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THE BEHAVIOUR OF TONIC α AND γ MOTONEURONES DURING STIMULATION OF RECURRENT COLLATERALS

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Emphasis in this paper is laid on the physiological significance of the Golgi recurrent collaterals. As pointed out by Cajal (1909), recurrent collaterals are common throughout the central nervous system and so must be important circuit elements. The problem is therefore general, but its limitation to the testing of a specific system, the small tonic α ventral horn cells of Granit, Henatsch & Steg (1956), has several advantages, among them that these motoneurones are entrusted with the relatively well-defined task of maintaining the γ -controlled postural stretch reflex (Eldred, Granit & Merton, 1953; Henatsch & Ingvar, 1956; P. Matthews & Rushworth, 1957*a, b*). Another advantage is the information accumulated by previous studies of motoneurone recurrent collaterals (Renshaw, 1941, 1946; Eccles, Fatt & Koketsu, 1954; Holmgren & Merton, 1954). The following facts seem particularly relevant.

When the reflex from one fraction of a ventral root is recorded, an antidromic volley through other filaments of the same root exerts an inhibitory effect on it, provided that it precedes the reflex by 1.5-2 msec. The inhibition is maximal some 5-10 msec later and lasts, on an average, 40 msec. Sometimes slight excitation is seen, but then always with a much longer delay. With micro-electrodes in the ventral horn the antidromic inhibition is found to coincide with a high-frequency discharge of small cells, now known as Renshaw cells, beginning at rates from 1500 to 1000 per sec and then gradually falling off. These facts were established by Renshaw (1941, 1946). Using intracellular recording from motoneurones Eccles *et al.* (1954) found the high-frequency discharge to coincide with hyperpolarization of motoneurones, and from this and the general agreement between the two phenomena concluded that the recurrent circuit was inhibitory and made use of the Renshaw cells as interneurones.

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Eccles *et al.* (1954) state that 'the functional significance of this inhibitory pathway from motor axons to motoneurons remains obscure'. Holmgren & Merton (1954) set up a sustained reflex discharge by scratching the animal's opposite flank and found that this discharge could be temporarily silenced by a volley backfired into a synergist nerve after ipsilateral de-afferentation. The effect was seen with a shock as weak as 25% maximal. The length of the silent period depended on the balance between excitation and antidromic inhibition (cf. Renshaw, 1941). They held that the recurrent collaterals exercise a stabilizing influence on the discharge of motoneurons (cf. also Hammond, Merton & Sutton, 1956). None of these workers saw Renshaw's excitation.

Both Renshaw and Eccles *et al.* failed to detect any definite organizational component in the recurrent inhibition other than that it was strictly segmental. Within any one segment it was found to operate between extensors as well as from flexors to extensors and vice versa.

In this paper we have not recorded intraspinally but have studied individual fibres from the small tonic α cells responding to stretch after appropriate potentiation (Granit, 1956; Granit *et al.* 1956; Granit, Philips, Skoglund & Steg, 1957). The temporal course of 'feedback' action, together with the results of Holmgren & Merton (1954), and several facts established below, suggested to us that recurrent collaterals are likely to be of greater importance for tonic than for phasic activity (cf. Hammond *et al.* 1956).

One leading question in this work has been: why should there be a high-frequency discharge interposed between cause and effect? From what particular functional aspects could it derive its significance; and what about the late excitation, lost since the early work of Renshaw? Or, again, assuming the feedback to deal with the small tonic α cells, would it also influence the γ cells? Golgi (1903) pointed out that the terminals of the collaterals spread widely and disappear in the network of the grey matter (cf. also Cajal, 1909) so that, as far as anatomy is concerned, they may very well connect to γ cells.

METHODS

All the experiments were made on cats decerebrated by suction between or just in front of the colliculi under Thiogenal (Merck, Darmstadt; sodium salt of 5-(2-methylthioethyl)-5-(L-methylbutyl)-2-thiobarbituric acid) anaesthesia. The spinal cord on the side used was de-afferented from L5 to the tail end. All leg muscles of the same side were denervated except the ankle extensors. These were attached to the isometric strain-gauge myograph without separation of components other than plantaris. (Three experiments were carried out without dividing more than half ventral L7 and S1. These are described in detail in connexion with Fig. 14.)

Functional single fibres were isolated in ventral L7 or S1 filaments. Stretch was used to activate such fibres reflexly. Invariably 10 mm stretch was employed in the standard preparations (exception Fig. 14, as described separately below). The rest of the root from which the single fibre was isolated was connected to the stimulator for antidromic shocks, triggered at variable intervals. Occasionally the antidromic shock was delivered to isolated filaments.

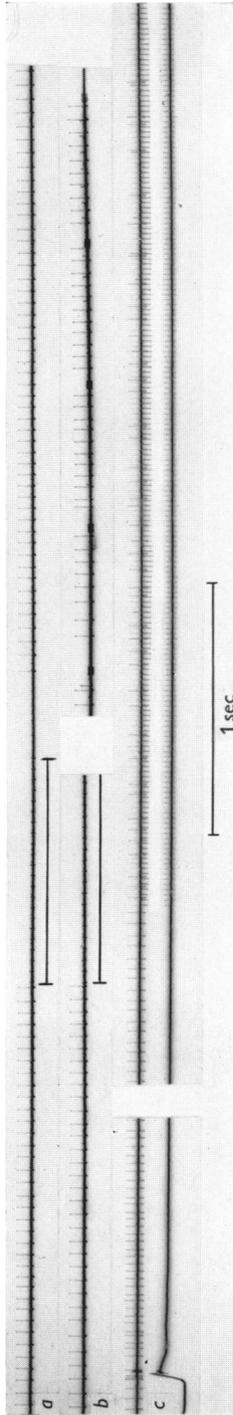


Fig. 1. Anaemic decerebration. Cut dorsal and ventral roots. *a*, suppression of spontaneous discharge in single L7 motoneurone by stimulating rest of L7 antidromically at rate 12/sec; *b*, repetition of same, then 0.5 sec cut out, followed by brief antidromic bursts of 13 shocks at rate 435/sec to show pauses; *c*, standard intercollicular decerebration and standard 10 mm stretch of ankle extensors, recorded on myograph by lower beam. Upper beam records tonic stretch reflex in single fibre after post-tetanic potentiation by repeated brief pulls as in Fig. 2*b*. Note immediate drop and irregularization of discharge when antidromic stimulus at a rate of about 50/sec is introduced; ultimately total suppression.

