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SURPLUS EXCITATION IN REFLEX ACTION OF MOTONEURONES AS MEASURED BY RECURRENT INHIBITION

BY R. GRANIT AND L. T. RUTLEDGE*
From the Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm 60, Sweden

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The antidromic or recurrent inhibition (Renshaw, 1941, 1946) of ventral horn cells was studied in decerebrate cats by Granit, Pascoe & Steg (1957) with tonic discharges to stretch from functionally isolated ventral root filaments belonging to knee extensors. The antidromic shock was applied to a portion of the same root. When triggering the antidromic shock from the discharging spike they found that the frequency of the firing cell often decreased to zero or to a very low value in a cumulative fashion (as here in Fig. 1). Quite often, however, the recurrent inhibition immediately reduced the frequency of discharge to a constant value and thus was non-cumulative. These two modes of behaviour of recurrent inhibition are strikingly different, and failure to understand why sometimes one, sometimes the other effect of recurrent inhibition occurred inspired us to undertake the experiments reported below.

It soon became evident that it was necessary to approach this problem —so essential for the understanding of both recurrent inhibition and the control of discharge frequency of motoneurones—by setting out from a hypothesis with assumptions rigid enough to make it possible to test them experimentally. These assumptions are formulated in the first section of Results. With their aid it has proved possible to develop a method of measuring what we have called 'surplus excitation' in reflex activity of motoneurones. This paper is mainly devoted to the experimental elaboration of this result and the formulation of some useful concepts. In a second paper (Granit, Haase & Rutledge, 1960) we use these concepts in an analysis of the general problem of control of frequency of discharge of motoneurones.

From the work of Eccles, Fatt & Koketsu (1954), confirmed in some essential aspects by Brooks & Wilson (1959) and Wilson (1959), it is known that recurrent inhibition acts by hyperpolarizing the motoneurone cell membrane via the Renshaw cells, which according to Szentágothai (1958)

* Visiting NIH Research Fellow. Present address: Department of Physiology, University of Michigan, Ann Arbor, U.S.A.

also can be identified histologically as small cells lying ventromedially to the motoneurones. These cells discharge spontaneously (Frank & Fuortes, 1956; Curtis & Eccles, 1958) and are more likely to do so in our decerebrate 'tonic' preparations than in the anaesthetized animals of most other authors. Besides the 'tonic' motoneurones are particularly well provided with recurrent collaterals and Renshaw cells (Granit, Pascoe *et al.* 1957; Kuno, 1959; R. M. Eccles, A. Iggo and M. Ito, personal communication) and so their natural tonic activity will maintain 'natural recurrent inhibition' as a permanent hyperpolarizing influence on the motoneurone cell membrane. This effect will increase when the number and rate of firing of the tonic cells increases.

METHODS

Experiments of the kind required for the present work on tonically discharging single motoneurones can succeed only with lively preparations capable of responding in this way. Therefore decerebrate cats have been used. In other respects the technique is essentially as described by Granit, Pascoe *et al.* (1957), i.e. denervation of the leg used with the exception of the medial and lateral gastrocnemius nerves, isolation of the knee extensors (triceps surae) sometimes with separation of gastrocnemius and soleus, laminectomy for operations on the spinal cord, and functional isolation of single ventral root fibres capable of delivering a tonic reflex discharge in response to pull on the muscle, as in Fig. 1*A*. In Arrangement *A* the antidromic shock stimulates a portion of the same root from which the functionally isolated fibre emanates. The tonically discharging fibre was generally localized in the ventral root of L 7, which was severed so that the antidromic shock could be delivered to a portion of this root while S 1 and L 6 were left intact in order to have some γ support of the muscle spindles which elicit the reflex to pull on the muscle (Eldred, Granit & Merton, 1953; Matthews & Rushworth, 1957; Matthews, 1959*a*, *b*). In spite of this precaution it is necessary to keep on stretching the muscle at regular intervals while the experiment is in progress, since the reflex tends to diminish after every prolonged pause.

In most experiments the discharging spike triggered the antidromic shock, as illustrated in Fig. 1*A*: in some, independent iterative stimulation of the ventral root was used. In the former case the stimulus was synchronized with the discharging spike, in the latter it was independent of its rate of firing. Unless recurrent inhibition is strong enough to silence the cell, synchronized stimulation offers the advantage of giving a more regular frequency of discharge to count. The original records below the diagram of Fig. 1 illustrate a control pull on the muscle (record 1) followed by the same pull (record 2) when the antidromic shock was locked to the spike in the manner described. Below each record is a tracing given by the electronic length-recorder used to record extension of the muscles. The 'cumulative' lengthening of discharge intervals with the triggered antidromic shock should be noted (record 2). Generally the spike triggered the shock without delay. A shock delay up to 10 msec was never found to have a significant effect on the recurrent inhibition obtained, suggesting that in a tonic preparation the average increase in the rate of discharge of the Renshaw cells is more important than the duration of firing in response to each individual shock.

Arrangement *A* permitted two further modifications of the experiment: (i) section of the muscle nerves and stimulation of them by repetitive electrical shocks instead of pull; (ii) section of all lumbar and sacral ventral roots.

The purpose of Arrangement *B* (Fig. 1) was to stimulate the cut dorsal roots and use the extensor muscle nerves for the antidromic shock, because this makes the latter influence extensor neurones only. For some problems this is of importance, since Wilson (1959) and

Wilson, Talbot & Diecke (1959, 1960) have found that recurrent stimulation sometimes has reciprocal effects on extensors and flexors. This means that recurrent inhibition from unidentified root filaments can be contaminated by recurrent excitation, even though in the experience of these authors the excitatory effect from flexors on extensors is small, and larger the other way round. The only reason for using Arrangement *B* was to make certain that the

