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RECURRENT INHIBITION IN RELATION TO FREQUENCY OF FIRING AND LIMITATION OF DISCHARGE RATE OF EXTENSOR MOTONEURONES

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Our previous paper (Granit & Rutledge, 1960) showed that recurrent inhibition silences the discharge of a motoneurone which is feebly supported by excitatory drive, even in the face of constant depolarizing pressure as defined in that work. One answer was also provided thereby to the general question of why sense organs and interneurones fire at frequencies which are in excess of immediate apparent needs. In the present study every precaution was taken to maintain afferent excitation in excess of that barely needed to keep up a given rate of reflex firing to electrical stimulation of muscular afferents. This is because the main question here concerns the relationship between normal firing frequency F_n of a motoneurone and its rate of discharge F_i under recurrent inhibition. F_i was now found to be a basically linear function of F_n. This finding proved to be of methodological interest in work on the physiological significance of recurrent inhibition.

Holmgren & Merton (1954), making use of an analogy derived from electronics, suggested that recurrent inhibition has a stabilizing effect on the discharge from motoneurones. Defining stabilization for our purpose as the integrated net effect of one or several processes engaged in holding the rate of firing within relatively narrow limits, we have also tried below to scrutinize this concept as a biological proposition. (It is not our intention to elaborate an electronic analogy which may or may not be valid.) This means that special attention will be given to the factors which determine the upper and lower limits of the frequency range. Thus limitation of discharge frequency is possibly a more accurate term than 'stabilization'.

We are not aware of any previous work concerned with the relation between F_n and F_i. The general problem of 'frequency limitation' may,

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however, be said to have arisen out of Adrian & Bronk's (1929) and Denny-Brown's (1929) work on the reflex activity of single motoneurones whose firing they proved to be restricted to fairly low rates. Out of the many papers confirming this finding, attention may be drawn to those of Alvord & Fuortes (1953), Lloyd (1957), and Margaria (1959) as being of interest for the present work. The question by what means the discharge frequency of motoneurones is limited (upwards) has not really come into the foreground until fairly recently. Further relevant contributions will be found in papers by Granit, Pascoe & Steg (1957); Eccles, Eccles & Lundberg (1958); Granit (1958); Matthews (1959*a*, *b*); Phillips (1959); Granit & Rutledge (1960) and Pompeiano (1960).

METHODS

Nothing technically new is introduced in this work. Arrangement *A* of the previous paper (Granit & Rutledge, 1960) was used. The precollicular decerebrate animals were deefferented, generally from L 6 to end of cord but always within the segments used. Stimulating electrodes were placed on the severed central stumps of the medial and lateral gastrocnemius nerves, tetanic rates of not less than 114/sec, rarely more, being used to elicit tonic firing from functionally isolated motoneurones in L 7 or S 1. In agreement with Alvord & Fuortes (1953) we think this a good way of setting up a central excitatory state, in the original sense of Sherrington. By chance our standard rate of stimulation fell near the optimal values (70–100/sec) which, while this paper was in preparation, were published by Curtis & Eccles (1960) for monosynaptic post-synaptic potentials in motoneurones. The isolated reflex spikes from one ventral root filament were made to trigger the antidromic shock to the rest of the root, as is described in the previous paper (its Fig. 1) and also illustrated with records from an original experiment in Fig. 1 of this paper.

The standard procedure consisted in measuring the frequency of discharge, F_n, during 5 sec of control, its rate, F_i, during 5 sec of locked antidromic stimulation, and finally F_n in recovery for 5 sec afterwards, the last in order to have a check on possible loss of drive. The two control values for F_n, before and after antidromic stimulation, were averaged. For special problems shorter times were used. This averaging means including a component of rebound (Granit, Pascoe *et al.* 1957) but since this process may have started already during stimulation it is safer to use two averaged control values. Different frequencies of discharge F_n were obtained by variation of stimulus strength and it was attempted to find motoneurones that could be made to vary in rate of discharge by these means. The time of onset of antidromic relative to onset of afferent stimulation was constant for any one series of observations at different frequencies, unless the effect of afferent stimulus duration had been specifically proved to be insignificant for the particular neurone used. This will be separately discussed below. The most common times for onset of antidromic stimulation fell between 20 and 30 sec after initiation of afferent stimulation.

In every case the isolated spike was tested from threshold or minimum frequency for tonic firing, F_n min., to maximum obtainable, F_n max., by increasing afferent stimulus strength. Stretch afferents excite a large number of interneurones (Kolmodin, 1957) and that interneurones actually contribute to reflex excitation of extensor motoneurones has been proved by Granit, Phillips, Skoglund & Steg (1957). The excitatory state thus obtained should not be confused with monosynaptic effects on motoneurones. The decerebrate animal is in a state of extensor release and flexor suppression, as is known from the work of the Sherrington school and recently elaborated by Job (1953), Eccles & Lundberg (1959) and Holmqvist & Lundberg (1959). Afferent stimulation of the extensor muscle nerves influences

this 'state' and does not specialize on monosynaptic effects, as was clearly realized by Alvord & Fuortes (1953).

Locking of the antidromic shock to the discharging spike ensures that the antidromic inhibition is forced to follow the dominant rhythm F_n of the spike studied. The rhythm F_i will emerge as a balanced state between orthodromic depolarization and recurrent (re)polarization (Eccles, Fatt & Koketsu, 1954) of the motoneurone membrane, evaluated as frequency of discharge.