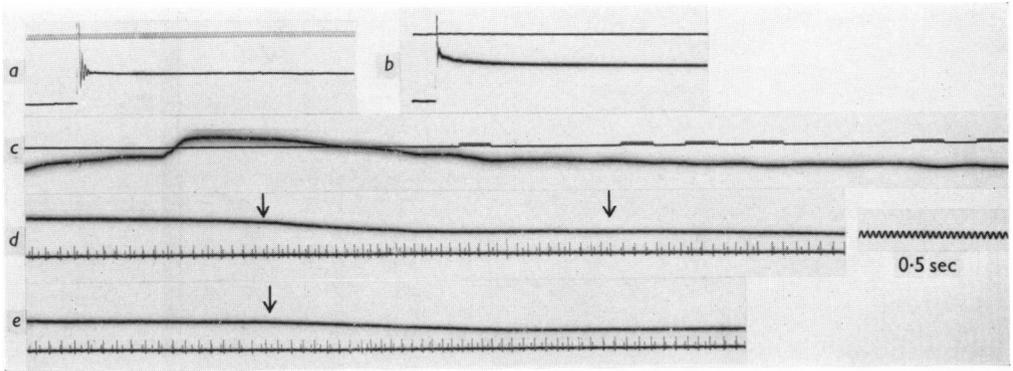


Fig. 14. Standard decerebration, but only half S1 and half L7 cut and placed on electrodes for antidromic shock. Falling weight adjusted to pull 8 mm and catch after initial free fall. *a*, control stretch of ankle extensors after severance of their nerves at end of experiment. Time, 100 c/s. Myograph excursion of plateau corresponds to 186 g; *b*, stretch reflex to same pull adds 86 g; *c*, same while loading quadriceps with pull while basic stretch maintained; some bursts of antidromic tetani inserted and marked on upper beam; *d* and *e*, similar loading of quadriceps, better maintained stretch, but now indicator spindle (large spike) recorded on second beam instead of shock artifacts. Hence latter not visible when between arrows of *d* and at arrow of *e* the cut roots are tetanized antidromically at rate 25/sec. Note slow diminution of stretch reflex with increase of spindle response. Time for *b-e* in 50 c/s.

(See p. 395)

Essentially the technique has been the one described by Granit (1956), the only new feature introduced being antidromic stimulation and standardization of the conditions as mentioned above.

The temperature of the preparation was under permanent control from thermocouples in rectum and below the skin of the leg used. It was kept between 38 and 40° C (rectal).

RESULTS

(1) *General observations.* The antidromic shocks were mostly delivered to what remained of ventral S1 or L7 when a number of thin filaments containing neurones discharging tonically had been dissected out from either root. As will be described below, post-tetanic potentiation was used to produce tonic discharges to maintained stretch. There was no exception to the rule that an antidromic shock to the rest of the root caused a silent period in this discharge, provided the rate of firing was fast enough to show it. The effect varied from filament to filament, but in these de-efferented animals it very often proved possible to stop the discharge by repetitive antidromic stimulation as in Fig. 1.

In a number of experiments we proceeded to dissect out thin filaments for the antidromic shock in order to find out how the effect was distributed over the root. Occasional filaments had no inhibitory effect, others as strong ones as the whole root. Between these extremes intermediate values were obtained. There is thus convergence of antidromic inhibition (Renshaw, 1941) in agreement with the convergence upon the Renshaw cells (Renshaw, 1946; Eccles *et al.* 1954; Frank & Fuortes, 1956).

The results suggested that a tonic discharge to stretch automatically is run with natural inhibition from recurrent collaterals. The experiment therefore consists in disturbing this process by superimposing additional recurrent inhibition. This can be done in two fundamentally different ways: (i) by neglecting the natural rhythm of the tonic discharge and thus stimulating antidromically at an independent rate 'out of phase' with it; or (ii) by locking the antidromic shock to the naturally discharging spike so that it always will be 'in phase' with respect to the latter. Both methods were used. Fig. 1 (*a, b*) is from an animal with cerebellar rigidity after anaemic decerebration which produces hyperactivity of α neurones, the ' α -cat' of Granit, Holmgren & Merton (1955). In this preparation, despite de-efferentation, one often finds spontaneously discharging neurones such as the one illustrated. The black line in *a* shows the period of antidromic stimulation at rate 12/sec. The discharge stopped and, when antidromic stimulation was withdrawn, started after a while. The length of the pause produced by antidromic stimulation varied considerably and frequently the discharge did not start again unless reflexly reactivated as by a pinna twist. Record *b* begins with a repetition of the same experiment. When the spike had regained its rhythmic activity, brief antidromic bursts were elicited in order to demonstrate the pause produced by each burst.

(2) *Cumulative effects.* Since all our cats were de-efferented the γ loop was interrupted. From the work of Eldred *et al.* (1953) it is known that the sustained or tonic stretch reflex is weak or absent without γ support and this has recently been confirmed by P. Matthews & Rushworth (1957*a*) by selectively cocainizing the γ fibres, and by Henatsch & Ingvar (1956) by suppressing the γ activity from the central end by chlorpromazine. How then produce the sustained discharge to stretch that was necessary for the analysis of antidromic inhibition of the small tonic α neurones which maintain the stretch reflex? The method was described by Granit (1956) and consists in setting up a state of post-tetanic potentiation by a high-frequency tetanus of the large muscular afferents. After this, slight pull on the muscle easily elicits a sustained discharge which in a later paper (Granit *et al.* 1956) was shown to be specifically restricted to the small tonic α motoneurones. Large phasic neurones were also potentiated, in spite of which they only responded to stretch with a few initial spikes. Stretch of the muscle, if repeated at intervals of less than about 5 sec, produced similar potentiations of the tonic cells. In developing this theme further it was found that quickly repeated brief stretches could set up equally long-lasting or even more enduring discharges than those obtained by electrical tetani (Granit *et al.* 1957). The interpretation of these authors was that the γ support, lost by de-efferentation, could partly, at least, be replaced by such means. For the present purpose, this experiment suggested an ideal method of activating the small tonic α neurones to deliver sustained discharges.

Since this procedure is essential for the development of our theme we reproduce as Fig. 2 a typical experiment. It thus illustrates how the long-lasting discharges to stretch were obtained that have been used throughout this work for the analysis of antidromic inhibition of the tonic cells. Record *a* is a single 10 mm pull, record *b* two pulls with the third maintained, and record *c* nine pulls with the tenth maintained. We know that a brief pull elicits a high-frequency discharge from nuclear bag endings which is silenced at the unloading of the muscle (Matthews, 1933, repeatedly confirmed). Thus, instead of a continuous tetanus, we have interrupted afferent bursts of high frequency. The pauses do not matter at these intervals. In this sense the process is cumulative over gaps of afferent silence and it has been found that post-tetanic potentiation is cumulative in the same sense (Granit, 1956).