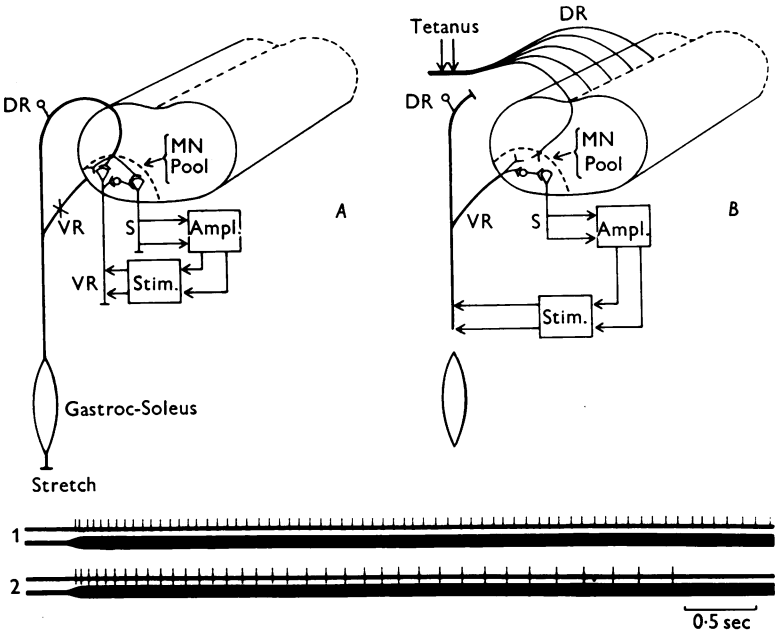

Fig. 1. Experimental arrangements *A* and *B* and sample of experiment with arrangement *A* below. Symbols, DR, dorsal root; VR, ventral root; S, single fibre from ventral root; MN, motoneurone. Arrangement *A*. Spike from single ventral-root fibre amplified and connected to start antidromic shock from stimulator to portion of VR of same segment. Recurrent loop shown. Cross on VR to indicate that in some experiments all lumbosacral ventral roots were severed: such experiments not illustrated in text. Arrangement *B*. Lumbosacral dorsal roots severed and spike in single fibre discharged from DR of its own segment. The amplified spike now triggers shock to the medial and lateral gastrocnemius nerves severed just above muscle. Recurrent path of antidromic impulse shown as in *A*.

Spikes activated by stretch of knee extensors in the manner shown by record 1 below. Record 2 is same strength of pull but with antidromic shock locked to tonically discharging spike (note artifact). Rate and length of pull (15 mm) indicated by length-recorder below.

effects described also occurred with pure recurrent inhibition (between extensor motoneurones of gastrocnemius-soleus). Since with this arrangement all dorsal roots from L4 or L5 downwards are severed, the antidromic shock to the gastrocnemius nerves (see Diagram) can only enter through ventral roots. Arrangement *B* does not afford an easy experiment, because stimulus strength and frequency have to be very carefully adjusted for the maintenance of a tonic discharge. If this required exceptionally strong stimuli, there was too much variation of background from trial to trial.

When delivering the antidromic shock to cut ventral roots it is often necessary to split

root filaments in order to find a portion capable of giving good inhibition on the tonic reflex discharge (Granit, Pascoe *et al.* 1957). Whenever gastrocnemius and soleus were separated, most fibres were found to respond to gastrocnemius pull alone. Soleus fibres, in accordance with previous experience (Granit, 1958) have to be searched for, as, indeed, one would expect from the relative size of the muscles and their fibre supply. Though soleus neurones respond much better to stretch (Denny-Brown, 1929; Granit, 1958), it is possible to activate some gastrocnemius neurones also, particularly if stretch need not be limited to the normal range.

RESULTS

Definition of problem

The individual motoneurones taking part in a maintained soleus stretch reflex tend to fire at a constant rate independent of extension (Denny-Brown, 1929; Granit, 1958). The steady state is upheld by what is called *excitatory drive*. In stretch the excitatory drive comes from the muscle spindles (the nuclear bag or annulospiral endings), directly by monosynaptic paths which have but few (10–20), large end-feet on the motoneurone (according to Szentágothai, 1958), and indirectly over polysynaptic routes. Without support from the latter there will be no maintained stretch reflex. The excitatory drive may be regarded as a barrage of impulses which activate a certain number of synaptic knobs per unit time, thus producing a depolarizing current P_{dep}. Opposed to this influence are inhibitory signals from structures such as Renshaw cells and Golgi tendon organs and also after-hyperpolarization (Brock, Coombs & Eccles, 1953); these generate a repolarizing current P_{pol}. By adding P_{dep} and P_{pol} algebraically one obtains the net depolarizing current, the one thought to determinate the firing rate of the cell. This net current is equivalent to the depolarizing pressure of Phillips (1959); we have simply added a more precise definition of the term.

According to this reasoning, the frequency of discharge (F_{n}) must be a function of the sum of P_{dep} and P_{pol}. Hence

$$F_{\text{n}} = f(P_{\text{dep}} + P_{\text{pol}}). \tag{1}$$

F_{n} in this equation is the only quantity which is directly measurable, but we shall proceed to keep F_{n} constant and to raise the question whether under such circumstances the right-hand term of (1) is always constant also. In order to test this proposition we inject a constant amount of recurrent inhibition P'_{pol} into a stretch reflex. This means adding to the right-hand member of equation (1) the term P'_{pol}. The experiment consists in testing its effect upon the discharge frequency F_{n}. The experimental problem is therefore: if the constant rate of firing of a maintained stretch reflex in a motoneurone signifies that the depolarizing pressure is constant, then also a constant P'_{pol} of recurrent inhibition ought to have a constant effect whenever applied. It might be added that, to direct electrical excitation of the motoneurone membrane, F_{n} is a linear function of P_{dep}

(Barron & Matthews, 1938; K. Frank & M. G. F. Fuortes, personal communication). This question will be considered in our second paper (Granit *et al.* 1960).

Recurrent inhibition into stretch reflex

The simplest approach is to start by testing a stretch reflex at regular intervals with a brief antidromic tetanus, properly adjusted in strength and frequency so as to give the necessary range to the test. We use

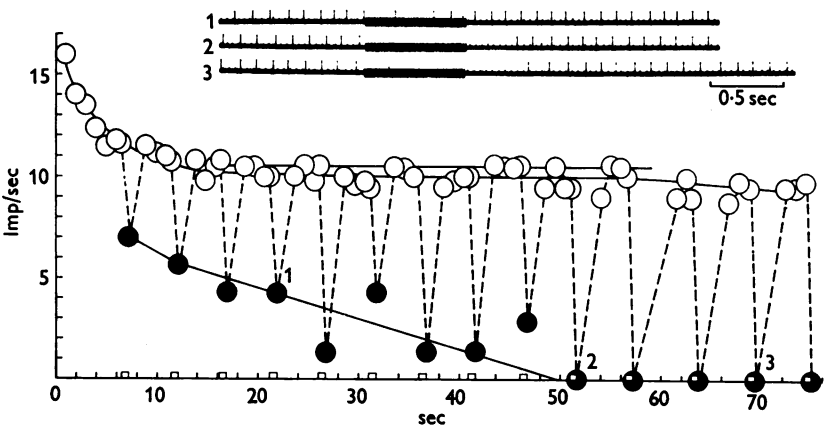

Fig. 2. Arrangement *A* and 15 mm steady pull on the knee extensors. Tetanic antidromic inhibition at 114/sec inserted for 0·7 sec at regular intervals as marked by rectangles on abscissa (running time). Frequency of discharge constant between the two parallel horizontal lines. ●, number of impulses (imp/sec) during the periods of recurrent inhibition. 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.

