

# AUTOGENETIC MODULATION OF EXCITABILITY OF SINGLE VENTRAL HORN CELLS

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MANY PROBLEMS would present themselves in greater clarity if the analysis of reflex mass effects were replaced by a study of individual ventral horn cells, and for this reason a few remarks on general principles may serve to introduce this paper.

It is well known that the concept of reflex facilitation in the sense of Sherrington (30; see also 5) was based on experiments dealing with reflex mass action. Similarly, massed activity is the index in the more recent work by others such as Renshaw (26) and Lloyd (19), utilizing the response recorded from the ventral roots or a muscle nerve. Motoneurons may enter into activity from the subliminal fringe (facilitation) or drop back into the silence of the fringe (inhibition) and thus add to or disappear from the reflex volley recorded. Thus the classical concept of facilitation is intimately tied up with that of the subliminal fringe and is less concerned with a variation in the level of excitability of the individual ventral horn cells participating in the response. Measurements of excitability in individual ventral horn cells would add a very different type of information to our stock of knowledge. Cells in the active zone and in the fringe zone could, for instance, be studied separately. To take but two examples: most important seems the necessity of knowing something about the properties of the neurones within the fringe, more than that they do or do not fire. Some interesting observations on this point have been made by Eccles and his collaborators (4) using restricted mass effects. Also, so much has now been hypothesized about the consequences of firing for "inhibition," recently again in the Bremer-Bonnet "théorie interneuronique" (2), that it has become a matter of first importance to find out what firing actually is doing to the level of excitability of individual ventral horn cells. Neurones in the subliminal fringe, as we shall see below, can often be made to fire in response to stretch but do not fire to a single test shock. If a motoneurone is silent, is this silence due to, or influenced by, the passage of an impulse and how can true inhibition be distinguished from refractoriness including subnormality? This problem requires a study of single ventral horn cells and, in addition, requires a situation in which the relative significance of facilitation, inhibition and refractoriness can be assessed. In examining the levels of excitability of individual horn cells we shall take up problems of this type, ending with the last one mentioned.

## MODE OF ANALYSIS

The principle of our mode of analysis consists in recording the monosynaptic reflex impulse in a single ventral root fibre while stretch of the gastrocnemius muscle is altering the "background." A monosynaptic test shock is set up in the gastrocnemius nerve. The arrangement is illustrated in Figure 1 and is seen to be but a repetition, in terms of individual ventral horn cell responses, of a type of experiment recently carried out in this laboratory (13, 14) with the monosynaptic mass effect as test of the excitability. The earlier experiments have provided the information necessary for balancing out facilitation against inhibition from the gastrocnemius muscle. Translated into single fibre test responses the experiment assumes a different aspect. During stretch of the gastrocnemius muscle, the individual horn cell, be it facilitated or inhibited, either fires or refuses to fire in response to the test shock. How, then, is the level of excitability to be gauged?