RESULTS

Relation between F_n and F_i

At the outset it is necessary to realize that the experiment deals with a complex situation. When stimulus strength is augmented in order to increase F_n, several other motoneurones are excited in parallel with the one studied and many of them will be provided with Golgi recurrent collaterals. Thus, to an unknown degree, 'natural' recurrent inhibition

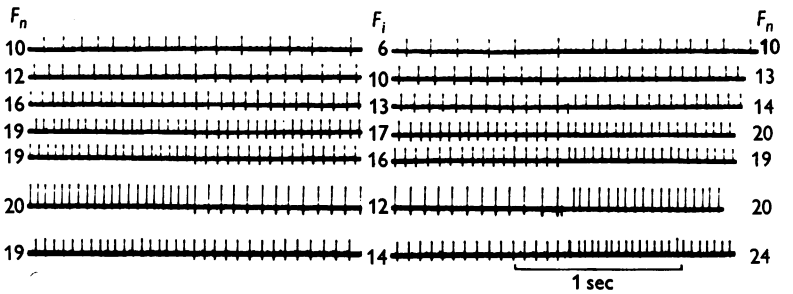

Fig. 1. Records from three experiments showing tonic reflex discharge of single fibre in ventral root to afferent stimulation at repetition rate 114/sec. F_n is normal frequency of discharge 1 sec before and after locking of antidromic shock to firing spike to obtain F_i. Values of F_n and F_i (impulses/sec) against the records refer to the cut out portions and not to total period of counting. The first five rows, from above downwards, refer to one experiment, the sixth and seventh to two other experiments; the seventh is put in to illustrate good rebound.

will compete with the experimentally injected component for the available number of Renshaw cells, and some rivalry will occur up to the saturation point (defined as the maximum number of maximally active Renshaw cells). More afferent inhibition will also be introduced by an increase of stimulus strength, but this need not concern us in the present work for which it is important merely to have a semistationary state of balance between excitatory and inhibitory forces, with consequent steady rate F_n of firing. It is immaterial what depolarizing and polarizing forces participate in building up the net depolarizing current—defined as depolarizing pressure ($P_{dep} + P_{pol}$)—as long as excess drive is present, and this should be obtainable by electrical stimulation above the threshold (for definitions, see Granit & Rutledge, 1960).

Figure 1 serves to introduce the actual experiment. The first five hori-

zontal rows are from one cat, the two lowermost from two other animals. In each row the last few control values before antidromic stimulation are shown on the left, then the initial portion of the 5 sec antidromic stimulation, the last portion of it, and finally the control afterwards. Strictly, 5 sec of each portion should be illustrated to give the full experiment with its greater accuracy. The figures indicating discharge rates in the records refer merely to the strips cut out for reproduction. The rebound in the lower records should be noted.

The curves *A* to *G* and *I* of Fig. 2 are from motoneurones isolated in one single animal; *H* is from another animal. The data have been plotted in terms of F_i as ordinates, against F_n as abscissae. Straight lines have been drawn through the points. Curve *H* was added to show how nearly the lower limit can be balanced: at $F_n = 15$/sec recurrent inhibition silenced the cell because it was difficult at the threshold to mobilize enough surplus excitation (see Granit & Rutledge, 1960). This value corresponds to F_n min. After a slight increase of stimulus strength the discharge rate went up to $F_n = 16$/sec, at which level a balanced discharge was obtained and thereby the first point on the curve. The many values, heaped around the top of several curves, bear witness to fruitless attempts to increase F_n max. by augmenting shock strength to the afferent nerve. Curve *I* is an interesting special case in which the linear relation between F_n and F_i was valid in spite of the fact that two different spikes were present and, possibly, three in the end. Complications are common in such cases. For instance, at a certain rate of discharge one of the two may actually be facilitated by the rebound of its predecessor and so, quite suddenly, adopt its rate of discharge. For this and other similar reasons all our analyses are based on single-fibre preparations. The results may then be conveniently summarized by a linear equation for which we have preferred the less common form

$$F_n = aF_i + b. \tag{1}$$

The values of the constants a and b will be found in the legend, b being the intercept for $F_i = 0$. Put in this form, a increases when recurrent inhibition increases and more often than not b is positive. The constants have been derived from curves drawn freehand, as no particular purpose was served by calculating the ideal straight lines.

The efficacy of recurrent inhibition is given by the difference between the normal and the inhibited rate of firing, which is wanted as a function of F_n and easily derived from equation 1. It is

$$F_n - F_i = F_n(a-1)/a + b/a, \tag{2}$$

from which it emerges that the plot of equation (1) is useful also because it represents a convenient way of deriving the values of the constants of equation (2). This, in fact, has been our procedure and in the present case

the curves of Fig. 2 have been replotted in Fig. 3 for $F_n - F_i$ as a function of F_n in accordance with equation 2. The simple formulas used should not be interpreted as a mathematical treatment of recurrent inhibition. Their