Arrangement *A* and pull on the muscle. In Fig. 2 a 0·7 sec antidromic tetanus at 114/sec is injected about every 5 sec. While stretch is being maintained the effect of the antidromic volley is seen to increase, ultimately depressing the discharge to zero. To begin with there was good recovery and even some rebound after each test but in the end, after a number of repetitions of the recurrent stimulus, the discharge frequency started to diminish and ultimately, when the series was completed (shown in the diagram), seven more seconds of waiting for the discharge proved useless. It did not return. It is seen that some variation occurred. The line drawn between filled circles in the diagram serves roughly to indicate the increasing efficacy of recurrent stimulation in spite of constant discharge frequency (between the two horizontal lines). The constant amount of recurrent inhibition P'_{pol} starting from the same basic level of discharge frequency reduces the

latter progressively more and more by an amount that depends upon time after application of stretch. Does this depend on temporal summation of the recurrent tests (P'_{pol}) from moment to moment?

In a good experiment, pull on the muscle can be repeated several times with the same effect and so it becomes possible to lock the antidromic shock to the spike at different moments during the repeated stretch reflexes (as in Fig. 1). Thus recurrent inhibition in each instance is inserted anew into a fresh pull and can be kept locked to the firing spike for any time desired. In such experiments each test with recurrent inhibition is independent and cannot possibly add to the after-effects of a previous test.

In Fig. 3*A* the experiments are of the type just described. There were probably two active fibres though the spikes were of the same size. Measuring began 1 sec after onset of stretch. Attending first to the lines drawn in full, it is seen that in the controls the spike frequency became stabilized at around 40 imp/sec (○). With two identical pulls the antidromic shock (●) was locked early (1) and late (2) in stretch. The very characteristic finding is that suppression to zero took some time in 1 and was practically instantaneous in 2. The interrupted lines refer to conditions to be discussed separately below.

Figure 3*B* refers to another experiment with a different animal. The discharge frequency in steady pull stabilized at 13 imp/sec (illustrated in Fig. 4 with records). The plot is now different. On the abscissa is indicated the moment in pull at which the antidromic stimulus was locked to the discharging spike, on the ordinate the duration required for complete suppression of the discharge. Both curves of Fig. 3*B* show that the later in the discharge the antidromic shock was locked, the more rapid the suppression of the firing. In the original records of Fig. 4 the final phase of suppression is illustrated for curve *B*2 of Fig. 3. The records 3 and 4 serve as controls for 1 and 2, in that they illustrate the discharge frequency at later stages in stretch, as it would have been without early onset of recurrent inhibition by locking the shock to the spike. Curve B 1 of Fig. 3 is a result earlier in the day with the same root filament as that used for *B*2. The discharge frequency of the motoneurone was the same as later (*B*2), but recurrent inhibition was more effective.

The general conclusion drawn from such results is that some factor responsible for the maintenance of a constant discharge had spent much of its force in the course of the stretch reflex but that this change had not yet found an expression in the frequency of discharge, which remained constant until tested by antidromic stimulation. This may be restated by defining this factor in a general way as *surplus excitation* with respect to any given discharge frequency, F_n (which by itself gave no sign of the existence of surplus excitation). Thus F_n is stabilized by a frequency-

limiter cutting out the surplus at some point in the system. More attention will be devoted to the problem of frequency limitation in our second paper (Granit *et al.* 1960).

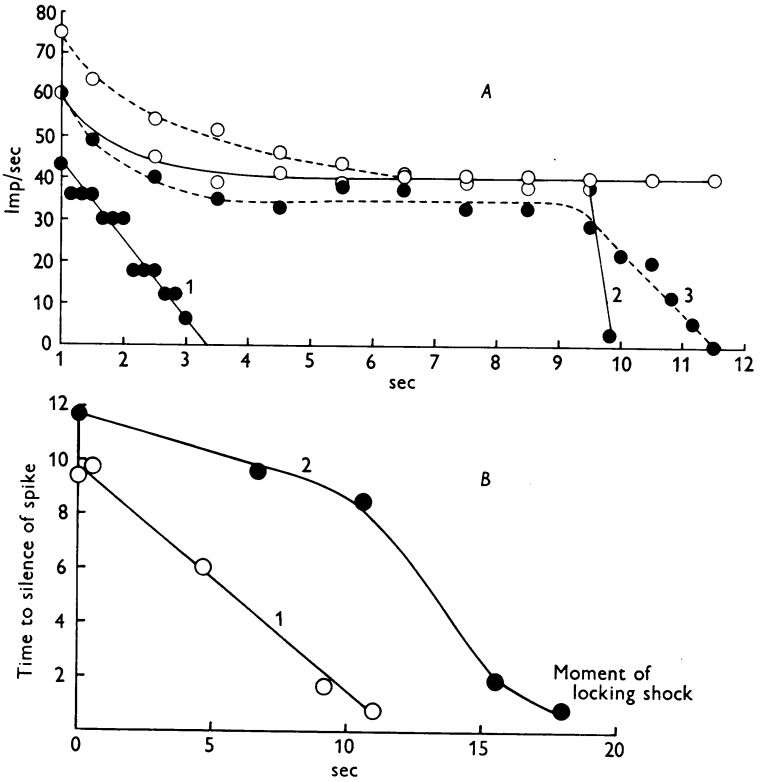

Fig. 3. Experiments with Arrangement *A* varying moment of locking antidromic shock within reflex discharge to steady stretch. Probably two efferent fibres though spikes undistinguishable. *A*. Pulls of 14 mm (lines drawn in full) and 16 mm (broken lines) which between the 6th and 7th sec begin to give the same output frequencies in the controls (○). ●, corresponding pulls with antidromic shock locked to spike from the beginning (1) and 9·5 sec later (2) in different 14 mm pulls, and from the beginning (3) in the 16 mm pull (broken line). In the latter case recurrent inhibition is non-cumulative for 9 sec. After this it suppresses discharge to zero in 2·5 sec. *B*. Another animal. Curves 1 and 2 from two experiments with the same spike, early and later in the day. Its reflex frequency of discharge to steady pull became stabilized at 13 imp/sec to 15 mm stretch in the controls. The time it took to silence this discharge is plotted against the moment at which the antidromic shock was locked. Experiment done with a series of pulls to 15 mm. Sample records of Fig. 1 refer to the same spike and show suppression of discharge to silence.