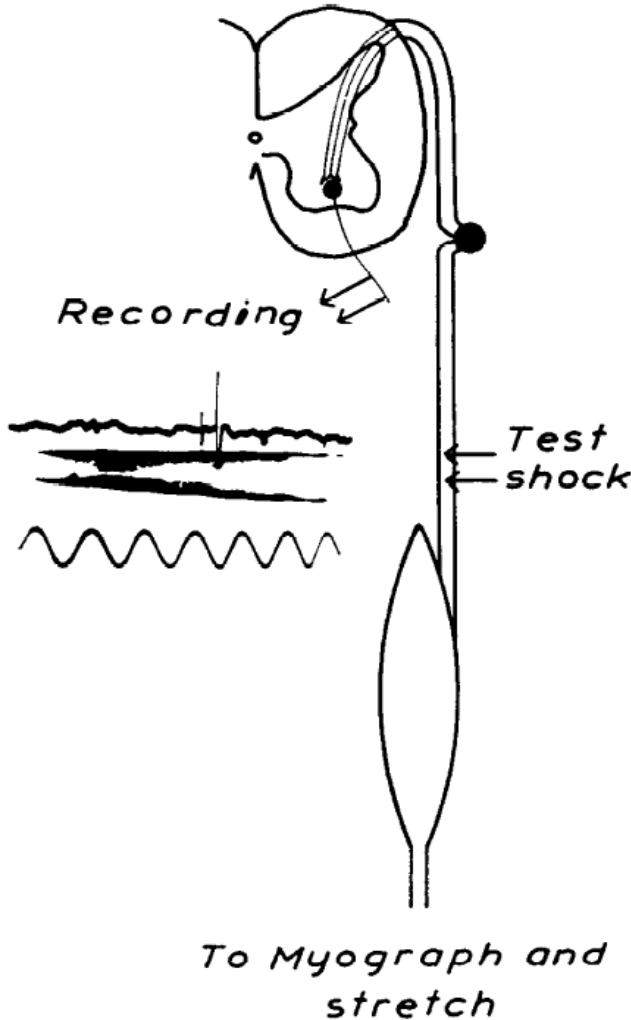


FIG. 1. Diagram illustrating experimental arrangement. Gastrocnemius muscle with intact afferent path and test shock electrodes on nerve. Recording from single fibre in ventral root; several fibres drawn converging upon ventral horn cell tested. Left (inset): Shock artefact and spike during stretch, latter recorded on second beam of cathode ray. Time 100/sec.

will now be 7/10 and we have a measure of facilitation in terms of a rise in the level of excitability and can, for instance, study for how long, during stretch, Pr has a measurable positive value. Vice versa, for inhibition shock strength may be chosen to give a Pr = 10/10, and inhibition adjusted to cause a reduction of this value. By properly adjusting tension and stretch we can augment facilitation or autogenetic inhibition and make the horn

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Consider the case of a gastrocnemius ventral horn cell being in the subliminal fringe with respect to the test shock which therefore cannot excite it. Ten trials are made to make certain and therefore the probability of response is 0/10. If, then, the gastrocnemius muscle is slightly stretched and the test shock properly placed with respect to phase of stretch it is now found to elicit a response in, say, 7 trials out of 10. The probability of response (Pr)

cell discharge to stretch and find out how it afterwards responds to a test shock, etc. This, therefore, is a plastic method equally as well adaptable for neurones in the subliminal fringe as for those in the active zone. Both facilitation and inhibition can be applied by stretch *with* and *without* an impulse fired by stretch alone, and the state of excitability gauged by a curve of Pr against time. Replacing "facilitation" and "inhibition" by "levels of excitability" provides conceptual clarity. Instead of "conditioning" by a single shock we are conditioning by adequate stimuli altering the level of excitability.

#### TECHNIQUE

The cats were generally kept in light dial anaesthesia, sometimes in addition were spinalized by a section at the level of Th 10. A number of spinal decerebrate cats was also used. The ventral roots from L5 downwards were severed; the corresponding leg was denervated except for the gastroc.-soleus nerves. Fibres were functionally isolated in L7 or S1 by division of filaments. Electrodes for the test shock were placed on both the medial and lateral gastroc. nerves. The techniques of recording from the root and of reproducing stretch on the cathode-ray screen were described in an earlier issue of this *Journal* (13). An important question concerns the criteria by means of which functional isolation was established. This, of course, is of less importance for inhibition because we are dealing with the probability of response, based on a decision between response or no response, and so it may often be immaterial how many neurones have been silenced together with the one supposed to be analyzed. The case is different for facilitation. Here it is essential to have one or, maximally, two well isolated axons. The usual criteria for a single fibre response gave excellent orientation whilst isolation was in progress. Far stricter criteria were obtained by applying stretch and changing muscle tension. The spike had to be of constant size also when facilitated during stretch, it had to disappear in an all-or-none fashion, and above all, to be identical with the spontaneous or natural impulse discharged by a sufficient increase of stretch and tension. Complex spikes made up of a few fibres were found to look like single fibres but sooner or later were almost without exception split up by stretch, which was repeated at intervals of about 5 sec. Figure 13 illustrates, for a spike delivered by two fibres, this splitting up of an apparent single fibre response.