In Fig. 3 the antidromic shock is locked to the spike, which is kept firing by stretch (as in Fig. 2). The five experiments show natural variations of drive, in their turn caused by variations in the amount of post-tetanic potentiation. It is seen that the silent period lengthens by cumulative inhibition from one spike to the next. When the recurrent inhibition in this manner acts 'in phase' with the natural discharge, it tends to be especially forceful, as clearly shown by these records. Resumption of the discharge by double spikes (see below) was a characteristic feature of all 'in phase' experiments, provided that the level

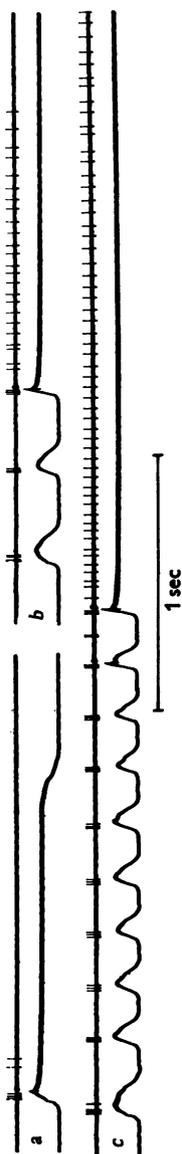


Fig. 2. Typical tonic spike in de-efferented, decerebrate preparation responding to standard 10 mm stretch. *a*, single stretch; *b*, two brief pulls and myograph then held against stretch; *c*, similarly maintained stretch after nine brief pulls. Note the enormous potentiation despite pauses between the pulls as well as the accumulation of this effect across the gaps of rest.

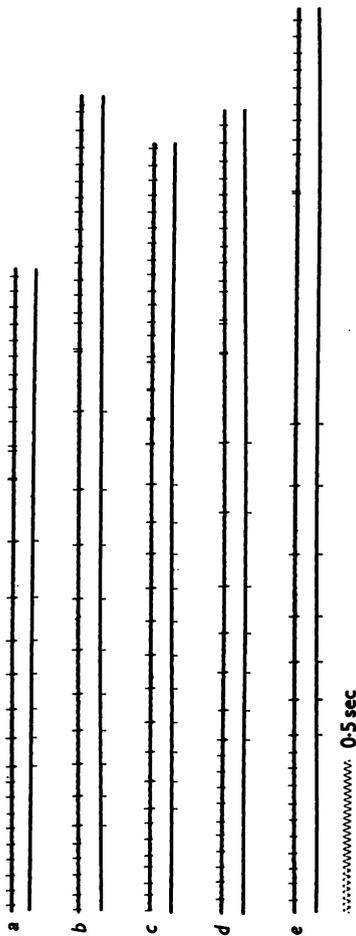


Fig. 3. Standard conditions, but each tonic spike without delay triggers an antidromic shock to another thin filament of the same root. Muscle stretch is maintained and spike potentiated after several pulls as in Fig. 2 and most figures below. Stimulus also directly connected to lower beam to demonstrate shock artifacts more clearly. *a-e*, repetitions of same experiment but variations in the number of previous pulls (not shown). Note cumulative lengthening of silent period and resumption of discharge with double spikes. As long as stimulus is triggered by the spike, the next spike is always single because the shock is triggered without delay and so the rapid feedback inhibition prevents second spike from appearing. With a delay of about 3.5 msec doubling of spikes occurred also during the stimulation period.

of excitation (see below) was high enough. (It might be observed that in a circuit kept in check by 'negative feedback', the discharge frequency is no absolute measure of excitation.)

Accepting the present evidence (Renshaw, 1946; Eccles *et al.* 1954) that the antidromic inhibition is mediated by the Renshaw cells which to each antidromic volley set up high-frequency bursts destined for the motoneurons, it seemed a reasonable working hypothesis to expect these bursts to inhibit the latter in a cumulative fashion, provided that the intervals between a series of antidromic shocks did not exceed a certain value (to be experimentally determined). Dealing, as we do, with a tonic discharge and assuming the Renshaw circuit to be operating also under natural circumstances it is clear that it would be a mistake to neglect the evidence for the cumulative nature of effects set up by interrupted high-frequency bursts (see Fig. 2), in trying to assess the physiological significance of the recurrent collaterals. It has furthermore been shown by Renshaw (1946), confirmed by Eccles *et al.* (1954) and Frank & Fuortes (1956), that many motoneurons converge towards the same Renshaw cell and so good activation of these cells should be ensured by our experimental arrangement.

Now, in the natural course of events, recurrent effects will both be 'in phase' and 'out of phase', varying with discharge pattern and the number of active recurrent collaterals. In the latter case the antidromic volley will elicit highly variable effects, owing to the continuously changing time relations between shock and tonic spike. However, inhibition should be cumulative over and above these variations. Fig. 1, record *c*, shows such variations together with slowly cumulative inhibition of a typical tonic stretch reflex. Fig. 4 is from an active animal tending to give grouped spikes which, besides, are discharged at higher frequency than in any other of our experiments with tonic spikes. Record *a* is the control, directly continued in *b*. Records *c* and *d* show with double shocks and brief tetani how antidromic stimulation repeated every 0.3 sec cuts short the discharge. The lengthening of the silent period again is cumulative. Very characteristic is the sudden stop. Rebound trebling of spikes after the silent periods should be noted.

Rebound will be discussed below. For the time being we conclude that the over-all effect of the Renshaw circuit is inhibitory, at least when dealing with this fairly homogeneous system of small tonic α neurones. In suitable cases complete inhibition of the discharge may be obtained by firing the antidromic shock at rates as low as twice per sec. Sometimes frequencies up to 20/sec are needed. At this rate, as shown by Eccles *et al.* (1954), there could be no post-tetanic potentiation at the first synapse, i.e. between motoneurone and Renshaw interneurone. On the contrary, the response to the second of two antidromic shocks tends to be depressed at intervals up to 100 msec (Renshaw, 1946; Eccles *et al.* 1954). The cumulative effect is so potent at low frequencies, particularly when 'in phase', that it is reasonable to place it at the synapse between the highly repetitive Renshaw cell and the motoneurone.