Now in the case of the stretch reflex we know that surplus excitation diminishes with time, if for no other reason, at any rate on account of the adaptation of the muscle spindles. Yet the output may remain steady for

quite a while. The salient point here is that to a first approximation the amount of surplus excitation may be taken to be proportional to the curves of Fig. 3*B*, i.e. to the time necessary for recurrent inhibition to exert a constant effect, as defined by silence of the cell.

It is important at this stage to draw attention to the experimental fact that in stretch the surplus excitation can actually be destroyed by recurrent inhibition. This change can be traced by studying time for recovery

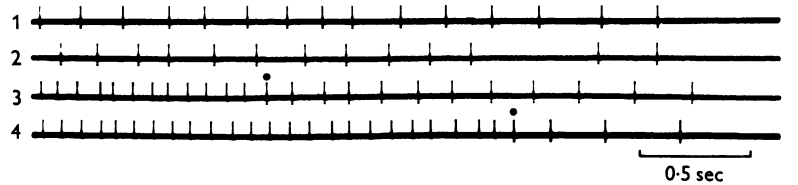

Fig. 4. Original records from which Fig. 3 *B*2 was plotted. Suppression to zero shown in each case. 1, antidromic shock locked from onset of stretch, time to silence 11·7 sec; 2, corresponding time from moment of locking antidromic shock (10·6 sec) to silence, 8·5 sec; 3, moment 15·5 sec, time to silence 1·9 sec; 4, moment 18·0 sec, time to silence 0·75 sec. Dots mark locking of antidromic shock in 3 and 4.

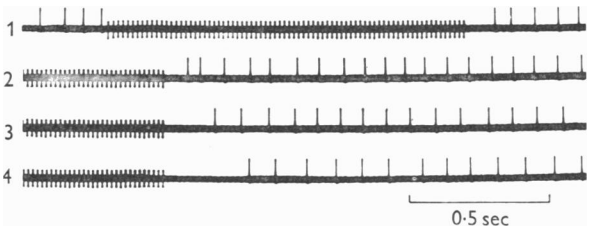

Fig. 5. Same experiment as in Fig. 3 *B*2, this time with repetitive antidromic stimulation at frequency 63 imp/sec inserted into stretch reflex at moments 11 sec (record 1), 13·4 sec (2), 19·1 sec (3), and 23·9 sec (4). Note tendency to 'doubling' of first pair of spikes after antidromic stimulation and the increasing time of recovery from 2 to 3.

after the tests. In Fig. 2, the inserted records 1–3 refer to corresponding moments in the graph. In them it is seen that time of recovery increased from 1 to 3. The discharging spike used for the graph of Fig. 3*B* (curve 2) was also tested by repetitive antidromic stimuli. The delayed recovery later in stretch, after recurrent inhibition, is well illustrated by the records of Fig. 5. The slightly accelerated recovery (rebound) early in stretch, previously described by Granit, Pascoe *et al.* (1957) as a sign of good excitatory drive, should be noted. It is a common phenomenon and will be taken up below. With tetanic tests, as in Fig. 2, rebound will contribute to the maintenance of the rate of firing between the tests.

Returning to Fig. 3*A*, but this time to consider the interrupted lines alone, the experiment also shows in another way why it is necessary to

conclude that the system possesses a frequency-cut with respect to excitation. We have made use of the fortunate circumstance that maintained soleus stretch reflexes of individual motoneurones may become stabilized at rates of firing which are independent of extension of pull (Denny-Brown, 1929; Granit, 1958, Fig. 1), a fact which in itself demonstrates frequency limitation (for full discussion, see Granit *et al.* 1960). Since for the steady state the input frequency of the afferents from muscle spindles increases linearly with extension (Eldred *et al.* 1953; Granit, 1958) a modest increase of extension (avoiding excess, which will introduce some inhibition) is bound to produce surplus excitation. Now, in Fig. 3*A*, the controls (○) show that ultimately the discharge frequencies become stabilized at much the same value around 40 imp/sec both for the original 14 mm and the second (interrupted lines) 16 mm pull. The curve marked 3 refers to recurrent inhibition, locked from the beginning with the 16 mm pull, and therefore comparable with the one marked 1 for the 14 mm pull. An increase of surplus excitation is demonstrated by the long period of non-cumulative inhibition that now preceded the much delayed drop of rate of firing to zero (cf. also curves 2 and 3). Experiments of this type provided further good evidence for our conclusion that the resistance to recurrent inhibition is determined by the excess of excitation beyond the amount barely capable of sustaining any given rate of firing. The surplus excitation in this case clearly comes from excitatory drive increased by harder pull.

Repetitive and locked antidromic stimulation are compared in Fig. 6, which was chosen also because of the exceptionally strong effect of recurrent inhibition, although in this case the discharge was unusually irregular. The rapid rise of the frequency curve from zero at onset of pull is not included. Antidromic repetitive stimuli at various frequencies (see legend) were compared with locked ones. The effects did not differ very much, in spite of the variations in the mode of applying recurrent inhibition. Yet in experiments with less marked recurrent inhibition the mode of application proved important. The general rule was that if repetitive antidromic stimuli were delivered at rates roughly corresponding to or below the cell's natural rate of firing in the reflex, then synchronized (locked) antidromic shocks were more effective than unsynchronized (cf. Granit, Pascoe *et al.* 1957, p. 398). Otherwise repetition rates from 40 to 50 imp/sec gave stronger recurrent inhibition than did synchronized shocks which were tied to the slow rates of discharge of tonic cells. However, during a tetanus, frequency of discharge tends to be very irregular. It is easier to measure F_n with triggered (synchronized) recurrent inhibition.