The readings plotted in the figures refer, practically without exception, to the probability of response (Pr) in 10 trials with stretch. Sometimes double readings for the same time interval have been averaged. For the curves Pr is given as a decimal. Time is given from beginning of stretch.

#### RESULTS

##### 1. *Monosynaptic thresholds of ventral horn cells. A- and F-neurones*

We define as a fringe neurone (F-neurone) a ventral horn cell that cannot be excited by a single supramaximal test shock. In our type of experiment only such F-neurones as can be raised to firing level by stretch are accessible to measurement. By an active or A-neurone is meant one that can be activated by the monosynaptic test shock directly without stretching the muscle. A-neurones may, of course, be put into the subliminal fringe by decreasing the strength of the test shock but their A-character nevertheless suggests a more active mode of participation in the response to certain large muscle afferents, probably because of greater end-feet density.

According to Lloyd (20), single shocks to each of the branches of the gastrocnemius nerve do not fire the same motoneurones. However, Eccles (8) and Lloyd (20) describe facilitation between them (cf. also 13, 21). Per-

haps the most common experience with isolated ventral horn cells is the one illustrated by Figure 2, in which Pr is plotted against relative stimulus strength (originally measured in microamps). The motoneurone is seen to have been an A-neurone for the lateral gastroc. nerve. The rapid rise of Pr is characteristic, similarly the great facilitation by stretch with the test shock placed in optimal position. For the medial gastroc. nerve the same neurone was an F-neurone and could be fired by additional stretch and even then only with the low Pr of 1/10. Such asymmetry is common, either of the

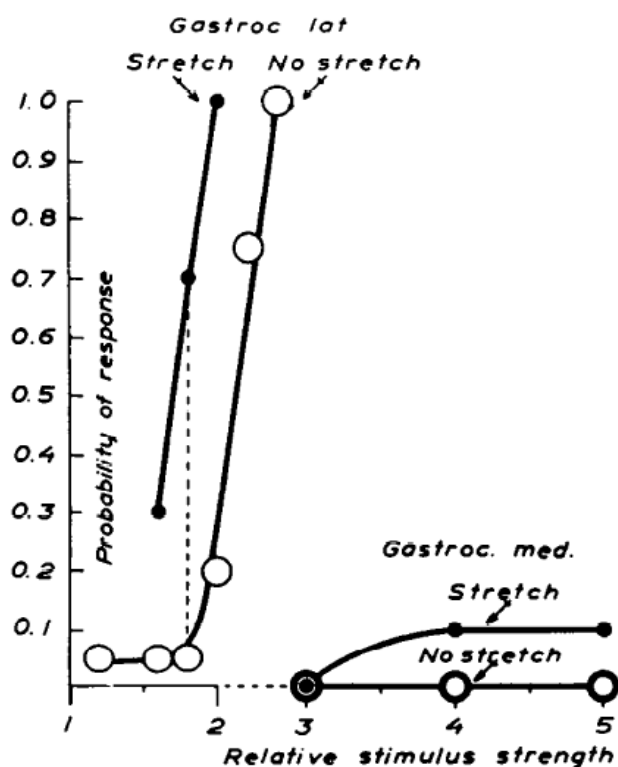


FIG. 2. Probability of response for neurone, tested separately from med. and lat. gastroc. nerves, as function of relative