(3) *Balance of input excitation against antidromic inhibition.* It has been found that the antidromic inhibition can be opposed by raising the level of excitation

(Renshaw, 1941; Holmgren & Merton, 1954). We have not found it possible to prevent antidromic inhibition of a sustained discharge of the tonic α cells, howsoever the latter are potentiated. Individual silent periods always follow each shock but, if a long-lasting electrical tetanus at high frequency is given

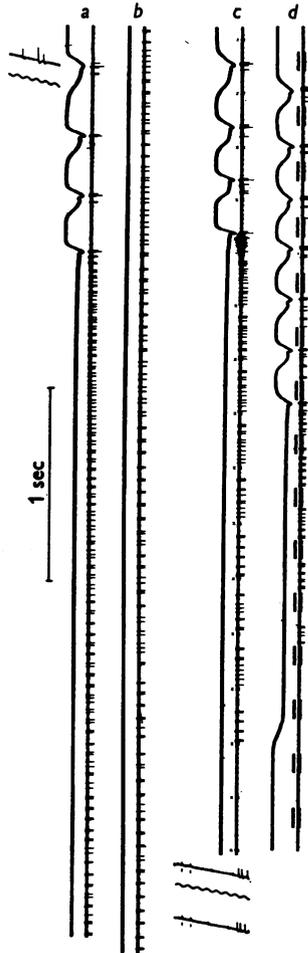


Fig. 4. Standard conditions. Characteristic discharge of large phasic and small tonic spike in S1, shown separately on sweep (time 100 c/s) alongside record *a*. *a* and *b*, response to stretch (see myograph record) maintained without interruption; *c*, repetition of same experiment with antidromic double-shocks every 0.3 sec. Note lengthening silent period and sudden suppression, also the trebling of the spikes after the silent period, as shown separately on cut-out sweep below; *d*, larger number of pulls precedes maintained stretch. The antidromic stimulus is now a brief tetanus, 44 shocks at rate 435/sec.

to the afferent nerve, the characteristic cumulative lengthening of the silent period disappears and the discharge cannot be stopped.

In Fig. 5 a tonic discharge has been produced in the control (large empty

circles) by ten brief pulls followed by maintained stretch. The two lower curves dropping to zero show the same experiment done twice with antidromic shocks repeated every 0.19 sec. The silent period lengthened by cumulative inhibition. Then the afferent nerves were tetanized at rate 330/sec for 30 sec while the antidromic shocks were elicited at the same rate as before, except for the brief period between the two arrows (large filled circles). Though each of the antidromic shocks was followed by its silent period there was no cumulative lengthening of the silent period. Nor did it help to increase the frequency of antidromic stimulation.

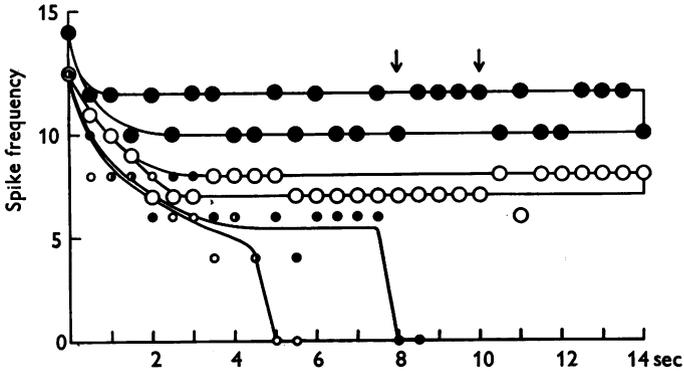


Fig. 5. Standard experiment. Spike frequency of single tonic motoneurone plotted against duration of maintained stretch. \circ , control after ten brief pulls; \bullet , two repetitions of same experiment with antidromic shock triggered every 0.19 sec. Note, while effect of antidromic shock accumulated, silent period increased from about 70–80 msec in the beginning to over 100 msec at the time when abrupt suppression occurred; \bullet , this time gastrocnemius nerves tetanized for 20 sec at rate 300/sec, afterwards maintained stretch as before. Continuous antidromic stimulation throughout experiment except for interval between arrows. No suppression this time, but the individual silent periods after each shock are still present, lasting 55 msec in the beginning and 65–70 msec at the time when plot ends.

Cajal (1909) stated that some ventral horn cells are lacking recurrent collaterals. This in itself may merely mean that they receive collateral effects from neighbours, considering the amount of convergence found above and previously demonstrated by Renshaw (1946), Eccles *et al.* (1954) and Frank & Fuortes (1956). But Eccles *et al.* also found one-fifth of the cells studied not to be influenced by antidromic stimulation. How do such motoneurones relate to the muscular afferents? They are not likely to be common among the small tonic α cells, or we should have missed antidromic effects often enough.

Now monosynaptic testing with shocks to muscular afferents activates several neurones which are phasic with respect to stretch and of higher threshold than the tonic ones. One might have expected these fringe neurones to be particularly inhibitable because of their low level of excitation when activated by stretch. Yet nothing is easier than to demonstrate that most of the neurones which lack recurrent inhibition must be among this group.

In Fig. 6 records *a* show a tonic neurone, monosynaptically activated and easily suppressed by timing the antidromic shock correctly (three lowermost records). Records *b* show a phasic neurone, similarly activated, which could not be inhibited by very strong optimally placed antidromic shocks. In *c* there is the monosynaptically elicited output of a thin filament containing both tonic and phasic fibres; in *d* the antidromic shock is put in. There is a remnant of monosynaptic activity inaccessible even to maximal antidromic inhibition.

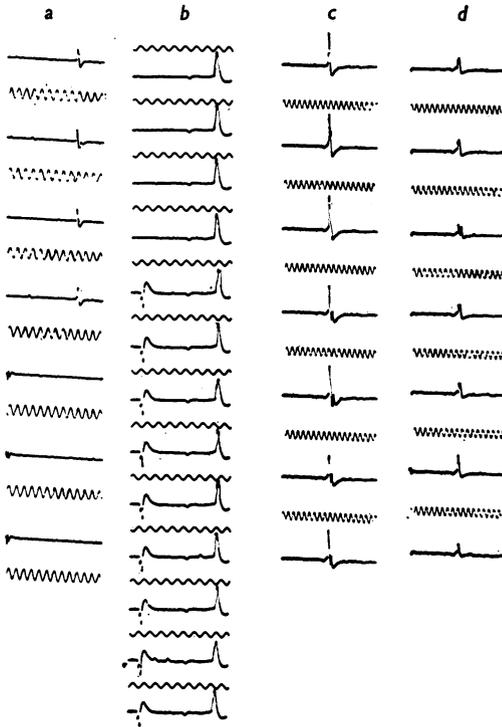


Fig. 6. Standard conditions. All responses monosynaptic and elicited by shock to gastrocnemius nerves. *a*, isolated small tonic spike as determined by previous test with stretch. When antidromic shock is put in (last three records), response inhibited; *b*, response of filament uninfluenced by strong antidromic shock (last nine records); *c*, filament with some tonic and phasic spikes; *d*, same with antidromic shock. Shows suppression of part of the response only. Time, msec.

From this experiment it should not be concluded that all neurones which are phasic in terms of muscular afferents necessarily lack antidromic inhibition. On an average phasic neurones are larger than tonic ones (Granit *et al.* 1956, 1957, recently confirmed by Eccles, Eccles & Lundberg, 1957*a, b*). They give rise to larger spikes of higher conduction velocity. Since silent periods are obtained with shocks 25% maximal (Holmgren & Merton, 1954) it is probable

that there are phasic neurones capable of influencing tonic ones antidromically. Phasic neurones with recurrent inhibition are also seen from time to time.