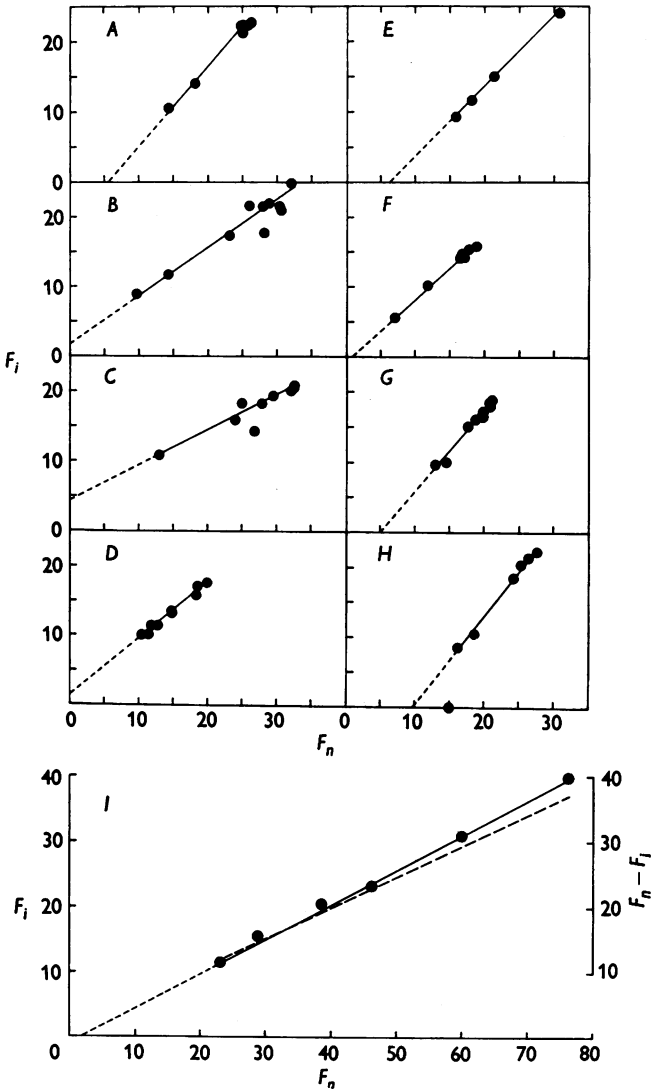

Fig. 2. Plot of F_i as ordinates against F_n as abscissae (imp/sec) for a number of isolated motoneurones *A–G* and for a pair of motoneurones *I*, all from single experiment, *H* being from another animal and put in to show the critical effect of lack of surplus excitation, as explained in text. Curves drawn free-hand to equation $F_n = aF_i + b$. The values of the pairs a and b from *A* to *H* are 0·96, 4·3; 1·47, −3·0; 2·01, −9·2; 1·22, −1·8; 1·00, 6·3; 1·10, 1·0; 0·86, 5·0; 0·79, 9·8. Ordinate scale on the right for curve *I* and interrupted curve refers to plot of F_n–F_i against F_n as in next figure.

purpose is merely to simplify and concentrate description of our findings to essentials.

When we focus attention on the derived constant $(a-1)/a$, it is seen that if $a = 1$, the amount of recurrent inhibition is wholly determined by b, and the curve (in the plot of Fig. 3) is a horizontal line at ordinate b, as in E of that figure. Recurrent inhibition then is constant and independent of rate of discharge F_n. If $a > 1$, then $F_n - F_i$ increases in proportion to the normal discharge frequency F_n, as shown by curves B, C, D and F of

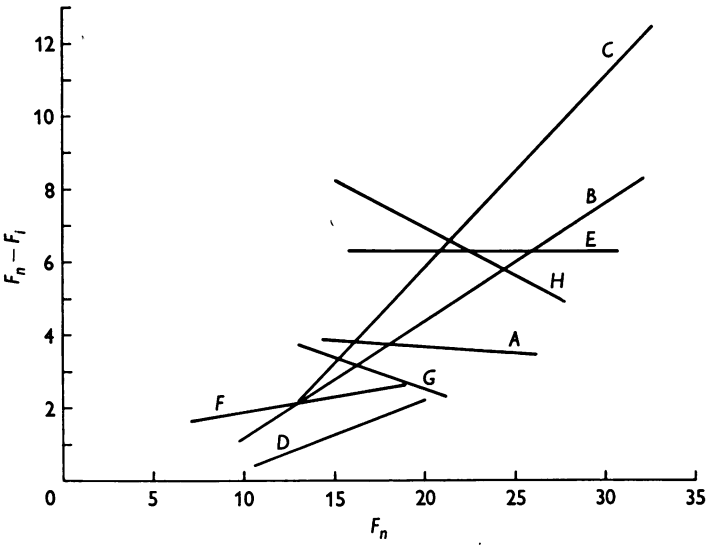

Fig. 3. Plot of curves derived from those of previous figure and similarly marked but using equation 2 of text. Ordinates, $F_n - F_i$; abscissae, F_n (imp/sec.). Each curve also shows the experimentally obtained range from F_n min. to F_n max.

Fig. 3. Finally, if $a < 1$, $F_n - F_i$ diminishes with F_n, as illustrated in A, H and G. Thus in this experiment all possibilities were sampled, though it is more common to find one particular animal to some extent specializing, as it were, on positive or negative slopes $(a-1)/a$.

As has been stated, all values for F_n were brought to the maximum obtainable by electrical stimulation of the muscle nerve. When the range within which F_n could be made to vary was very small, below 6 imp/sec, we have not included the data in our survey of Fig. 5, because in such cases the question of linearity hardly arises. Actually small ranges were quite common, but being interested in the relation between F_n and F_i we have naturally tried to select spikes capable of varying in frequency of discharge. In order to demonstrate that linear curves do provide a good description of our findings it is of interest to reproduce seven curves ($A-G$ of Fig. 4) comprising large ranges in addition to the two (B and C)

already shown in Fig. 2. Occasional single values may fall outside but we shall provide evidence below for the conclusion that recurrent inhibition is under supraspinal control, and decerebrate animals are well known to have occasional fits of activity.

Sometimes recurrent inhibition has a threshold above the minimum maintained value of F_n, but generally F_n and recurrent inhibition start

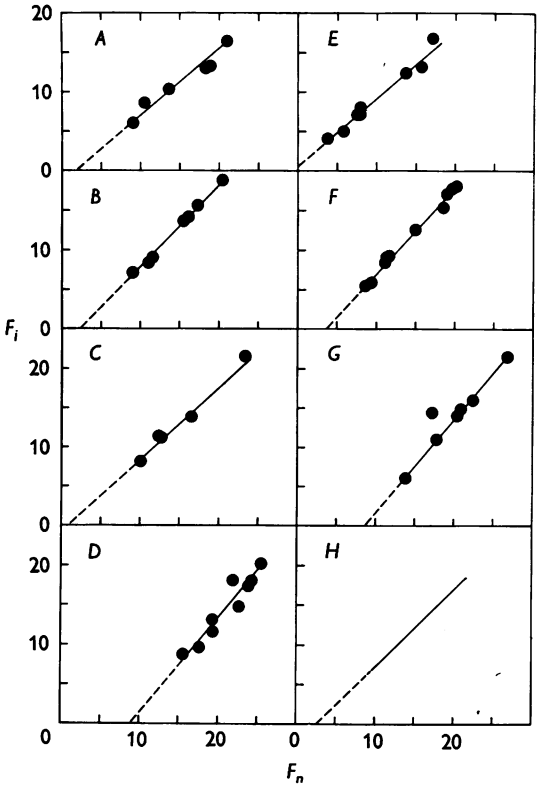

Fig. 4. *A–G* are curves plotted as in Fig. 2 but from experiments in which range of F_n was large, as in curves *B* and *C* of that figure. The constants *a* and *b* are from *A* to *G*: 1·25+1·0; 0·97+2·4; 1·08+1·0; 0·84+8·9; 1·17–0·6; 0·92+3·7; 0·84+8·8. Curve *H* is the average from 33 experiments; $a = 1{\cdot}043$, $b = 2{\cdot}6$ imp/sec.

abruptly together at the threshold and the former has to be raised a little before balanced states can be obtained, as exemplified in Fig. 2*H*, especially when $a < 1$. This means a negative slope in the graph of Fig. 3 and hence recurrent inhibition is largest at the low frequencies of discharge. At the upper end recurrent inhibition may approach saturation before F_n has reached its maximum value and so the slope will be changed because now $F_n = F_i$ and recurrent inhibition is gone. It was never supplanted by recurrent excitation. With some spikes the curves for F_i against

F_n (equation (1)) have flattened out at the upper limiting values. Repetition of long-lasting afferent stimulation at the maximum stimulus strengths is likely to undermine reliability (see below).