Figure 6 also serves to emphasize that, in spite of maintained stimulation by an amount of extension which in the controls was fully capable of keeping up frequency of firing for minutes in the fashion shown, insertion

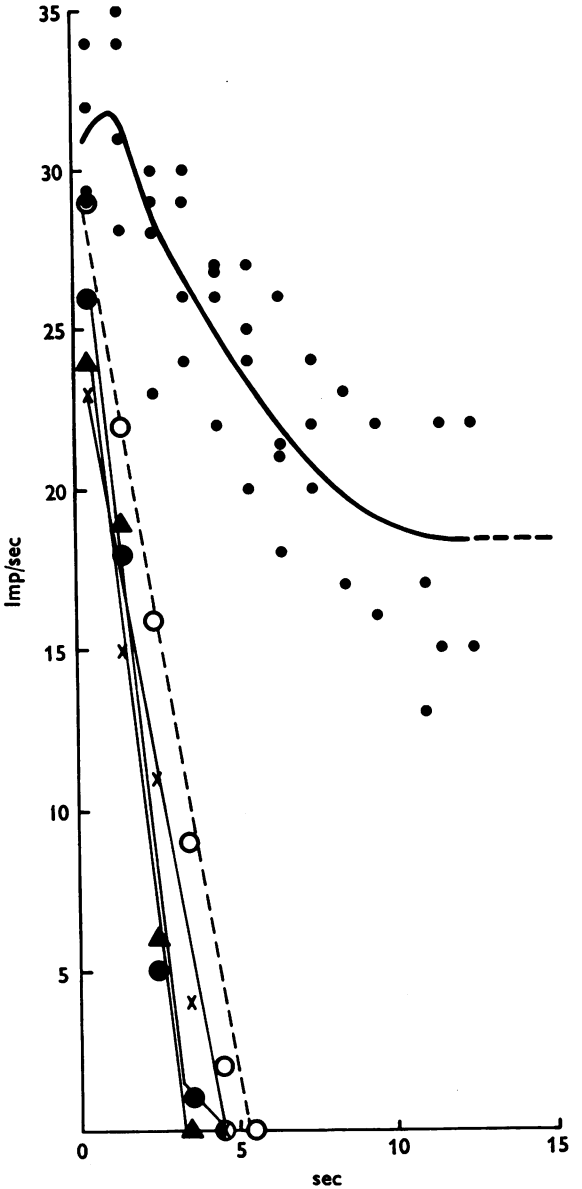

Fig. 6. Arrangement *A*. Control discharge to 14 mm pull (uppermost, small dots) was well maintained as long as pull lasted but irregular, possibly owing to the presence of two indistinguishable spikes. Chosen because of the very powerful recurrent inhibition from onset of stretch. Locked and repetitive antidromic stimulation compared, both maintained until spike was silenced. ○, Locked; ●, rate 14 imp/sec; ▲, 24 imp/sec; ×, 64 imp/sec.

of recurrent inhibition sufficed to do away with the discharge in 5 sec, and very thoroughly at that, since it did not return for the 3 sec the camera was kept running. An amount of recurrent inhibition which is constant (or even diminishing owing to its own effect on rate of discharge) therefore is capable of removing surplus excitation.

To sum up: (i) Somewhere in the discharging system there exists a frequency limiter. (ii) As a consequence any available surplus of excitation is prevented from augmenting frequency of discharge, F_n. (iii) By adding recurrent inhibition it is possible, within limits, in such cases to estimate the amount of surplus excitation by using a constant index, in our case suppression to silence (Fig. 3). (iv) When any given F_n is upheld by a sufficiently small surplus of excitation, recurrent inhibition acts as if it were capable of destroying it altogether with consequent fall of depolarizing pressure to a value from which firing fails to recover. (v) With maintained stretch a progressive loss of surplus excitation is concealed behind a 'screen' of steady frequency of discharge, but its existence can be revealed by testing with recurrent inhibition.

Electrical stimulation of afferents

It is clear that whatever the nature of the function defined by equation (1), a decrease of depolarizing pressure can be produced by adding to the inhibitory quantity P_{pol} of the right-hand member, e.g. by steady pull on an antagonist flexor muscle during electrical stimulation of the extensor afferents. When pull is hard enough, frequency of discharge of the extensor motoneurones goes down, even when a constant electrical tetanus of the severed extensor afferents (efferent roots being cut) maintains a constant drive. The experimental question is therefore: is it possible, with maintained drive, to reduce depolarizing pressure without making non-cumulative recurrent inhibition cumulative, i.e. without silencing the discharge of the cell? It is, of course, necessary to have a good recurrent inhibition to begin with.

The experiment of Fig. 7 has been designed as an answer to this question. The upper record is the control followed by locking of antidromic stimulation, the lower one the corresponding experiment during maintained stretch of the flexor tibialis anterior. There were in all 130 periods of measurement, 1 sec each, pull on flexor and no pull alternating. Control frequency of discharge without pull was $32 \pm 1{\cdot}6$ imp/sec. It was reduced by non-cumulative recurrent inhibition to $18 \pm 1{\cdot}2$ imp/sec. The corresponding values during flexor pull were $25 \pm 1{\cdot}7$ and $13 \pm 0{\cdot}8$ imp/sec. The suppression ratios (control frequency:inhibited frequency) with and without pull were therefore 1·8 and 1·9 respectively, hence equal within the limits of error. Thus, in spite of a definite reduction of depolarizing

pressure, the cell could not be silenced by recurrent inhibition as long as a constant excitatory drive was maintained. A good amount of surplus excitation relative to the value of F_n was thereby maintained. The recurrent inhibition in the present case was very potent, causing a reduction of depolarizing pressure which in terms of frequency of discharge amounted to 45 %. The orthodromic inhibition had reduced depolarizing pressure by an amount corresponding to 7 imp/sec. Thus this type of experiment (of which there were four in all) is an important supplement to those on stretch, in which, however, a slight drop of depolarizing pressure (frequency) was tantamount to loss of surplus excitation with consequent