The experiment of Fig. 7 illustrates a phasic neurone *a* which in record *b* could be made to discharge for a brief while to a pinna twist. To phasic stretch it produced maximally three spikes in the potentiated state. In record *c* the same spike is elicited by a shock to the gastrocnemius afferents. When in *d* it was preceded by an antidromic shock to adjacent fibres, complete inhibition occurred. Next followed 10 sec tetanization of the gastrocnemius nerves at a frequency 330/sec. Record *e* shows that it then resisted antidromic inhibition. Somewhat later, when the post-tetanic state had declined, a control *f* was taken.

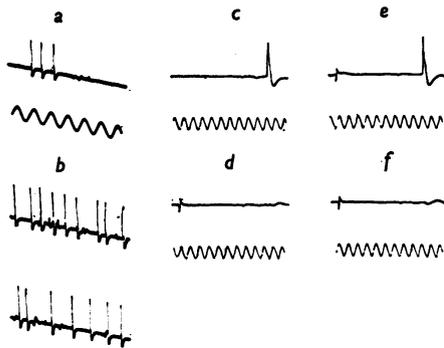


Fig. 7. Standard conditions. Phasic neurone, as defined by repeated pulls, giving triple responses to fast stretch in *a* (stretch not shown), but no lasting discharge; *b*, same spike gives brief high-frequency discharge to twist of pinna. Time, 100 c/s. *c*, same spike elicited by shock to gastrocnemius nerves, inhibited, in *d*, when antidromic shock is put in. *e*, same spike but now after potentiation by a 10 sec tetanus at 330/sec to gastrocnemius nerves—in this state not inhibited by antidromic shock; *f*, control, somewhat later, when antidromic inhibition again effective. Time in msec.

A considerable number of experiments on antidromic inhibition of monosynaptically elicited volleys in the post-tetanic state were made using electrical tetani of various rates and durations. The large synchronous post-synaptic potentials obtained in such cases (Eccles, 1953) very effectively counteract antidromic inhibition, as is to be expected from the later results of Eccles *et al.* (1954) according to which the Renshaw inhibition is a hyperpolarization that should be easily overcompensated by a large post-synaptic potential.

Usually strong phasic stretch provides enough rising excitation to prevent antidromic inhibition. Nevertheless, exceptionally one succeeds in suppressing a phasic reflex of a thin filament by a suitably placed antidromic shock as in Fig. 8, fully described in the legend.

(4) *The γ cells.* An important function of the γ cells is to set up high frequencies from the nuclear bag (annulospiral, A2) afferents through the spindle loop and thus to maintain the excitability of the small tonic α cells which have

been found to possess especially rich projections of such afferents (Eccles *et al.* 1957*b*). Without γ support tonic stretch reflexes do not come through (Eldred *et al.* 1953; Henatsch & Ingvar, 1956; P. Matthews & Rushworth, 1957*a*).

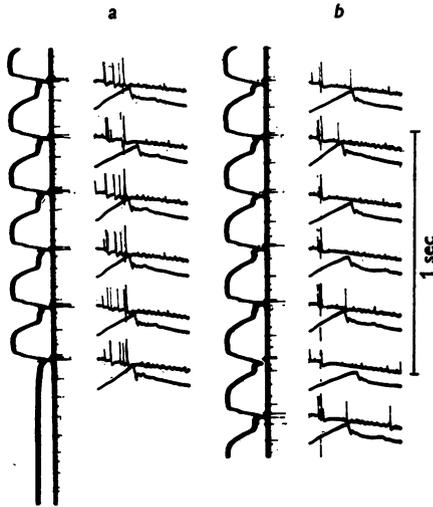


Fig. 8. Standard conditions, tonic and phasic spikes, but this time stretch triggers antidromic shock and this phase, including myograph record, expanded on sweep. In *a*, serving as control, antidromic shock occurs after the phasic discharge; in *b*, it is shifted into earlier position relative to stretch and now inhibits.

Fig. 9 shows typical results whenever amplification was high enough to show up the γ fibres. Record *a* is an α discharge to stretch with a triggered antidromic shock to demonstrate that the antidromic effect is present. Then amplification was increased and the muscle kept slack in order merely to record the tonic γ discharge (*b*). Even though a brief tetanus of five shocks was used, there was no effect on the spontaneously discharging γ spike. Record *c* is from another experiment in which both the α and the γ spikes fired spontaneously. From the fourth sweep on the antidromic shock was inserted. The α spike was pushed out towards the end of the sweep by the recurrent inhibition while the γ spike, as before, occurred in all positions relative to the shock.

Clearly the γ system is designed to exercise its effect across the spindle loop without demonstrable interference on the part of the recurrent circuit. The γ cells control the afferent, the Renshaw cells the efferent part of the reflex arc.

(5) *The rebound component.* In our experiments an excitatory component has appeared as rebound with doubling or trebling of the first spikes after the silent period. For this effect to occur it is necessary to have excitable preparations. Fig. 10 shows a stretch reflex with slowly repeated antidromic interruption. Initially (*a*) the rate of firing is 18/sec and there is no doubling. In records *b* a larger number of brief pulls has increased the average rate of firing and

some doubling occurs. In records *c* additional reflex activation (pinna, crossed extensor skin reflex) has raised the frequency of firing to 37/sec, and doubling is now a regular feature of the response. (Though different cells cannot be compared on the basis of frequency, an increase of frequency in any one cell is likely to be significant as a sign of increased excitation.)

When the imposed antidromic inhibition is out of phase, doubling will also depend upon the time between the preceding spike and the antidromic shock. Fig. 11 illustrates the general fact that doubling is considerably facilitated by the antidromic shock falling soon after the spike and less regular in appearance

