the basis of our approach that recurrent inhibition repolarizes the motoneurons across an internuncial cell and that it in this respect resembles other polysynaptic inhibitions, a parallel emphasized by Eccles (1957). Most of the work to be considered below is from three papers from the Nobel Institute (Granit *et al.*, 1957, 1960; Granit and Rutledge, 1960). They will be referred to as nos. 1, 2 and 3, respectively.

In paper no. 1 the decerebrate preparation was used and antidromic stimulation of a number of efferent filaments was made to influence the tonic discharge of a functionally isolated cell in a thin ventral root filament. This technique has been used also in papers nos. 2 and 3. To produce a tonic discharge we stimulate by pull on the gastrocnemius-soleus muscle or cut the nerve to this muscle and tetanize its central stump electrically at a

rate around 114/sec. In the former case truly tonic cells discharge, in the latter case stimulation will be strong enough to activate tonic responses in cells which normally—to muscular afferents—would respond phasically. With electrical tetanization of muscular afferents the efferent roots have to be cut.

Now there are two ways of stimulating antidromically. In both cases the stimulating electrodes are on the root delivering the filament whose spike we analyze; in one case on the whole remaining root, in the other only on some antidromically active filaments. With extensor reflexes it does not seem to matter much if one selects the most strongly inhibiting adjacent filaments or the whole remaining root.

Let us first consider the tonic stretch reflex as exemplified by the single-fibre preparation responding reflexly to pull on the gastrocnemius-soleus. This situation requires some conceptual clarification. The muscle spindles are responsible for what we call "excitatory drive" which may be regarded as a barrage of impulses that activates a certain number of synaptic knobs per unit time. The result emerges as net depolarizing current P_{dep} across the cell membrane. This process is opposed by repolarizing forces such as orthodromic inhibition from afferents over internuncial cells and natural recurrent inhibition initiated by the firing tonic ventral horn cells themselves. Let the sum total of these opposing forces be P_{pol} . As stated above, antidromic stimulation was proved by Eccles *et al.* (1954) to repolarize the ventral horn cell. The normal frequency of discharge F_n will be some function of the net depolarizing current which is the algebraic sum of the two opposite forces.

$$F_n = f(P_{\text{dep}} + P_{\text{pol}}) \quad (1)$$

Long ago Barron and Matthews (1938) were interested in this function whose right-hand term from now on I shall call *depolarizing pressure*, defining thereby more precisely a term taken from a paper by Phillips (1959). The experimental difficulty is, of course, how to eliminate P_{pol} or to keep it constant. Barron and Matthews tried to stimulate the spinal cord from the outside with a depolarizing current and they published figures for one cell in which F_n was proportional to strength of depolarizing current. The ideal technique for elimination of P_{pol} from equation (1) is to stimulate through an inside microelectrode in the manner of Araki and Otani (1955). Systematic measurements by this technique have been made by Fuortes and Frank who kindly have allowed me to quote unpublished results. The firing frequency of single motoneurons was found to be a linear function of depolarizing current. Slope constants varied from cell to cell and their range of variation was as wide as from 4 to 13.6 imp/sec per $\text{m}\mu\text{A}$. With many cells linear curves running up to 100 imp/sec were obtained. We recall that normal tonic firing of motoneurons is at rates which are but a fraction of this theoretical maxi-

imum and later we shall consider the general problem of frequency limitation. For the moment let us return to depolarizing pressure ($P_{\text{dep}} + P_{\text{pol}}$) and use recurrent inhibition as our instrument of analysis.

The simplest approach is to study situations in which depolarizing pressure is constant which means that frequency of discharge F_n is held constant. Assume that we pull out gastrocnemius-soleus to an extension of 10 mm and leave it stretched. In a good, tonic decerebrate animal we shall then find spikes which discharge for minutes at practically constant rates. By definition this means that depolarizing pressure is constant. We then proceed to gauge depolarizing pressure by a brief tetanic burst of antidromic stimulation repeated at regular intervals. This means adding to equation (1) a term P'_{pol} of recurrent inhibition by means of which we measure if the sum $P_{\text{dep}} + P_{\text{pol}}$, as reflected by a constant rate of discharge F_n , really is constant at different moments after onset of pull.