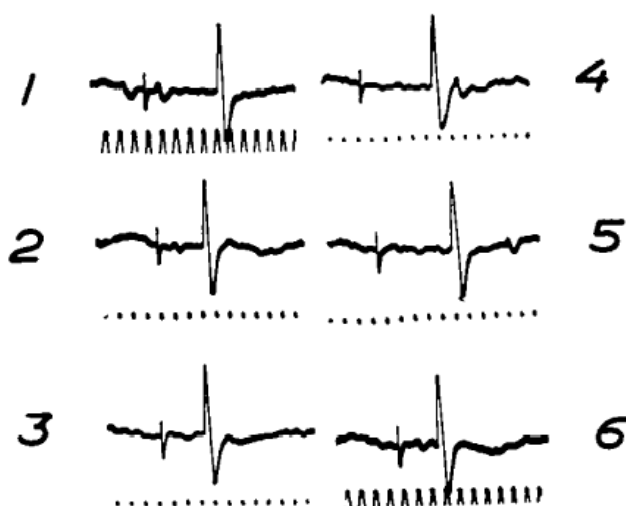


FIG. 3. A-neurone. 1-5: Variations in latent period at threshold of monosynaptic single fibre response at relative strength 1.1, probability of response at 1.0 being 0/10. 6: Stimulus strength increased to 1.8. No stretch. Time 1,000/sec.

stimulus strength. Testing carried out with muscle at rest as well as during light stretch with test shock placed in optimum position for facilitation. See text.

two giving very much lower probability, 1/10-3/10, and requiring stronger test shocks. The case of both nerves firing the same neurone without the necessity of facilitation by stretch must be rare but has once been encountered. Very often the ventral horn cell, by our tests, belongs exclusively to either nerve. The relative effect of the medial and lateral gastrocnemius nerves must also depend on the unknown amount of background facilitation and inhibition from sources not tried in these experiments. By our method one may make an occasional mistake if both nerves happen to fire exactly identical spikes in different fibres but one does not make this mistake every time and so we conclude that with a good excitatory "background" both nerves can fire the same ventral horn cell, generally, however, with the asymmetry illustrated in Figure 2.

The variations of latent period at the threshold sometimes stand out fairly well by our method if one succeeds in making the fine adjustments of stimulus strength that are necessary for this purpose. Thus records 1-5 in Figure 3 show latency variations at relative strength 1.1, a slight decrease of stimulus strength to 1.0 giving a Pr of 0/10. All variations of latency in 1-5 were not equally probable. The values centered around 3.2-3.5 msec. and 5.2-5.5 msec. During stretch the latent period shortened to a constant value of 3.0 msec. The long values around 5.0 msec. in the second group are

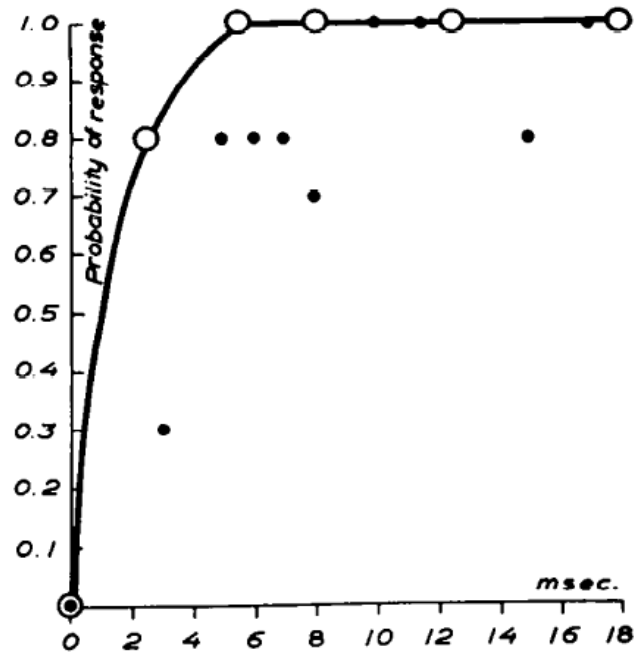


FIG. 4. F-neurone tested during 3 mm. stretch at light tension. Curve joining circles illustrates level of excitability in terms of probability of response as function of stretch. Test shock at relative strength 3.0. Dots show values obtained in same experiment when stimulus strength decreased to 1.7.

almost certainly due to cross-excitation in the muscle or stimulation of muscle end-organs by the contraction. Hagbarth and Naess (unpublished observations) have seen long values of this order in the massed response. They could never be obtained after severance of the nerve between muscle and stimulating electrodes.