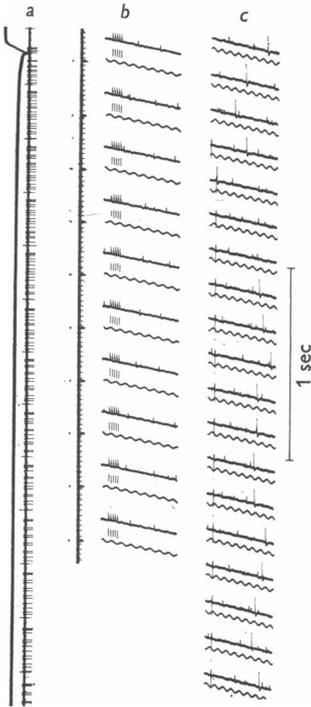


Fig. 9

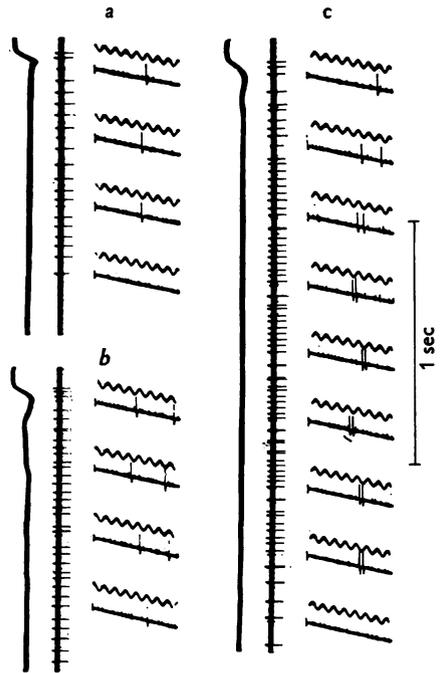


Fig. 10

Fig. 9. Standard experiment. *a* shows that tonic discharge in stretch is opposed by recurrent inhibition at each antidromic shock; *b*, same experiment without stretch but amplification increased to show the spontaneously firing γ spike on both side beam and sweep. Note that it occurs in all positions relative to the antidromic tetanus of five shocks; *c*, another similar experiment but filament selected with a view to bring in a γ spike together with the tonic α spike discharging to stretch (myograph record not shown because side beam removed to save space). When in the fourth sweep antidromic shock is inserted the α spike shifts towards end of sweep but γ spike is independent of shock. Sweep time, 100 c/s.

Fig. 10. Standard experiment. Antidromic shock initiates every sweep. *a-c*, tonic response is intensified in three steps to show how the occurrence of double responses after silent period increases in frequency when discharge rate increases. Regularly present in *c* when spike fires at rate 37/sec. Sweep time, 100 c/s.

when the interval is longer, as noted long ago by Eccles & Hoff (1932), though the effect, of course, also is subject to natural variations of excitability. When the antidromic shock is locked to the spike, doubling is practically always obtained, provided that it is locked with a delay not less than about 3·5 msec. If the delay is shorter, as in Fig. 3, the inhibitory action of the recurrent circuit is fast enough to prevent the appearance of the second spike, as is to be expected from the previous measurements of the central delay in the Renshaw cells, the latency of the silent period (Renshaw, 1941, 1946) and of the inhibitory hyperpolarization (Eccles *et al.* 1954). All results hitherto described may also be obtained by sending in the antidromic shock to the filament discharging the spike instead of into a suitable neighbouring filament.

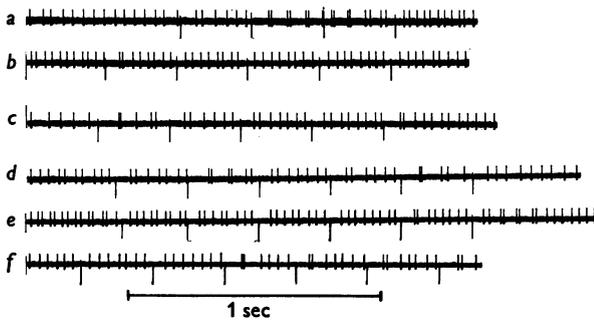


Fig. 11. Standard experiment, but with very thin filament for antidromic shock. Large artifacts show antidromic shocks out of phase with natural rhythm. *a, b*, stimulus strength 0·2 V; *c*, strength 0·32 V; *d-f*, strength 0·5 V. Maintained stretch (myograph cut out). Note doubling of response after silent period is favoured when shock occurs near a preceding spike.

The question next arose of whether or not there would be filaments exhibiting doubling unconnected with previous inhibition. The ventral roots were split into fine filaments which in turn were applied to the electrodes delivering the antidromic shock. When doubling or inhibition was seen, strength of the antidromic shock was systematically varied in order to find out whether the two phenomena had different thresholds. An example is shown in Fig. 12. It is an out-of-phase experiment, but the same result was obtained by locking the antidromic shock to the spike. Doubling was always preceded by inhibition. If the inhibitory effect was weak or absent, there was no doubling.

We conclude that doubling produced antidromically is a consequence of inhibition. Doubling, of course, requires good excitability, as shown by the fact that any sudden strong reflex excitation may start with double spikes. This is seen both with pinna, scratch and stretch reflex, if their onset is sudden enough.

If the antidromic shock really makes excitable cells swing between inhibition and excitation, it should be possible to demonstrate such changes of excitability also by setting up a discharge from the afferent muscle nerves and timing

the antidromic shock precisely with respect to it. The experiment requires considerable patience because if the orthodromic stimulus is too strong or the antidromic too early or too late, the cell fires anyhow and what is aimed at is to find a discharge the tail end of which can be accelerated or prolonged by an antidromic shock. This is shown in Fig. 13. The discharge was produced by carefully grading a brief afferent tetanus whose shock artifacts are shown in the uppermost record of *a*. Both sets of records begin with some controls. It is

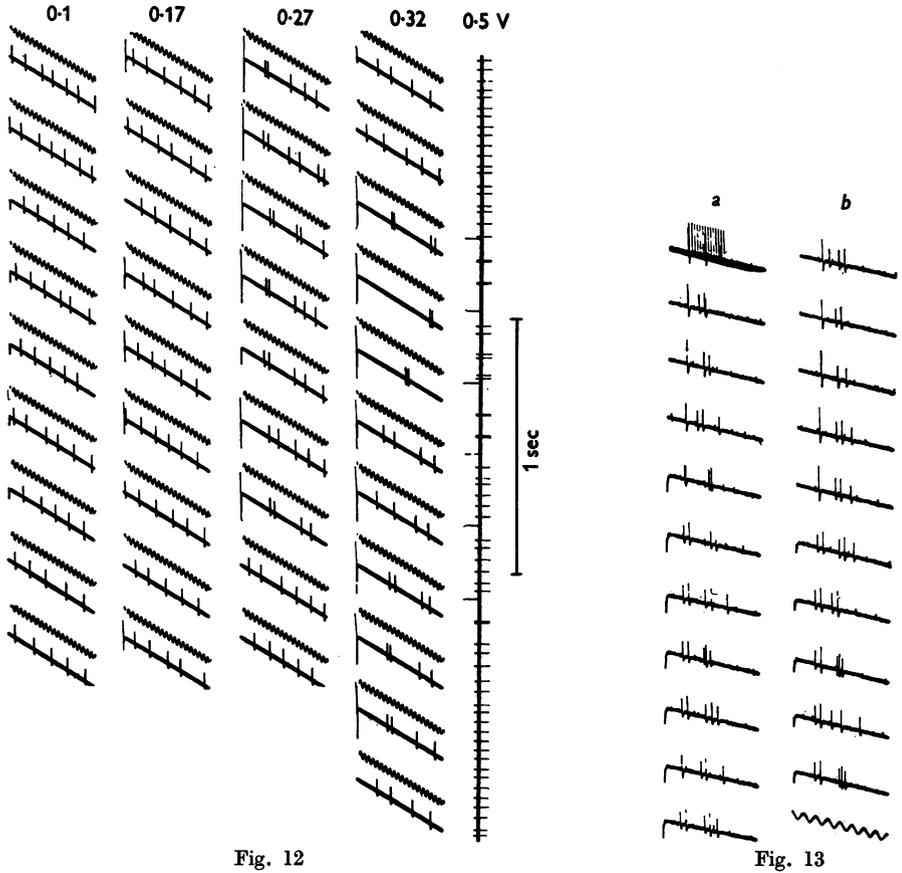


Fig. 12

Fig. 13

Fig. 12. Standard experiment. Tonic spike potentiated by stretch. Thin filament for antidromic shock, discernible by shock artifacts. Some controls without antidromic shock occasionally inserted. Curves show effect of stimulus strength (V) above each record. Threshold is at 0.27 V when doubling and inhibition appear together. Sweep time, 100 c/s.