Analysis of an experiment is shown in Fig. 1 from paper no. 3. The reflex rate of discharge of the tonic cell was approximately constant between the two horizontal lines, from 20 to 55 sec after onset of stretch. The tests by constant brief tetani of antidromic stimulation vary in efficacy from moment to moment but the general trend of the result is perfectly definite: as time

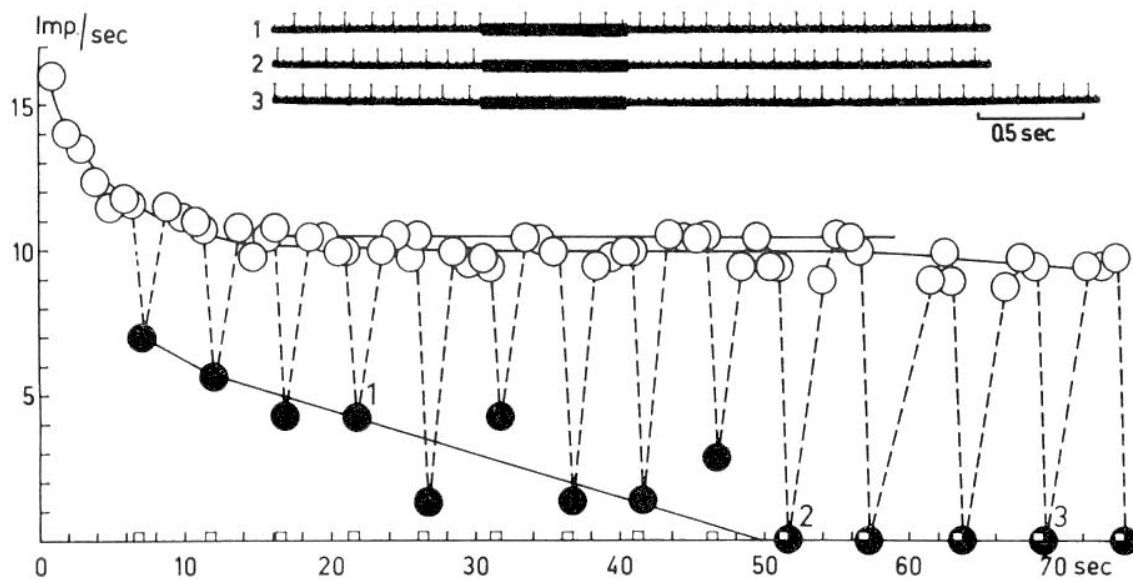


FIG. 1. A 15-mm steady pull on the knee extensors. Tetanic antidromic inhibition at 114/sec inserted for 0.7 sec at regular intervals as marked by oblongs on abscissa (running time). Frequency of discharge constant between the two parallel horizontal lines. Black circles show number of impulses during the periods of recurrent inhibition evaluated in imp/sec. (*Inset*)—Original records at moments marked 1, 2 and 3 in the diagram. Note, that when delayed recovery after recurrent inhibition begins, then discharge frequency fails to reach its original level (at this rate of repetition of antidromic stimulation periods). Discharge stopped for good with last period of stimulation having been five times temporarily silenced (Granit and Rutledge, 1960).

goes on, recurrent inhibition becomes increasingly effective, in fact, inserted at moment 52 sec it succeeded in blocking the cell altogether. The moments 1, 2 and 3, marked in the figure are reproduced in the inset from the original records. We note that the time needed for recovery after a burst of antidromic stimulation increased from 1 to 3 even though between tests the discharge rose to the same level as before.

It is concluded that, since for some reason a constant depolarizing pressure, as gauged by a constant amount of recurrent inhibition, does not deliver a constant inhibitory effect, some concealed factor must be present which is not included in the simple formula (1) relating frequency of discharge to depolarizing pressure. The very first question is whether or not this concealed factor might be a temporal summation of the effects of the individual antidromic bursts so that for this reason the inhibitions actually increase in strength from test to test though—physically speaking—identical as stimuli.