## 2. Sensitivity of method. Effect of light tension and stretch

Figure 4 shows experiments during stretch with an F-neurone, only accessible from the lat. gastroc. nerve, and by definition only during stretch. For the upper curve (empty circles) a test shock at relative strength 3.0 was used and it is seen that during stretch a ceiling was reached after 5 msec. The experiment was therefore repeated with a weaker test shock of strength 1.7 and the points marked by dots obtained. This time there were some periodic fluctuations in excitability. Such variations are common with sufficiently weak test shocks. The test shock had therefore been too strong to exhibit them in the upper curve, a fact to be observed when using the probability method. The experiment provides the information that neurones in the subliminal fringe, and deep enough in the fringe never to be accessible

to the single-shock technique, may during stretch possess raised excitability and be on the verge of firing for several msec.; indeed, we have cases in which the raised excitability has outlasted the stretch. Such neurones, if not too "deep" in the fringe, may even be made to fire by stretch alone. In the case shown in Figure 4 it was necessary only to increase initial tension a little in order to make the ventral horn cell discharge an impulse exactly identical in appearance with the one set up by the test shock.

In Figure 5 it should be noted that time scale is logarithmic and duration of stretch indicated. The dots refer to an F-neurone which is seen to maintain high excitability up to the point where the record of the muscle contraction ended. The circles illustrate the behavior of an A-neurone for which the test shock had been made subliminal. At relative strength 1.1 it gave a

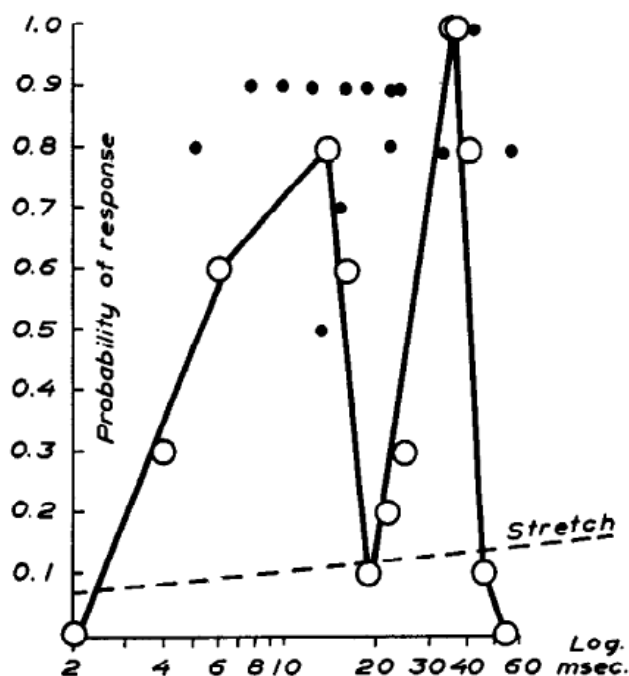


FIG. 5. Circles, joined by curve, refer to A-neurone, tested during light stretch at light tension by shock, subliminal for muscle at rest. See text. Dots, F-neurone from another experiment, tested during light tension in similar experiment. Note logarithmic time scale.

Pr = 10/10, while at 1.0 it was Pr = 0/10. In order to determine the curve, shock strength was placed as low as to be at 0.8. The large dip in the curve is its most interesting feature. This is followed by a secondary rise of excitability. Such curves are quite common. Occasionally a third late increase of excitability has been noted. The two neurones in Figure 5 were from different animals. There was no discharge in response to stretch alone at this tension and degree of stretch. Whilst fluctuations of excitability are almost the rule with a properly adjusted test shock, we believe that dips in the curve of the order of magnitude seen for the A-neurone in Figure 5 always suggest autogenetic inhibition. The next section will present the evidence for this view.