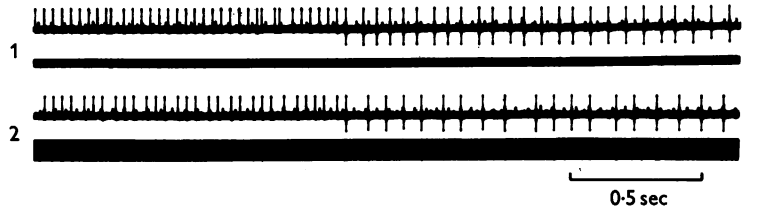

Fig. 7. Arrangement *A*, but electrical stimulation from severed medial and lateral gastrocnemius nerves at 64 imp/sec instead of pull. Nerve to antagonist tibialis anterior preserved intact for 13 mm inhibitory pull in record 2 (see length-recorder below). 1, frequency of discharge 32 imp/sec, suppressed by non-cumulative recurrent inhibition to 18 imp/sec; 2, during pull on tibialis anterior frequency of discharge diminishes to 25 imp/sec and recurrent inhibition now suppresses it to 13 imp/sec. These values refer to averages of measurements for in all 130 periods, each of 1 sec, with and without pull. Contralateral dorsal lumbosacral roots cut in this experiment.

delayed recovery after recurrent inhibition. The experiments on stretch thus differed from the present one in that any fall in the steady rate of firing led to an apparent increase of potency of recurrent inhibition, generally ending in silence of the discharge.

It now remains to use Arrangement *B* in order to have some experiments in which extensor reflex spikes are tested with recurrent inhibition limited to extensor fibres (see Methods). These experiments were restricted to constant discharge frequencies and it was attempted to make use of the frequency-limiter in the system by varying stimulus strength so as to approach the minimum excitatory drive necessary for maintaining a constant discharge frequency.

In Fig. 8 the records 1*a* and 1*b* are continuous and it is seen that recurrent inhibition was non-cumulative. Reduction of stimulus strength in record 2 with little effect on average rate of discharge immediately made inhibition cumulative. In *D*, the records 1*a* and 1*b* refer to the same run. Antidromic stimulation triggered at moments 1·3 sec (1*a*) and 8·7 sec (1*b*) after initiation of repetitive stimulation of the dorsal roots gave

non-cumulative inhibition in the first instance and cumulative inhibition in the second when the discharge had been maintained for a longer time (cf. the counterparts in Fig. 2 with stretch reflexes, first section). Stimulus strength was then slightly reduced without significantly influencing frequency of discharge (= depolarizing pressure). Record $D2$ is the control; record $D3$ shows that recurrent inhibition now was cumulative.

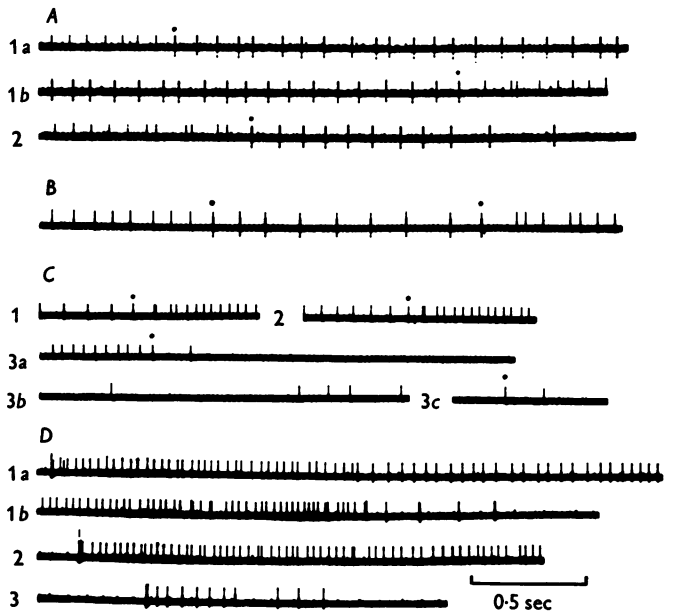

Fig. 8. Arrangement *B*. *A–D* from different experiments. In *A–C* locking and unlocking of antidromic shock marked by dots. Locking identified in *D* by shock artifacts. *A*. 1*a*, directly continued in 1*b*, illustrates non-cumulative recurrent inhibition which by slight decrease of stimulus strength in 2, turns cumulative. *B*. Non-cumulative recurrent inhibition on and off to show tendency to doubling of spikes at cessation of antidromic stimulation. *C*. 1 and 2 show similar doubling of discharge after cessation of locked antidromic stimulation. 3*a–c*, same experiment after reduction of stimulus strength to reduce afferent excitation. 0·5 sec cut out between *a* and *b*; *c*, 0·8 sec after last spike in *b*. Spike kept locked to stimulus until dot in 3*c*. Note in this case that recovery is delayed and feeble, and no doubling in 3*c*. *D*. 1*a* recurrent inhibition non-cumulative when inserted 1·3 sec after initiation of DR stimulation; 1*b* shock, locked to spike in the same stimulation period at moment 8·7 sec from initiation of dorsal-root driving of spike. Recurrent inhibition now cumulative; 2, control after slight reduction of stimulus strength; 3, same stimulus with antidromic shock locked to spike from the beginning.

Like the previous experiments with stretch, these, too, show that with a sufficiently potent recurrent system there is no fundamental difference between cumulative and non-cumulative recurrent inhibition (which are purely descriptive terms). It is all a matter of whether any given moto-

neurone is provided with enough surplus excitation to be able to recover from antidromic inhibition. The experiments also show that in spite of electrical stimulation surplus excitation tends to diminish with continued stimulation. In this respect animals differ very much, as do individual cells in one and the same animal. (This finding has been studied in greater detail by Granit *et al.* 1960).