Fig. 13. Standard conditions. Reflex discharge of a number of fibres regularly elicited by a brief tetanus to the gastrocnemius nerves. Shock artifacts separately photographed in first record of *a*. In both *a* and *b* antidromic shock inserted after some controls and marked by large shock artifacts. Note that antidromic impulse tends to split response into two portions and to lengthen or increase frequency of tail end of discharge. In *b* orthodromic stimuli a little later than in *a*. Sweep time, 100 c/s.

seen that the antidromic shock splits the reflex into two parts and tends to lengthen or accelerate the tail end of the discharge.

The likeliest explanation of the rebound excitation is that a cell kept near its firing level of depolarization returns from a transient hyperpolarization at a faster rate than when action starts from an already depolarized state. Under such circumstances the crayfish stretch receptor produces rebound excitation after a transitory repolarizing inhibition set up by its specific inhibitory fibre (Kuffler & Eyzaguirre, 1955). The receptor had in their experiment been maintained near its firing level by slight stretch. For similar results with *Aplysia* ganglion cells, see Tauc (1956). Our results could be attributed to the same type of membrane change. No specific excitatory fibres are needed to explain this variety of rebound.

(6) *Application to stretch reflex.* Since the Renshaw circuit is an efferent antagonist of the tonic stretch reflex and, on our results, does not interfere with the γ neurones, successful antidromic suppression of the stretch reflex should immediately be counteracted by increased spindle activity, provided that the spindles are well enough supported by the γ system. If they are too slack for lack of γ support the Renshaw inhibition might easily gain the upper hand. In fact, this has happened in the cases we have seen above of complete suppression of the stretch reflex by cumulative action. It should be noted that our deafferented animals were artificially raised to the state of post-tetanic potentiation that normally would have been better taken care of by the γ -spindle organization. In view of this it is of some interest to test our conclusions on a stretch reflex with some γ support. The conclusions tested are (i) that the antidromic shock has no effect on the γ system, and (ii) that a spindle with some γ support should increase its rate of firing during antidromic inhibition.

In the experiment of Fig. 14 (facing p. 383) half of the roots L7 and S1 are intact, the other half cut and placed on electrodes for antidromic stimulation. Record *a* is the control taken after the experiment when the nerves of the gastrocnemiussoleus muscle used in stretch (falling weight) had been cut in order to show the myographic muscular change alone. Record *b* is a stretch reflex to the same rate and distance of fall. Here is now some γ bias, as without it there would be no tonic stretch (Eldred *et al.* 1953; P. Matthews & Rushworth, 1957 *a, b*). This basic amount of stretch in our experiments could be but little if at all influenced by additional recurrent stimulation (beyond what it may contain in itself). Starting from this basic amount of stretch, we proceed, in record *c*, to load quadriceps which activates the ankle extensors (Granit, 1950) and particularly the small tonic neurones (Eccles *et al.* 1957 *b*). There is now a superimposed synergic reflex slowly dwindling away and definitely influenced by bursts of antidromic tetani. Each burst is followed by slight recovery suggesting active spindle antagonism. To prove this, records *d* and *e* were taken in the same way but with an indicator spindle. The existence of γ bias is clearly demonstrated

by the fact that the spindle fires *during* the stretch reflex which should silence it (owing to unloading) if it were without γ support. Between the arrows of d and at the arrow of e , antidromic stimulation is put in. It is seen that the antidromic stimulation suppresses the stretch reflex and that the spindle antagonizes this effect by an increase in discharge frequency. This it could not have done very well if the antidromic stimuli also had inhibited the γ neurones.

Experiments were also carried out with tendon jerks while firing the antidromic shock at rates of 140/sec or higher, but it was never found possible to demonstrate a definite effect on them, whatever the rate of antidromic stimulation. Whatever the timing of the tendon tap with respect to the antidromic shock, the jerk was not inhibited. On the other hand, with the de-efferented animals, i.e. without γ support (as shown in Fig. 8), it is occasionally possible to influence phasic stretch and the monosynaptic response.

DISCUSSION

A number of new facts have been established in this paper. We shall summarize them and then consider what the recurrent collaterals can do to the system we have been studying. This system can be briefly described as the γ -operated spindle loop which by its large nuclear bag afferents drives the small tonic α motoneurones to give a sustained discharge to stretch (= the tonic stretch reflex). For a review of the earlier literature, see Granit (1955). The later contributions have proved (Granit *et al.* 1956, 1957) that there are special tonic α cells which tend to be smaller than the phasic α cells (when 'tonic' and 'phasic' are defined with respect to muscular afferents). The dependence of this system upon γ activation has been demonstrated by Eldred *et al.* (1953), Henatsch & Ingvar (1956) and P. Matthews & Rushworth (1957*a, b*). Finally Eccles *et al.* (1957*a, b*) have demonstrated that the small tonic α motoneurones are characterized not only by slow conduction velocity but that they tend to run preferentially to well-known tonic muscles (Denny-Brown, 1929), and that they receive particularly large monosynaptic projections from the nuclear bag afferents. These neurones also have long-lasting after-hyperpolarizations, when studied by the intracellular technique. Although our de-efferented preparations have been lacking the γ support required for stretch reflexes, we have been able to imitate decerebrate spasticity by electrical post-tetanic potentiation or by repeated natural bursts of impulses to stretch (Granit, 1956; Granit *et al.* 1956, 1957). Taken as a whole this work seems to have established beyond doubt that the small tonic α cells do constitute a specific motor system with well-defined properties.