In Fig. 5 the results with 33 fibres are summarized. The peak of the minimum values for F_n is between 6 and 10 imp/sec. F_n min. should, of

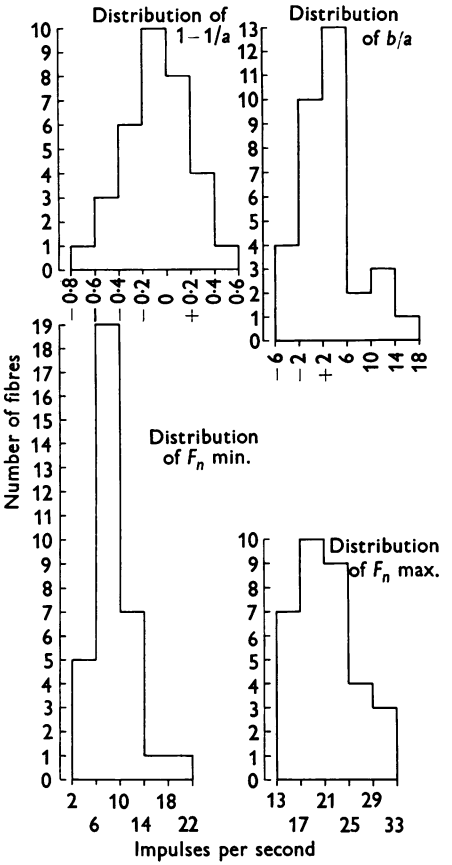

Fig. 5. Histograms showing distributions, for 33 fibres, of the various quantities indicated: class intervals correspond to the intervals marked on abscissae.

course, be taken to refer to the threshold for a maintained discharge and not to the phasic burst by which many fibres respond to very weak stimuli. The maximum is spread over a considerable range with a broad peak between 13 and 25 imp/sec. The average range ($F_n$max. $-$ $F_n$min.) is 12 imp/sec. The other two distribution curves show $(a-1)/a$ and b/a. In the present material a averaged out at about unity (1·04), so that the average difference between F_n and F_i would be largely determined by the average value of b, which was 2·6 imp/sec. The average curve based on

these values is plotted in Fig. 4 as H. While this may serve as a descriptive landmark for the present data—a selection based on spikes that actually could be made to vary in F_n—it cannot at the moment be used as representative mean (cf. below). The really significant finding is not the average curve but the facts that (i) the rate of discharge under recurrent inhibition, F_i, tends to be proportional to the normal or control rate of discharge F_n when forced to act at that rate; (ii) the slopes in the plot of $F_n - F_i$ against F_n (Fig. 3) vary as much as they do and (iii) some of the curves for $F_n - F_i$ as a function of F_n even slope downwards (when $a < 1$ or $(a-1)/a$ is negative) so as to approach a minimum $F_n = F_i$ which corresponds to $b/(1-a)$, as seen from equation (1). The variations in slope of $F_n - F_i$ represent gradations of recurrent inhibition which we do not yet fully understand. However, being reasonably well defined by the use of a simple formula, they are easily reproducible under various conditions and so, in the end, must become explicable.

Time factors

Several experiments were devoted to investigating the possible significance of the moment of onset of recurrent inhibition relative to time of onset of afferent stimulation. Durations of afferent stimulation up to 300 sec were used, the durations of afferent stimulation in the standard experiments having been kept within 30–60 sec. During maintained iterative stimulation of the afferent nerves the spike frequency slowly declined, even though temporarily raised a little by the rebound after each inserted period of antidromic stimulation. Post-tetanic potentiation will increase during the first 10 sec (Lloyd, 1949; Curtis & Eccles, 1960) and a steady state would therefore have to be tested after a minimum of 10 sec of afferent stimulation. This also was our minimum time and most tests actually fell between 20 and 30 sec after onset of afferent tetanization. At stimulus rate 114/sec potentiation of individual cells would be modest (Curtis & Eccles, 1960). In many, perhaps in most, cases the temporal variation of the value of F_i was a simple consequence of the change of F_n and when this was so all observations fitted on to the same straight line for F_i against F_n in our plot of equation (1). With longer durations of stimulation a real change commonly took place in the sense that recurrent inhibition increased in potency. This is illustrated with a characteristic example in Fig. 6, fully explained in its legend. After some 30 sec of afferent stimulation the genuine increase of recurrent inhibition would become measurable, when present. The opposite effect that recurrent inhibition was stronger early than late in afferent stimulation was rarely encountered.

Supraspinal control

This is too large a subject to be broached in the present work but, on the other hand, it is too important for an interpretation to be wholly left out. The method of approach elaborated in the previous sections is useful in studying supraspinal control because a check of the whole curve in the plot of Fig. 2 before and during supraspinal stimulation provides a more reliable criterion than individual values can give.

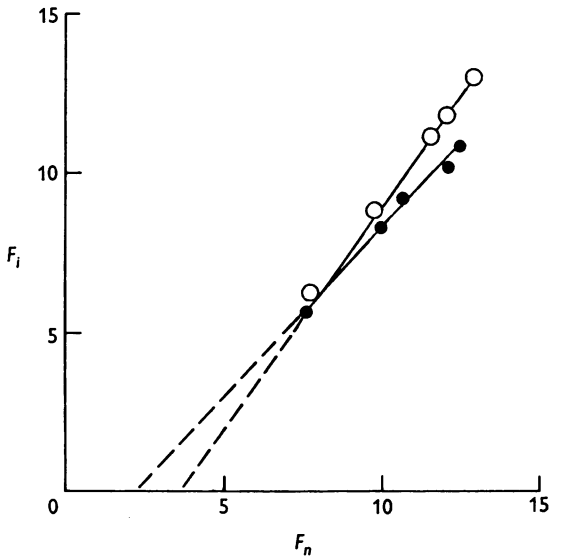

Fig. 6. Plot as in Fig. 2 for two periods of locking antidromic shock to spike at moments 30 sec (○) and 60 sec (●) after start of afferent stimulation. Co-ordinates scaled in impulses/sec.