Of particular interest are the results of records *B* and *C* 1 and 2 (Fig. 8) which show that 'doubling' of spikes (rebound) also occurs with extensor spikes tested by an extensor recurrent inhibition which therefore is

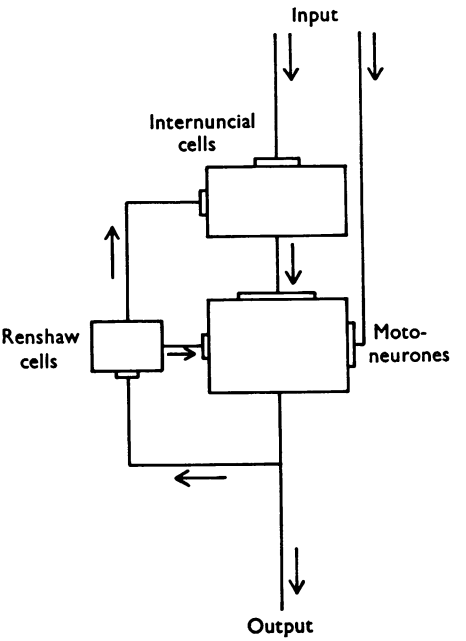

Fig. 9. Block diagram to show recurrent circuit (see text).

contaminated by excitatory effects from flexors (cf. Wilson, 1959; Wilson *et al.* 1959; and Methods). Weakening stimulus strength, as in records 3*a* and 3*b* of Fig. 8*C*, so as to reduce surplus excitation, removed 'doubling'. The results are therefore in line with the interpretation of Granit, Pascoe *et al.* (1957), according to which doubling is due to rebound in the presence of good excitatory drive, rather than with that of Wilson & Talbot (1960) who regard the phenomenon as a genuine excitatory component of recurrent inhibition.

Sometimes, when a powerful recurrent inhibition was combined with a good excitatory drive, one could, when the antidromic shock was locked to the spike, find inhibition cumulate towards a low value for the rate of discharge without ever being able wholly to silence firing. The long pauses

between the spikes allow time for some recovery from recurrent inhibition, and so the firing rate of the spike increases anew. This, in turn, increases repetition rate of the locked antidromic shocks and so cumulation of inhibition is reinstated. In this manner, then, the circuit closed artificially by locking stimulus to spike could go on hunting for its balance point. Such cases are rare compared with those in which the antidromic shocks sum their effect to give a steady or but slightly fluctuating reduction of firing frequency.

DISCUSSION

A block diagram of the possible circuits we have been studying is given in Fig. 9. We regard the connexion recurrent collateral–Renshaw cell–motoneurone to be established (Renshaw, 1941, 1946; Eccles *et al.* 1954; Brooks & Wilson, 1959; Wilson, 1959). The second circuit from Renshaw cells to internuncial cells may start from the recurrent collaterals themselves, though we have drawn it so as to suggest that (some) Renshaw cells may inhibit internuncial cells. Frank & Fuortes (1956) have shown by direct recording from interneurones farther inside the spinal cord that antidromic shocks may elicit discharges also at this site (cf. also Koizumi, Ushiyama & Brooks, 1959). This is in accordance with the original observations of Golgi (1903) who saw recurrent collaterals disappear into the cord. The results of Frank & Fuortes make direct internuncial effects from antidromic stimulation a reality but do not show if such recurrent branches are common or rare.

There is definite evidence for the conclusion that an internuncial excitatory path is of importance for the stretch reflex. Thus, for instance, it was proved by Granit, Phillips, Skoglund & Steg (1957) that the potentiating effect of repeated brief stretches on tonic discharges of extensor motoneurones likewise was potentiated by two polysynaptic reflexes, 'pinna' and 'crossed extensor'. It is also well known from the early work of the Sherrington school that extensor stretch reflexes disappear in acute spinalization, a fact confirmed innumerable times (cf. Alvord & Fuortes, 1953). Internuncial cells therefore contribute to the excitatory drive and the depolarizing pressure. In fact, it is doubtful whether any normal tonic discharge to stretch is ever possible without a substantial fraction of internuncial excitatory support. Pompeiano (1960) has recently devoted a study to the effect of internuncial 'release' phenomena on the stretch reflex.

On activating the input (Fig. 9) at increasing strength in the presence of sufficient internuncial support, the point is soon reached when the discharge frequency of motoneurones becomes practically constant. Thus a surplus of excitation is obtained. This may be regarded as an available reserve just above or a great deal above what is needed to maintain a certain firing frequency F_n. It proved possible in the experiments on stretch to measure this surplus by the time it took to silence the cell under recurrent inhibition. We have been able to show that excitatory drive (which determines surplus excitation) in some types of experiment is

destroyed by recurrent inhibition. We have also found that reduction of depolarizing pressure by itself (by flexor pull creating inhibition) need not necessarily augment the effect of recurrent inhibition, provided that the excitatory drive is well maintained by electrical stimulation. In our second paper (Granit *et al.* 1960) we discuss frequency limitation and depolarizing pressure. For the time being it is concluded that the polarizing effect of recurrent inhibition can balance out with depolarizing pressure as a steady (non-cumulative) state only when there is enough surplus excitation available. This comes from presynaptic excitatory drive. 'Cumulative' and 'non-cumulative' recurrent inhibition are merely descriptive terms. Non-cumulative inhibition turns cumulative when surplus excitation barely suffices to maintain any given frequency of discharge.

From the work of Eccles *et al.* (1954) it is known that recurrent inhibition has a hyperpolarizing action at the motoneurone membrane. It will thereby oppose depolarization. Thus it will reduce depolarizing pressure (see diagram of Fig. 9). Whatever form we assume for equation 1 (which is likely to be linear, as discussed in our second paper, Granit *et al.* 1960) it is clear that aur results show that the net effect of recurrent inhibition on F_n cannot be deduced from depolarizing pressure without taking into account excitatory drive. This is at least partly, and probably largely, a presynaptic influence maintaining P_{dep}, and is not itself included in the terms of equation (1). The excitatory drive, on this view, determines the rate at which the motoneurone cell membrane can compensate by depolarizing currents for the hyperpolarizing effects of recurrent inhibition.

The finding that recurrent inhibition can under some circumstances be used as an instrument for gauging the available amount of surplus excitation by which in reflex action any given depolarizing pressure is maintained, is one we find a little difficult (though not impossible) to understand on the basis of a recurrent inhibition solely directed towards the motoneurone membrane. The experiments presented have shown with considerable uniformity, and particularly clearly with natural stretch reflexes, that at times recurrent inhibition destroys excitatory drive and silences the motoneurone for good in spite of maintained stretch. We must therefore seriously consider the hypothesis that the recurrent process also destroys it at its source in the interneurones, acting, say, in the manner of a temporary acute spinalization. This process is often heralded by signs of delayed recovery after recurrent inhibition and ends in complete failure of the discharge to reappear, though the latter is well enough maintained in control pulls run for the same or greater lengths of time.

It has been pointed out by Matthews (1959*a*, *b*) that stretch reflexes for any given extension produce the same amount of static reflex tension independently of whether they are started by very slow or by fast pull.