### 3. General effect of tension. Autogenetic inhibition

The degree of autogenetic inhibition varies greatly from animal to animal and for reasons which generally cannot be determined (13). It was shown by

one of us (13) that this inhibition increases with an increase of initial tension and that separate fibres are responsible for it (cf. also 15). The further conclusion was reached that the autogenetic inhibition arose in the Golgi tendon organs. In an altogether different type of experiment McCouch and his coworkers (23) have also arrived at the conclusion that the Golgi tendon organs are inhibitory. Now these organs, according to Matthews (22), are likely to go on firing for a long time in a muscle at high tension and so it should be possible in favourable preparations to show an effect of these long-lasting discharges.

The uppermost record in Figure 6 shows the response to the test shock, the muscle being at rest in moderate tension. Tension was next increased by the simple expedient of pressing with the finger on the string joining muscle

FIG. 6. Same 2-fibre preparation of A-neurones (Pr 10/10) as in Fig. 13, consisting of two spikes which mostly (without stretch) were well synchronized. Top record: Muscle at rest in light tension. Permanent pressure then exerted by hand on string joining muscle and myograph before centre record was taken. Bottom record: after release of pressure. No phasic stretch. For records during stretch see Fig. 13.



and myograph. The response to the test shock disappeared, as shown by the record in the middle. It returned when the string was released. Controls showed that it also would have returned if pressure had been maintained for some time. This experiment succeeds with many preparations exhibiting good inhibition and then provides a clear-cut demonstration of the favourable effect of tension on autogenetic inhibition. It is often possible to demonstrate that firing from the neurone, set up by stretch, is prevented by a great increase of initial tension.

Occasionally one finds a ventral horn cell which, to properly adjusted tension and stretch, responds only with inhibition. The case presented in Figure 7 was one in which the test shock was elicited from the medial gastroc. nerve. The lateral branch could neither by itself nor supported by afferent stretch impulses from both nerves elicit an effect to a supramaximal test

shock. The upper curve illustrates that, with light tension and 2 mm. stretch, there was facilitation as long as the medial gastroc. nerve was intact, the Pr of the control without stretch being 0/10. Then, in order to curtail the afferent inflow, the medial gastroc. nerve was severed and at the same time the muscle was put under high initial tension to increase inhibition. With the curtailed inflow this proved possible without eliciting a natural impulse to stretch. The Pr of the control was adjusted to give the value 10/10. Now the sole and immediate effect of stretch was inhibition. (Slight facilitation