The new facts are: (i) That with respect to this system inhibition by recurrent collaterals is cumulative. We explain this by reference to the Renshaw interneurones which deliver high-frequency bursts carrying post-tetanic potentiation over the intervals between slowly repeated antidromic shocks, exactly

as excitation cumulates in response to several brief bursts set up by stretch (see Fig. 2), or as post-tetanic potentiation, tested monosynaptically, can be shown to cumulate over intervals of separated brief tetani (Granit, 1956). Eccles *et al.* (1954) have shown that potentiation of the Renshaw cells themselves requires 100 times faster rates of antidromic stimulation than those used above and still found to be cumulative. The state of post-tetanic potentiation must therefore lie at the terminations of the Renshaw cells upon the α cells. (ii) With sufficient excitatory drive, inhibition is regularly succeeded by a brief phase of rebound excitation, definitely related to the preceding inhibition. (iii) The γ cells in the ventral horn are not influenced by the recurrent collaterals. (iv) Inhibition from recurrent collaterals 'in phase' with the natural tonic discharge is especially potent and highly cumulative. (v) We have not encountered tonic cells capable of escaping inhibition from recurrent collaterals, though such cells may exist. (vi) Cells lacking recurrent inhibition have been phasic with respect to stretch but many phasic cells have also been inhibitable by recurrent collaterals. Our experiments throw no light upon the significance of these two categories of phasic cells. (vii) The tendon jerk is not influenced by inhibition from recurrent collaterals. (viii) Intense potentiation antagonizes the cumulative effect of the recurrent collaterals but does not remove the inhibition caused by individual antidromic shocks pitted against a tonic discharge. We have no explanation to offer of why cumulative inhibition sometimes permanently prevents resumption of the tonic discharge.

Apart from the significance of these observations for the understanding of recurrent collaterals as common circuit elements in most nervous centres, they also suggest an interpretation of the role of such collaterals in the specific system studied. Our interpretation must remain hypothetical, inasmuch as it is necessary to reason from a superimposed exaggeration of the effects to their role in normal reflex control. Our basic assumption, in agreement with Holmgren & Merton (1954), is that the same events occur on a normal scale which here have been over-emphasized by our mode of approach.

The recurrent collaterals, in our view, are the natural efferent antagonists to the γ -driven tonic system just described. They do not operate at the level of γ control. It should be recalled that the system studied is tonic throughout and thus its limitations and danger zone should be considered from this very point of view. The tonic γ fibres can force the tonic spindles to discharge at extremely high rates (Eldred *et al.* 1953) and these effects recruit slowly and similarly vanish slowly (Granit & Kaada, 1952). A consequence of this are the states of post-tetanic potentiation (here imitated by repeated stretches). The danger zone is created by the very slowness and cumulative nature of all these effects. The intercollicularly decerebrated cat is an example of such states created by γ release, known to the neurologist as rigidities and spasticities. Its exaggerated stretch reflexes disappear after γ suppression (see above).

Nevertheless, for a while, the hypersensitivity to stretch will remain, owing to the slowness of decay of post-tetanic potentiation of the small α cells. This is where an efficient efferent inhibition from recurrent collaterals, cumulative with the sustained discharge, comes in as a natural corrective. It emphasizes the slow after-hyperpolarization of the tonic cells which as such tends to keep down the rate of firing to the low values characteristic of most tonic α cells (Eccles *et al.* 1957 *a, b*).

This interpretation thus agrees with the general view of Holmgren & Merton (1954), Granit (1955) and Hammond *et al.* (1956) that the recurrent collaterals serve to stabilize the discharge, and shows how stabilization can be achieved. From our results it is clear that effects from inhibitory collaterals 'out of phase' will aid 'in phase effects', though the latter are more potent. Possibly the greater potency of 'in phase' antidromic inhibition is due to summation with the large after-hyperpolarization of the tonic cells (Eccles *et al.* 1957 *a, b*). Our interpretation is considerably strengthened by the fact that there is much convergence of many motor fibres upon the same Renshaw cells. When reflex drive increases, a transition from pure inhibition to inhibition-rebound can serve no other purpose than maintenance of the slow rhythm of discharge with double spikes instead of single spikes. The Renshaw cells are accessible also to orthodromic excitation (Renshaw, 1946; Eccles *et al.* 1954; Frank & Fuortes, 1956). There may thus be connexions for switching the Renshaw cells on and off and they may also cause central effects which at the moment are unknown.

In view of the low threshold of recurrent inhibition (Renshaw, 1941, 1946; Eccles *et al.* 1954; Holmgren & Merton, 1954) one might also consider specific suppression of small tonic α cells by large phasic ones, but the extensive literature on electromyography gives no support for this view, at least not with respect to muscle stretch, which is the only aspect we have been considering. Until such evidence has been produced this hypothesis must remain extremely doubtful, and it is safer to assume that the 'out of phase' effects from large phasic synergists do no more than similar activations of Renshaw cells from antagonists within the same segment, i.e. aid the general suppressor effect.

Incidentally our results throw fresh light on the origin of double spikes from motoneurones, a subject on which there is a large literature (Eccles & Hoff, 1932; Gilson & Mills, 1941; Hoff & Grant, 1944; Denslow, 1948; Toennies & Jung, 1948; Gordon & Holbourn, 1949). They do so by demonstrating the connexion of this phenomenon with level of excitability and with some recent intracellular observations.

SUMMARY

1. A study has been made of the effects of the Golgi recurrent collaterals upon the tonic motoneurones. In decerebrated, de-efferented cats sustained reflex discharges of the small tonic α cells of Granit *et al.* (1956, 1957) have been

set up by post-tetanic potentiation of muscular afferents while slowly repeated antidromic shocks have been given to adjacent fibres of the same ventral root.

2. Antidromic stimulation from rates of about twice per sec upwards has been found to cause cumulative inhibition of the tonic discharge.

3. The cumulative inhibition often succeeded in suppressing the tonic α discharge altogether, especially when the antidromic shock was triggered by the discharging spike.

4. Some motoneurones, phasic with respect to stretch, were also found to be influenced by recurrent collaterals, others proved to be independent of antidromic stimulation, while all tonic cells responded by the characteristic inhibition or silent period.

5. With enough reflex drive of the tonic system each inhibitory or silent period, elicited from the recurrent collaterals, was succeeded by rebound excitation leading to double discharges. It never occurred without previous antidromic inhibition. This effect also was more potent when the antidromic shock was triggered by the repetitive 'tonic' spike.

6. The γ cells were found to lack recurrent collaterals because no amount of antidromic stimulation had any influence whatsoever on them.

7. The tendon jerk was not inhibited by antidromic stimulation.

8. Intense post-tetanic potentiation antagonized inhibition from recurrent collaterals, particularly its cumulative form, but never succeeded in preventing silent periods in discharging tonic α cells.

9. These observations on the effect of recurrent collaterals upon the tonic α cells are synthesized into an interpretation of the Golgi recurrent collaterals as a system for stabilizing the sustained output of impulses in the stretch reflex at a slow rate, thereby serving as an efferent antagonist of cumulative, presynaptic excitatory states produced by the γ -driven nuclear bag afferents.

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