Thus in Fig. 7, after some hours of experimentation, a spike was located which was but feebly influenced by recurrent inhibition, as is shown by curve *B* in comparison with curve *A*, which is the 45° line from zero for absence of effect or $F_n = F_i$. This spike could be made to respond by increased inhibition to antidromic stimulation when the brain was stimulated from Horsley-Clarke co-ordinates P6, H2, 1 mm contralateral to the mid line, as well as from the pyramid at the bottom. The order in which the stimulation periods followed is marked in the figure and it is seen that the effect was progressive from *C* to *D*. The underlined points were taken without simultaneous central stimulation, but apparently the change was slow enough to persist in the absence of such stimulation, which in these types of experiment was inserted together with brief periods of antidromic stimulation while afferent tetanization was maintained.

The main difficulty in evaluating findings of the kind shown in Fig. 7 is clearly that the change may depend upon loss of surplus excitation, but when the readings fell so well on a line as did the early ones, marked *C*, this error does not appear likely. However, no such criticism can be directed against the opposite suppressive effect of supraspinal stimulation, illustrated in Fig. 8. The open circles refer to averaged readings taken *before* and *after* a period of stimulation of the cerebellar point shown in the figure,

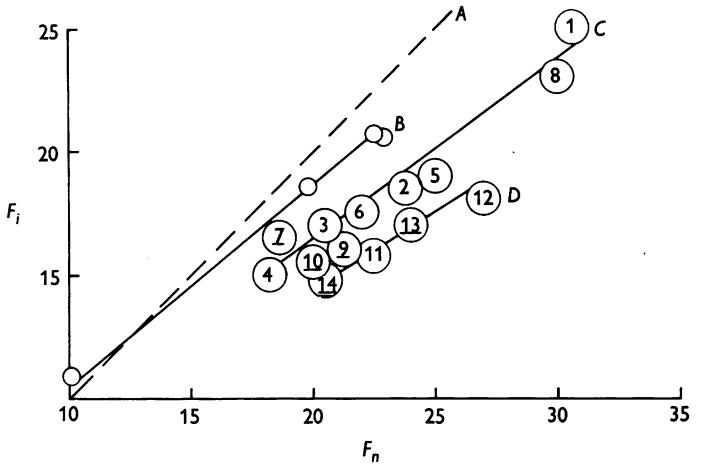

Fig. 7. Plot as in Fig. 2. All data from one fibre. *A*: to show graph for $F_n = F_i$. *B*: initial control for fibre drawn to fit $F_n = 1{\cdot}22\ F_i - 2{\cdot}8$. *C* and *D* successive periods of lower frontal portion of anterior lobe of cerebellum at Horsley-Clarke co-ordinates P 6, H 2 just contralateral to mid line at frequency 300/sec and 9·0 V; coaxial electrode, insulated tip referred to its shield. Order of observations marked on graph; underlined numerals are observations without stimulation of cerebellum. Curve *C* drawn to $F_n = 1{\cdot}39\ F_i - 2{\cdot}8$, *D* to $F_n = 1{\cdot}58\ F_i - 2{\cdot}8$.

the filled circles to readings taken during such stimulation. The difference in slope is not significant but the constant *b* is shifted from 8·2 to 4·0 imp/sec during stimulation of the inhibitory region. With a slope of the order of 1·0 this means that at each frequency the effect of recurrent inhibition, $F_n - F_i$, has been diminished by roughly 4 imp/sec owing to co-stimulation of the brain stem.

It is therefore clear that by our index there are neural or (possibly) hormonal governors of the Renshaw cells and so averaging of our data can only be of interest for the sake of surveying them conveniently and not of disclosing any meaning in them that would be lost among individual variations. At the moment we are engaged in studying supraspinal effects by recording from Renshaw cells. Koizumi, Ushiyama & Brooks (1959), using this approach, found spontaneously active cells, which they held to be Renshaw cells, to be inhibited by stimulation of the reticular formation.

More experience by this and the present method is needed to ascertain whether or not Renshaw cells and thus recurrent inhibition are controlled merely indirectly by variations in type, number or discharge rate of their motoneurones, or directly as are so many other internuncial cells. Our results suggest the latter alternative. Holmqvist & Lundberg (1959) did not find any evidence of supraspinal control of Renshaw cells by monosynaptic testing but their curves do differ for spinal and decerebrate cats.