It is not difficult to refute such arguments. To this end we make use of the regularity with which a good tonic motor cell responds to stretches repeated at regular intervals. Then it is possible to make each stretch an individual experiment and throw in the test by recurrent inhibition at any chosen moment in the reflex. Such experiments showed that it really is something behind the discharge of the tonic cell that undergoes a gradual weakening with time leading to a loss of resistance to inhibition. Thus, although the frequency of discharge remains the same and outwardly everything is as before, the stretch reflex in the end has lost the excitatory drive necessary to enable it to withstand a suitably chosen dose of recurrent inhibition.

From this result it is finally concluded that any given frequency of discharge of the motoneuron (F_n), corresponding to a certain depolarizing pressure ($P_{\text{dep}} + P_{\text{pol}}$), may be run on a greater or lesser amount of surplus excitation. The concept of "surplus excitation" which now is introduced is, as it were, another aspect of what was considered above under the term "frequency limitation". As soon as there is frequency limitation, it is possible to have a surplus of excitatory drive for which there is no equivalent increase of output. Long ago it was shown by Denny-Brown (1929) with the stretch reflex that the output frequency of discharge may be largely independent of the degree of extension of the muscle and I have confirmed this result in a recent study (Granit, 1958). We know very well that the muscle spindles discharge in proportion to extension (Eldred *et al.*, 1953; Granit, 1958) and so, the more the muscle is extended, the greater the excitatory drive. Yet the output frequency is limited to a constant value. If, at different extensions of the muscle, one tests with recurrent inhibition, it is easily shown that the greater the extension and hence the excitatory drive, the better the reflex resists recurrent inhibition. Throughout such experiments F_n can be kept constant. Similarly antidromic stimulation is held constant. In this manner then it is proved that the decisive factor is not the depolarizing pressure, as

assayed by constant rate of discharge, but the amount of surplus excitation or excitatory drive by which it is upheld. When the excitatory drive is low the reflex is destroyed by recurrent inhibition. This is what we measure. Early in a stretch reflex there is a good surplus behind the steady frequency of discharge and therefore every loss of depolarizing current resulting from recurrent repolarization is quickly replaced; later on the excitatory drive may be barely sufficient to maintain a given depolarizing pressure (steady output frequency) and so the cell falls an easy prey to repolarization by recurrent inhibition.

One broad generalization following from this work is that in problems of regulation the important intracellular approach which has led to so much conceptual clarification has definite limitations. This is when our concern is with the rules of the game by which frequency of output is determined. It is probably true that in the steady state condition depolarizing pressure determines impulse frequency in accordance with equation (1) and with the results of Fuortes and Frank, mentioned above, with inside stimulation of motoneurons. But up to a point the efficacy of an intercurrent inhibitory force depends on how well any particular depolarizing pressure is defended by excitatory drive. Naturally, efficacy of recurrent inhibition—or any other repolarizing variety of inhibition for that matter—must also depend upon the slope constant by which impulse frequency is related to net depolarizing current. Let us consider the extreme values of Fuortes and Frank, 4 and 13.6 for this slope constant and assume that we are studying two motoneurons adjusted to discharge at the same rate. Assume further that it would be possible to test them by identical amounts of P'_{pol} of recurrent inhibition. Merely because of the different slopes of the two curves (in a diagram relating their impulse frequency to depolarizing current), the effects of recurrent inhibition on the two cells would have to be in the ratio of 4 to 13.6, other things equal. This is clearly because equal amounts of repolarization will reduce frequency of firing in proportion to the constants mentioned.

One might think it unnecessary to introduce this distinction between excitatory drive and depolarizing pressure and instead try to explain our results by an uncertainty in the measurement of firing rate of the motoneurons. In order to reply to this criticism it is possible to design an experiment in which excitatory drive is maintained in spite of a reduction of depolarizing pressure. Thus, with extensor motoneurons, the tonic reflex can be elicited electrically by a maintained afferent tetanus. The depolarizing pressure can at the same time be lowered by pulling on the antagonist flexor muscle tibialis anterior. Exceptionally, in this experiment, the nerve to the flexor must be left intact. By these means it is easy to reduce the firing rate of the extensor motoneuron, used as indicator, in excess of any variation in rate during maintained stretch. When this is done, recurrent inhibition does not

silence the firing cell, the reason being that by the electrical tetanization of the extensor afferents excitatory drive is well maintained in spite of the lowered depolarizing pressure. Such experiments show that it is necessary to distinguish between excitatory drive and depolarizing pressure.