Hence it is difficult to understand why a muscle, kept extended, could not reproduce its reflex tension after temporary recurrent inhibition (Figs. 2, 3*a*, 4 and 5), assuming the latter to be directed merely on to the motoneurone cell membrane. To explain on the basis of the findings of Eccles *et al.* (1954) the fact that it cannot do so, it would be necessary to add the assumption that a high afferent starting frequency is necessary for activating the internuncial pool. Alternative hypotheses on similar lines could be formulated to account for the failure of a cell, blocked by recurrent inhibition, to regain its active state. Thus, Granit, Pascoe *et al.* (1957) suggested that post-tetanic potentiation at the synapse between Renshaw cell and motoneurone might strengthen the effect of recurrent inhibition by temporal summation. There is also accommodation to consider (Araki & Otani, 1959) and a firing cell, as we have seen, is supported by rebound.

Be this as it may, with regard to the physiological problems of regulation of rate of firing and general control the essential point is that recurrent inhibition—when well developed anatomically—powerfully influences those particular motoneurones that are kept firing on too small a supply of surplus excitation. The actual frequency of discharge of the motoneurone is immaterial. If barely maintained, recurrent inhibition will stop the firing. Thus it can be understood why Phillips (1959) often found what was interpreted as recurrent inhibition in the pyramidal cells with high firing frequencies, whereas Granit, Pascoe *et al.* (1957), found it to be strong in tonic ventral horn cells which fire slowly (cf. Kuno, 1959; R. M. Eccles, A. Iggo and M. Ito, personal communication). We have often seen high-threshold, rapidly firing cells silenced before low-threshold tonic ones run on a greater surplus of excitation. If with electrical stimulation spikes were kept actively discharging just at threshold strength, then, whatever their rate of firing, they tended to disappear as soon as antidromic stimulation was triggered to the spike, provided that they had recurrent inhibition. This was well seen whenever functional isolation of ventral root filaments for single spikes was unsatisfactory so that high threshold neurones were seen to be brought in at increased stimulus strength. In this case the feebly supported high-threshold repetitive discharges were usually silenced by recurrent inhibition.

One definite role of recurrent inhibition will therefore be to hold down the subliminal and just liminal fringe around any reflex action and to stop feebly supported discharges from lingering on. Since its effect depends upon how well any particular depolarizing pressure on a motoneurone is supported by surplus excitation, different forms of physiological activity may vary very much in this respect. Inasmuch as organizational features are important for recurrent inhibition, they will determine its effect in accordance with the principle now established.

Granit, Pascoe *et al.* (1957) regarded the recurrent collaterals as the 'natural efferent antagonists to the γ-driven tonic system' (p. 397) meaning that, since spindle activation was slow and was succeeded by long-lasting states of post-tetanic potentiation, a 'danger zone' of lingering effects was created which, in the end, was neutralized by recurrent inhibition. This deduction is included within, and not an exception to the generalization of the present work as given in the previous paragraph. They also noted that (what has been called here) surplus excitation, as obtained by post-tetanic potentiation from the muscle afferents, made cumulative recurrent inhibition non-cumulative, in fact, the urge to formulate and study the present problems came from their observations. Eccles *et al.* (1954) held recurrent inhibition to be a kind of safeguard in violent motor activity, a role which it could play well only with motoneurones from the subliminal fringe.

Wilson (1959) and Wilson *et al.* (1959) have found recurrent inhibition coupled with reciprocal excitation. This is evidence for its role in functional differentiation of reflex patterns, but further work will have to decide how strictly it follows the principle of reciprocal action. As a matter of fact, R. M. Eccles, A. Iggo and M. Ito (personal communication) did not find reciprocity to be the decisive organizational feature, but after testing with very many different nerves decided upon 'proximity' in the cord. The excitatory effect we have not seen with tonically discharging extensor motoneurones and so recurrent inhibition must be vastly more potent in them. Also in these workers' results by the intracellular technique excitatory effects were rare and very small. Brooks & Wilson (1959) and Brooks (1959) concluded from the greater effect of recurrent inhibition on heteronymous than on homonymous reflexes that it served to concentrate the reflex field, acting much like Hartline's lateral inhibition (Hartline, 1949; Hartline & Ratliff, 1956). This deduction also falls under the generalization of the present work as a special case. Whenever there is a discrepancy between excitation actually used and excitation necessary to run any particular reflex against recurrent inhibition, the result can be predicted in its main outlines from this generalization. However, we show in our second paper (Granit *et al.* 1960) that the Renshaw cells are under central control and so, in the end, the scope of their influence will ultimately be determined by the extent to which they are mobilized.

SUMMARY

1. Tonic reflexes in single motoneurones of decerebrate cats have been elicited by extensor stretch and by electrical stimulation of muscular afferents or dorsal roots and tested by antidromic stimulation at constant strength.

2. When care is taken with steady stretch to maintain a steady reflex frequency of discharge, the effect of recurrent inhibition of constant strength and duration nevertheless increases, when it is injected later and later in the maintained reflex discharge. This is shown to signify that the steady discharge is kept up by a slowly diminishing surplus of excitation, not visible in the rate of firing, but gauged in this experiment by the relative increase in efficacy of recurrent inhibition. Hence motoneurones are provided with a frequency-limiter (cf. Granit *et al.* 1960).

3. By making use of the frequency-limiter to obtain stretch reflexes at the same rates of discharge in spite of different amounts of excitatory input, it is shown that recurrent inhibition actually remains constant or increases but slowly in potency, as long as there is evidence for surplus excitation on the input side. A cumulative or increasing effect of recurrent inhibition, under various conditions, is always found to signify that the steady rate of discharge of the cell is inadequately supported by the prevailing excitatory input.

4. Adequate support of any reflex discharge can be obtained with electrical stimulation of afferents. If under such circumstances the tonic extensor reflex is partially inhibited by steady pull on the antagonist flexor so as to reduce firing rate, this need not augment recurrent inhibition as does a negligibly small reduction in firing rate due to loss of surplus excitation. Reduction in motoneurone frequency of discharge is not as such decisive, but rather the amount of presynaptic excitatory drive by which any discharge is supported.

5. One major physiological role of recurrent inhibition is therefore to suppress feebly supported motoneurone activity from the 'fringe' and to prevent after-discharges from lingering on.

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