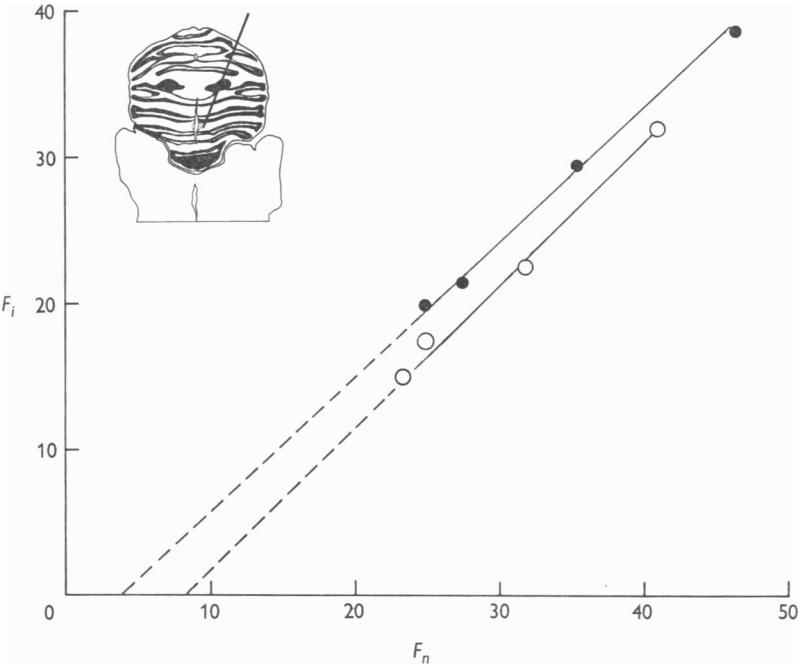

Fig. 8. Afferent stimulation maintained for 50 sec during which time antidromic shock was locked to discharging spike for brief intervals only, as tests. Values from such tests were averaged over four consecutive 10 sec periods from control runs (○) before and after one series during stimulation (●) of the point in the frontal part of the anterior cerebellum illustrated as inset. This well isolated spike had an exceptionally high initial frequency of discharge of around 40/sec, falling during maintained afferent stimulation to values between 20 and 25 imp/sec. The point stimulated proved to be in the anterior lobe of the cerebellum (electrocoagulated) as shown by inset where it is marked by pointer. Stimulus frequency during experiment was 300/sec, strength 0·45 V through tip of thin coated needle against ground. On increasing stimulus strength the discharging spike itself was ultimately inhibited. The difference between constants a is not significant (1·07 and 1·03) but the constant b is 8·2 in the control (○) and only 4·0 (●) during stimulation. Co-ordinates scaled in impulses/sec.

Frequency limitation

We recall from the previous paper (Granit & Rutledge, 1960) that F_n is a function of the depolarizing pressure, defined as $(P_{dep} + P_{pol})$, the latter

factor including all 'natural' inhibitions (ortho- as well as antidromic ones) and also after-hyperpolarization. It would be easier to measure F_n as a function of depolarizing pressure if these inhibitions, undesirable here, were absent or could be kept constant. Barron & Matthews (1938) tried to avoid them by stimulating motoneurones from outside. The only figures they publish (one motoneurone) suggest that F_n is directly proportional to net depolarizing current (= depol. pressure). Pascoe (1957) and M. G. F. Fuortes and K. Frank (personal communication) have stimulated cat motoneurones from the inside, using the bridge technique of Araki & Otani (1955). They confirmed the old findings, Fuortes & Frank in particular

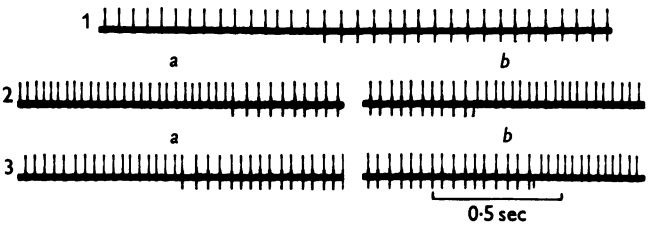

Fig. 9. Records showing maximum frequency obtainable by afferent stimulation of gastrocnemius nerves (1) and same when in addition fore part of body was squeezed, twice repeated (2, 3). Antidromic stimulation locked to discharging spike in all cases between *a* and *b* as controls.

stating explicitly that 'five normal-appearing motor horn cells gave the following slopes: 4, 5, 6·2 and 13·6 imp/sec/mμA' and that many cells went up to rates of 100 imp/sec along straight lines. Thus, if depolarizing pressure is allowed to rise, F_n rises in strict proportion, how far depending upon the cell and thus upon a factor of selection.

With stimulation across synapses there is an unknown amount of P_{pol} from inhibition and therefore we have to write

$$F_n = \text{const} \times (P_{dep} + P_{pol}). \quad (3)$$

From this follows directly that there are two fundamentally different possibilities for limitation of frequency of discharge: (i) F_n itself may be limited and the cell may therefore fail to respond to progressively increasing depolarizing pressures, or (ii) the depolarizing pressure may be the regulated quantity. For both alternatives we are free to hypothesize mechanisms (see Discussion).

Our results uniformly show that the controlled quantity is the depolarizing pressure but they cannot wholly exclude alternative (i). Thus the experiment of Fig. 9 is a simple but instructive case presenting a motoneurone whose F_n could not be raised beyond 20–22 imp/sec by stimulation of muscular afferents (record 1) while to stimulation by manipulation of forelimbs and their skin it fired (in 2 and 3) at rates of

30–34 imp/sec. Clearly, therefore, the depolarizing pressure was limited for the muscular afferents only (representing one particular combination of excitation and inhibition). The discharge mechanism itself was perfectly capable of raising F_n in response to a rise of depolarizing pressure.

As all experiments in this work were run from $F_n$min. to $F_n$max., they can all be reconsidered here from the point of view of frequency limitation. Since recurrent inhibition is a controlled event it may well be governed so as to become the limiting factor in many cases. This is a different proposition, however: in the present work, we have to keep within the precincts of our own measurements and from them some general conclusions emerge which are of interest for the general problem of stabilization.

Thus, when $a = 1$ and recurrent inhibition is independent of F_n, being equal to b, it is immediately clear that recurrent inhibition cannot have been the decisive limiting influence. Within the range from $F_n$min. to $F_n$max. depolarizing pressure has risen and the effect of the rise is stopped by the sum total of restricting factors (see p. 314).

When $a > 1$ the effect of recurrent inhibition increases with F_n, i.e. depolarizing pressure, but there is nothing to suggest that this process could not have gone on beyond the actual maximum F_n obtained, had it been possible to increase depolarizing pressure from the muscular afferents. In both these cases natural recurrent inhibition may be assumed to have added its contribution to frequency limitation, but it has not by itself had a decisive influence on determining $F_n$max.

However, when a is a good deal below unity, the slope of the curves for $F_n - F_i$ against F_n is negative and this means that recurrent inhibition is at its best at low rates of firing of the motoneurone. It thereafter approaches a limit $F_n = F_i$, as F_n increases. The limit is defined from equation 1 as $b/(1-a)$. In such cases, if this value agrees with the maximum F_n obtained for the same motoneurone, recurrent inhibition may well have been the decisive limiting factor. On this interpretation F_n approached F_i because 'natural' and experimental recurrent inhibition together set a limit for F_n. In this case F_n max must not exceed the calculated limit $F_n = F_i$, given by $b/(1-a)$. Results comparing calculated and observed values of F_n max. for 8 fibres are tabulated in Table 1.