In experiments in which the motoneuron has been silenced by recurrent inhibition it is often observed that maintained stretch does not succeed in reactivating the cell although antidromic stimulation is stopped. This is difficult to explain unless one assumes that recurrent inhibition penetrates into the spinal cord beyond the circuit completed with the projections of Renshaw cells upon motoneurons. Frank and Fuortes (1956) showed that neurons located further inside the spinal cord are influenced by antidromic stimulation and this has since been confirmed. It is therefore possible that recurrent inhibition does something to the interneurons which leads to removal of excitatory drive, provided that drive is low.

A general theory of the physiological role of recurrent inhibition follows from the results obtained. The recurrent control will preferentially be directed towards removal of discharges or states of excitation which are badly supported by excitatory drive, lingering after-discharges, subliminal fringes, near-threshold activity in general, and so, as it were, will hold the reflex to its task. The present author has often wondered why interneurons fire at such high rates and why afferent activation often is so much in excess of what is the immediate apparent need (see e.g. in Granit, 1955, p. 247) but it is clear that if excitatory drive is as important functionally as depolarizing pressure, then what superficially looks like excess activity is merely what is required to maintain low-rate operations of neurons provided with recurrent collaterals. As is well known most nervous centres possess recurrent collaterals. The motoneurons are by no means an exception. The views of ourselves (paper no. 1) and Brooks and Wilson (1959) with regard to special functions of recurrent inhibition fit well into this general theory. Also, whatever organizational features be ascribed to the recurrent system, the inhibitory effect will have to be in accordance with the general rule that has emerged from the work now reviewed.

Recurrent inhibition on a tonic discharge can, as we have seen (paper nos. 1 and 3), be made cumulative in the sense that it generally silences the discharge, the intervals between the efferent impulses increasing from spike to spike. This is done by reducing the amount of excitatory drive by which anyone depolarizing pressure is maintained. However, assuming drive to be sufficient, what is then the relation between (control or) normal frequency of discharge F_n and that during recurrent inhibition F_i ?

In order to be able to reply to questions of this type it is necessary to be able to vary the firing frequency F_n of any given cell and try recurrent inhibition on it. Many motoneurons are so heavily stabilized in firing rate that they cannot be used in a study of this particular kind. They simply refuse

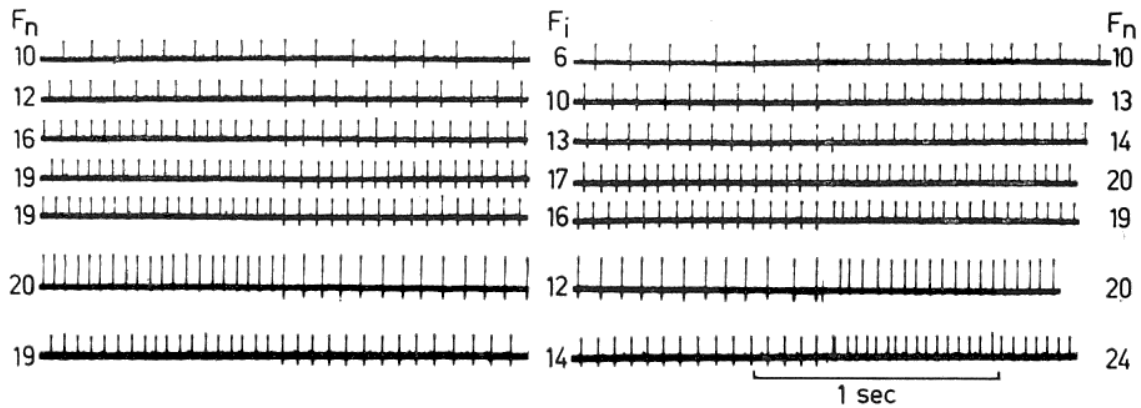


FIG. 2. Records from three experiments showing tonic reflex discharge of single fibre in ventral root to afferent stimulation at repetition rate 114/sec. Normal frequency of discharge F_n 1 sec before and after locking of antidromic shock to firing spike to obtain F_i . Values of F_n and F_i against the records refer to the cut-out portions and not to total period of counting. The first five rows refer to one experiment, the sixth and seventh to two different experiments; the seventh put in to illustrate good rebound (Granit *et al.*, 1960).