Table 1 shows that the upper limit of F_n may well have been determined in these cases by recurrent inhibition. Incidentally it throws doubt on the view that the recurrent excitation of Wilson (1959) and Wilson, Talbot & Diecke (1959, 1960) is much of a complication in our type of experiment, as there is no obvious reason why in its presence F_n should be limited to the low values obtained and, moreover, to the very values required by recurrent inhibition as a major limiting factor. One would expect to observe the recurrent excitation occasionally in the experiments, if it were

a really important mechanism in extensors, in particular in the region of F_n max. As a matter of fact one either finds inhibition with rebound or no effect whatsoever. The relation to rebound was dealt with in the preceding paper (Granit & Rutledge, 1960).

Stabilization of rate of firing should also be considered from the point of view of how motoneurone discharges are limited downwards. This at least is a situation that should give ample play for recurrent inhibition, if for no other reason because excitatory drive is reduced and with it the resistance of the motoneurone to the inhibitory barrage from the Renshaw cells (Granit & Rutledge, 1960). In fact, when firing at slow rates is a desideratum, as in the slow soleus, it can only be obtained if heavy afferent projections antagonize recurrent inhibition or else, at these slow rates, there would not be enough surplus excitation to counteract recurrent inhibition.

TABLE 1

Frequency range	$(a-1)/a$	F_n max. Observed	F_n max. Calculated
7	−0·43	13	12
9	−0·22	17	19
10	−0·41	18	20
10	−0·50	29	35
11	−0·22	21	24
7	−0·57	13	13
4*	−0·23	10	11

* Not included in summary of Fig. 5 on account of the small frequency range.

In the present experiments cells were commonly encountered among the triceps motoneurones which it proved possible to drive at slower rates with recurrent inhibition than without it. No doubt rebound also aids in stabilizing of the rhythm at these low frequencies. Now Granit, Pascoe *et al.* (1957) found that the small tonic α motoneurones of Granit, Henatsch & Steg (1956) were especially well provided with recurrent inhibition. The cells wholly lacking it tended to be found among the phasic motoneurones, though many of these also possess this mechanism. Since the γ spindle control is so strong on the small tonic motoneurones, Granit, Pascoe *et al.* (1957) drew the same conclusion as we now have arrived at from other points of view, namely that slow steady rates of discharge require good drive and strong recurrent inhibition. Eccles, Eccles & Lundberg (1957) next proved by the intracellular method that the soleus motoneurones, as the typical representatives of the small α cells, had excitatory projections from the large spindle afferents some 25 % stronger than had the phasic alphas. Finally Kuno (1959) and R. M. Eccles, A. Iggo and M. Ito (personal communication) have confirmed by testing different types of motoneurones that recurrent inhibition is particularly strong on soleus motoneurones compared, for example, with gastrocnemius neurones.

This series of interconnected experiments and deductions give recurrent inhibition a definite role as stabilizer of the slow rhythm of the stretch-sensitive tonic α motoneurones and thereby also serve to characterize the latter functionally defined system by properties other than size of efferent fibres (Granit *et al.* 1956, Eccles *et al.* 1957, 1958; Granit, Phillips *et al.* 1957; Henneman, 1957), sensitivity to post-tetanic potentiation (Granit *et al.* 1956) and after-hyperpolarizations of long duration (Eccles *et al.* 1958) It is known that soleus motoneurones can be stabilized at rates as low as 5 or 6/sec (Denny-Brown, 1929; Granit, 1958, fig. 1). To prevent misunderstanding it should be noted that by electrical stimulation, as in this work, or by activating the muscle spindles with succinylcholine (Granit, Skoglund & Thesleff, 1953) α motoneurones can be made to discharge tonically which normally do not do so (Henatsch & Schulte, 1958).

In several animals we have encountered the combination of heavy stabilization with failure of recurrent inhibition. Some but not all of them might be called unsuccessful preparations. Considering that the Renshaw cells are controlled, excessive stabilization may be one aspect of the activity of such governors and so, practically speaking, discharge frequency might become an invariant. On the other hand, as we shall see (cf. Discussion), many factors may contribute to stabilization, and with regard to recurrent inhibition itself it is not always possible to decide in individual cases whether it fails to appear because the mechanism is over-engaged or because it is feeble. At the moment we do not know why in some preparations excessive stabilization occurs, in the sense that the range F_n max. $- F_n$ min. is compressed to a few impulses per second.

DISCUSSION

The main finding of this work is that recurrent inhibition is proportional to discharge frequency, which in its turn is proportional to depolarizing pressure (cf. p. 322). This being so, the efficacy of recurrent inhibition, defined as $F_n - F_i$, will depend not only upon the number of overlapping Golgi recurrent collaterals and Renshaw cells taking part in regulating the discharge of any one motoneurone but also upon the extent to which one motoneurone differs from another in respect of the constant of equation 3. Fuortes & Frank (cf. p. 320) found this constant to vary from 4 to 13·6 imp/sec/mμA. To understand what this means, assume that we have isolated two motoneurones both of which discharge to afferent stimulation at identical rates, F_n. Assume further that in the two cases recurrent inhibition as such produces exactly identical repolarizing currents P'_{pol}. In terms of discharge frequencies the effects of recurrent inhibition (F_i) will then have to be in the ratio of 4 to 13·6 for the two motoneurones. By the

present technique we cannot measure this factor, but only suspect that it is of major importance inasmuch as small constants are likely to be found in tonic slowly-discharging cells and large ones in cells that are firing rapidly. However, fundamentally this prediction is verifiable by experimentation.

At the moment it is well to realize that terms such as 'strong' or 'weak' recurrent inhibition can only apply to one and the same motoneurone in different states, since no absolute measure is available. Clarification of this point, however, is merely a matter of further experimentation.