to vary their F_n . Others can by electrical afferent stimulation (at 110/sec) at different strengths be made to fire at different rates. By connecting electronically the tonic firing spike to the antidromic shock one can make recurrent inhibition act at the average rate of motoneuron output as in Fig. 2 (paper no. 2). Averaging the rate of discharge over 5 sec before and 5 sec after a 5 sec period of recurrent inhibition one obtains the basic frequency of discharge F_n . The value during recurrent inhibition is the average from the 5-sec period during which it acted. Plotting F_i against F_n gives straight lines of the type shown in Fig. 3 (paper no. 2). F_i is proportional to F_n and this relationship is reminiscent of the results of Hartline and Ratliff (1956) with Hartline's (1949) lateral inhibition in the *Limulus* eye.

This type of experiment also provides us with a method of measuring the potency of recurrent inhibition. In the record of Fig. 4 (paper no. 2) the dashed line is drawn at an angle of 45° in the F_i - F_n diagram to show the theoretical case of absent recurrent inhibition or $F_n = F_i$. B is the result actually obtained. Then concurrent tetanic stimulation of a point, low in the anterior cerebellum, was began and the readings repeated. They are now numbered in the order in which they were taken. The earliest ones fell on a good straight line C with a slope signifying a strong increase in the efficacy of recurrent inhibition. The last values fell better on line D and the effect was not merely visible during concurrent stimulation of the cerebellar point but rose and disappeared so slowly that the numbers underlined, which represent intercurrent controls without simultaneous central stimulation, did not separate out from the others. We also found central inhibitory points in this manner. Koizumi *et al.* (1959) in their work on spinal cord interneurons described one cell which they held to be a Renshaw cell and whose

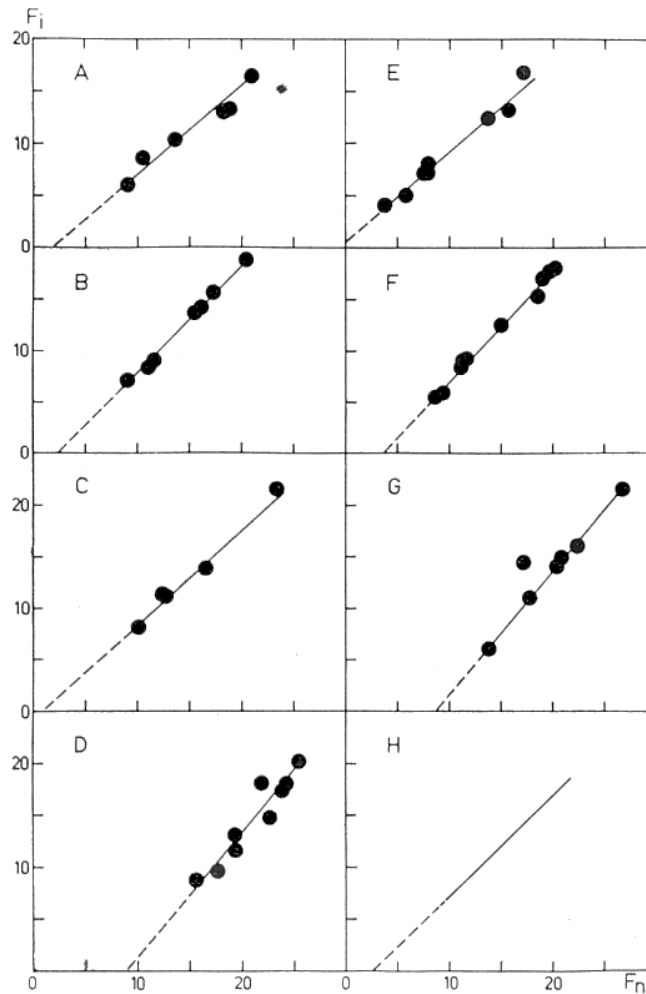


FIG. 3. A–G are curves plotted from experiments in which range of F_n was large. The straight lines have been drawn to reproduce formula $F_n = aF_i + b$. The constants a and b are from A to G: 1.25, 1.0; 0.97, 2.4; 1.08, 1.0; 0.84, 8.9; 1.17, -0.6; 0.92, 3.7; 0.84, 8.8. Curve H is the average from thirty-three experiments, $a = 1.04$; $b = 2.6$ imp/sec (Granit *et al.*, 1960).