An interesting parallel may be drawn with the results of Hartline & Ratliff (1956) obtained with Hartline's (1949) lateral inhibition in the eye of the horseshoe crab, *Limulus*. This process was found to be proportional to discharge frequency, implying that if one ommatidium A is steadily illuminated to deliver a constant rate of firing (F_n) from its eccentric cell, the illumination of a nearby ommatidium B will inhibit that discharge to F_i in strict proportion to the rate F_x at which B is made to discharge. There are no internuncial cells in *Limulus*, the contacts are held to be axo-axonic (H. W. Miller, personal communication), and the individual factors can be better isolated than in our case where the effect is competing with 'natural' recurrent inhibition for the output from the Renshaw cells. Nevertheless, the fact that the linear frequency rule is so well obeyed in our case also must reflect some basic similarity. It is clear that the degree to which the Renshaw cells are controlled from above and the circumstances in which they are switched on and off are important data, and that without this information it would be unwise to carry interpretation too far. At the moment the greatest difficulty in the way of a uniform explanation of the results of measuring $F_n - F_i$ as a function of F_n is that some curves have negative slopes. We would at the moment interpret such variations of slope by assuming different degrees of competition on the part of natural recurrent inhibition rather than by ascribing them to complications from recurrent excitation, as discussed in the previous section.

With regard to 'stabilization' or frequency limitation, either term is little more than a collective name for the integrated effect of several processes engaged in the regulation of the depolarizing pressure. Without further discussion of details we would like to mention (A), anatomical limitation of afferent terminals, (B), afferent inhibition, (C) recurrent inhibition, considered above in the Section of stabilization and hardly deserving a lengthier discussion before further data on regulation and control of the Renshaw cells have become available. (D) Desensitizing to repetitive stimulation by the postulated transmitter (see e.g. Thesleff, 1959) should be considered but does not seem important in our case with cells firing for many minutes at slow rates, and should more likely be considered with

alternative (i) of the preceding section (see also below). (*E*) After-hyperpolarization was noted by Brock, Coombs & Eccles (1953) and especially discussed by Eccles *et al.* (1958). The idea that after-hyperpolarization sets the upper limit of frequency of discharge finds its strongest support in the fact disclosed by the latter authors, namely that the slowly firing soleus motoneurones have especially long after-potentials. On the other hand, it is perfectly clear that after-hyperpolarizations can be overcome by sufficient depolarizing pressure, or else the results with direct electrical stimulation of motoneurones could never have been obtained. Also, large α motoneurones and small γ motoneurones (J. C. Eccles, R. M. Eccles, A. Iggo and A. Lundberg, personal communication) have after-potentials of the same order (70 msec). Yet the latter can fire at 10 times faster rates than the former. Finally, after-hyperpolarization is generally measured within the soma, whereas the firing region equally generally is held to be the area between axon hillock and medullated portion of the motoneurone (Araki & Otani, 1955; Coombs, Curtis & Eccles, 1957*a*, *b*; Fuortes, Frank & Becker, 1957). After-hyperpolarization is considerably shorter for the spike that arises in this segment (Brock *et al.* 1953). We are convinced that after-hyperpolarization alongside with other factors decisively contributes to frequency limitation (upwards), but would think factors *B* and *C* (above) more important. However, the slowly firing soleus motoneurones must make use of the added effects of recurrent inhibition and after-hyperpolarization suggesting that for extreme degrees of stabilization this combination, as again R. M. Eccles, A. Iggo and M. Ito (personal communication) have found, is likely to be of dominating importance. Perhaps, indeed, recurrent inhibition stabilizes only inasmuch as it co-operates with after-hyperpolarization.

While the factors hitherto enumerated belong to the governors of depolarizing pressure, the alternative ((i) in preceding Section), that F_n itself could be cut in range in spite of increasing depolarizing pressure, might be realized by accommodation. Pascoe (1957) and Frank & Fuortes (1960) in their experiments on stimulation of motoneurones from the inside found the discharge to undergo some accommodation leading to lower rates of firing during maintained stimulation. We have seen the same effect regularly in the present work, though in our case the slow decrease of discharge frequency could also be otherwise explained. Now Araki & Otani (1959) have reported that, with intracellular electrodes in the motoneurones of toads, the firing region around the axon hillock accommodates considerably faster than the soma. If this were so in the cat also, the consequence would be that more depolarizing pressure would be needed to maintain any given frequency of discharge. Thus, in this case, F_n would be cut and not depolarizing pressure. In

terms of our measurements this would mean that recurrent inhibition, removing a constant fraction P'_{pol} of the depolarizing pressure, would have a stronger effect later in the discharge than in the beginning. This finding was actually fairly regular and it was illustrated in Fig. 8. In many neurones this effect of stimulus duration would be late in appearance or absent but in some it could be seen within the first 30 sec of afferent stimulation.

'Stabilization' as a biological concept in relation to discharge rate of motoneurones may seem complex, but it is in the nature of this concept to be tied up with the influences that occur as components of the general problem of regulation in its widest sense and so it must always reflect their integrated complexity. It is perhaps better supplanted as a term by 'frequency-limitation', as this would avoid unjustified electronic analogies. Can such a complex concept serve as a useful entity deserving experimentation on its own account? The best case that can be made out for a positive answer is the existence of strong recurrent control and long-lasting after-hyperpolarizations in the small tonic α neurones, which for theoretical reasons were assumed to be highly stabilized and actually proved to be so by experimentation, as discussed in this and the previous Section.

SUMMARY

1. Tetanization of gastrocnemius afferents at rate 114/sec at varying strengths was used to set up a tonic reflex discharge from extensor motoneurones, isolated in root filaments. Selecting motoneurones that were capable of varying in discharge rate, the firing spike was made to trigger an antidromic shock to the rest of the root, and thus recurrent inhibition could be studied as a function of frequency of firing.

2. The discharge rate under recurrent inhibition was found to be related linearly to the average rates of discharge in the controls just before and after antidromic stimulation.

3. The constants of the linear curves were determined for 33 motoneurones varying in range of frequency by more than 6 imp/sec.

4. These constants could be modified by supraspinal stimuli.

5. The effect of recurrent inhibition tended to increase as a function of duration of maintained afferent stimulation.

6. Maximum and minimum maintained rates of discharge were determined for the 33 motoneurones and the factors responsible for 'frequency limitation' discussed on the basis of the data obtained.

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