rate of discharge could be inhibited by reticular stimulation. I mention this chiefly to show that our method is convenient for studies of this type and to underline that some of the discrepancies in work with Renshaw cells may well have been due to influences of this kind. Considering how little we know about supraspinal mechanisms of control, even for cells which have been studied extensively, much experimentation will be required before we know when and how in complex events Renshaw cells are excited or suppressed. For the time being it would be wrong to look upon them merely as automatic at the spinal level. We know that in truly tonic cells, such as those of soleus, recurrent inhibition is particularly strong (paper no. 1; Kuno, 1959; Eccles *et al.*, 1960) and in those cells it is likely to work in close cooperation with their long-lasting after-hyperpolarization, found by Eccles *et al.* (1958).

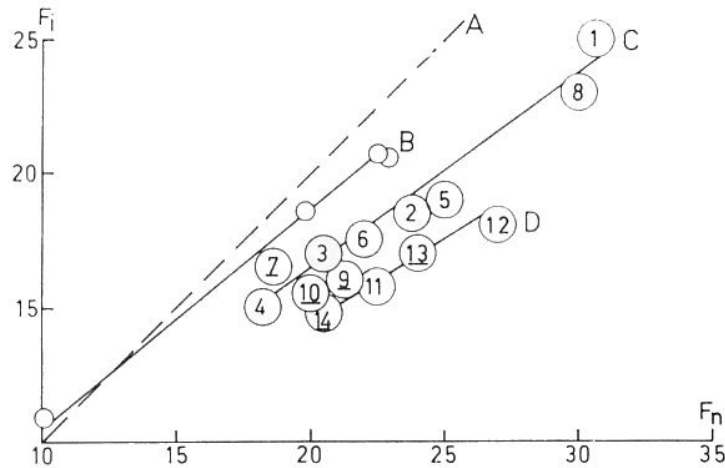


FIG. 4. Plot as in Fig. 3. A, to show graph for $F_n = F_i$. B, initial control of spike drawn to fit $F_n = 1.22 F_i - 2.8$. Stimulation of lower frontal portion of anterior lobe of cerebellum at Horsley-Clarke co-ordinates P6, H2 just contralateral to midline at frequency 300/sec and 9.0 V, coaxial electrode and insulated tip against its shield. Order of observations marked on graph; underlined numerals are observations without stimulation of cerebellum. Curve C drawn to $F_n = 1.39 F_i - 2.8$, D to $F_n = 1.58 F_i - 2.8$ (Granit *et al.*, 1960).

As to other organizational features, we have the results of Brooks and Wilson (1959), Wilson (1959) and of Wilson *et al.* (1959) which show that there is recurrent excitation which is better developed from extensors to flexors than the other way round, because inhibition is the dominant feature between extensors and from flexors to extensors. This is a kind of asymmetrical reciprocal innervation. Eccles *et al.* (1960) emphasize in the first instance nuclear proximity in the spinal cord as the leading organizational feature for recurrent inhibition.

Thus this brief review shows that from many points of view recurrent collaterals deserve to be studied. Recurrent inhibition seems to be dominating in studies of extensors. We have only seen facilitation as rebound.

I might end by saying a few words about limitation of discharge frequency. We have no evidence that recurrent inhibition is decisive, except for the tonic ventral horn cells where it can co-operate with after-hyperpolarization. If we consider equation (1) it is clear that there are two fundamental possibilities: (i) F_n may be cut, as in some of the *Carcinus* fibres of Hodgkin (1948), or it may be cut by accommodation. (ii) Alternatively depolarizing pressure $P_{dep} + P_{pol}$ may be the regulated quantity. It can be shown that the latter generally is the case. Depolarizing pressure is limited by many factors such as limited number of afferent terminals, afferent inhibition, natural recurrent inhibition, after-hyperpolarization. These factors are not easily disentangled; however, it is easily shown that in many cells depolarizing pressure is limited in response to muscular afferents when it still is capable of rising in response to many other types of stimuli. The rule seems to be

that depolarizing pressure rather than frequency of discharge is the quantity that the organism in the first instance holds in check.

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