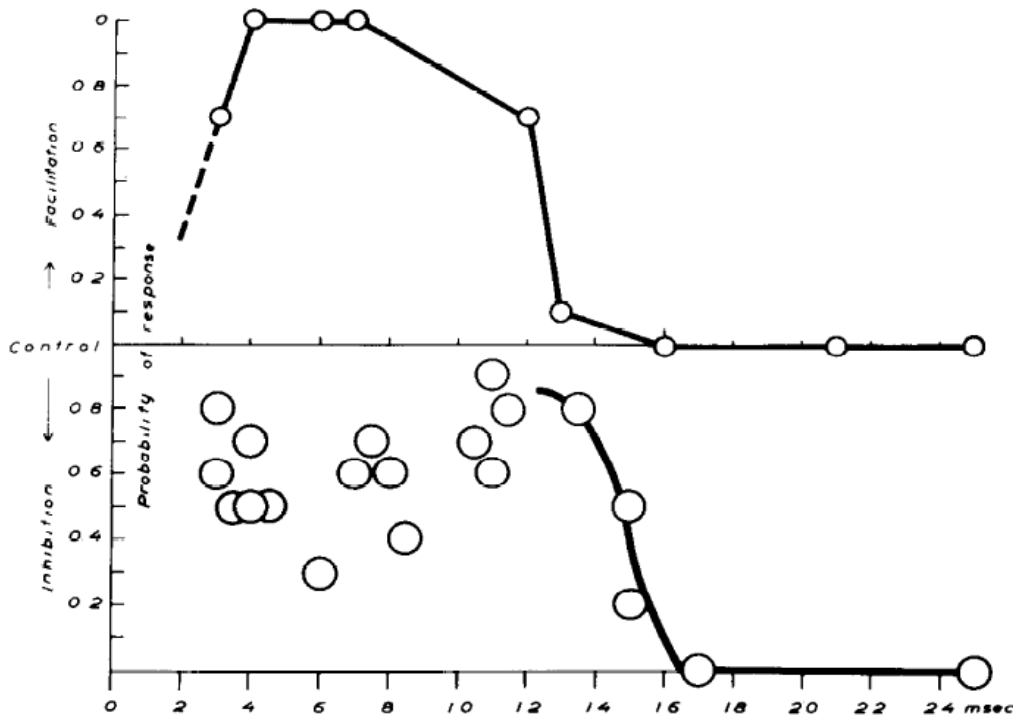


FIG. 7. A-neurone tested during stretch by shock from med. gastroc. nerve. *Upper curve*: light tension and stretch. Test shock (Pr = 0/10 at rest) subliminal so as to show up level of excitability during facilitation by stretch. *Lower curve*: med. gastroc. nerve now tied below electrodes, and muscle at high initial tension. Pr put at 10/10. Afferent discharge (through lat. gastroc. nerve), as shown by large number of observations taken (200), now gave inhibition from beginning.

had been noted at low initial tension.) Pr of the test shock was repeatedly controlled. This is a very important precaution. The experiment demonstrates that the inhibitory organs require high tension in order to overcome the effect of the excitatory ones and that under such circumstances they may succeed in blocking the discharge just as quickly as the excitatory ones can facilitate it (13). Stretch is not ideal for more accurate timing. Nevertheless, this result is in perfect agreement with the idea that the inhibitory end-organs are the Golgi tendon receptors which, so far as we know, have large fibres (1, 27, 28, 29). From as yet unpublished measurements by Hagbarth and Wohlfart (16) we know that there is a very much greater number

of large afferents in the gastroc. nerve than can be accounted for by the number of muscle spindles in the gastroc. muscle.

The inhibition noted from one head of the gastrocnemius upon the other by Sherrington (30), Liddell and Sherrington (18) and O'Leary *et al.* (24) could not be confirmed by Lloyd (19) who ascribed it to antidromic effects. However, since it now actually has been proved to exist with single fibre preparations, it would seem a reasonable proposition that, depending upon the state of the reflex centres, it actually may be demonstrable with the mass response also. It is clear, then, that (i) a motor neurone may suffer profound inhibition from autogenetic sources without firing a single impulse in response to stretch and (ii) that some inhibitory impulses are conducted in fibres of large size. This makes it highly dubious, indeed, whether it is possible ever to be certain that a shock to the large muscle afferents merely sets up excitation. For our particular type of experiment we can, on the contrary, be certain that the ventral horn cell nearly always is subjected to mixed excitatory and inhibitory influences, the latter being more prominent the greater is the initial tension, sometimes to the extent of being capable of curtailing excitation.

4. *Effect of autogenetic inhibition on F-neurones*

The experiments reported in the previous section were carried out with A-neurones. It would seem to be of importance to find out whether F-neurones show evidence of inhibition even though the fact that, by definition, they cannot be put on a control Pr around 10/10 makes it difficult to demonstrate the inhibition as satisfactorily as above. In Figure 8 the F-neurone was deep in the subliminal fringe and therefore standing a great deal of tension and stretch without firing in response to stretch alone. The upper curve was obtained at light tension with a supramaximal test shock (Pr = 0/10 for the control). The same test shock was used for the lower curve (dots) taken at high initial tension immediately afterwards. The increased tension is seen to have removed a large portion of the facilitation. After a brief initial rise in excitability the curve dropped to zero Pr.

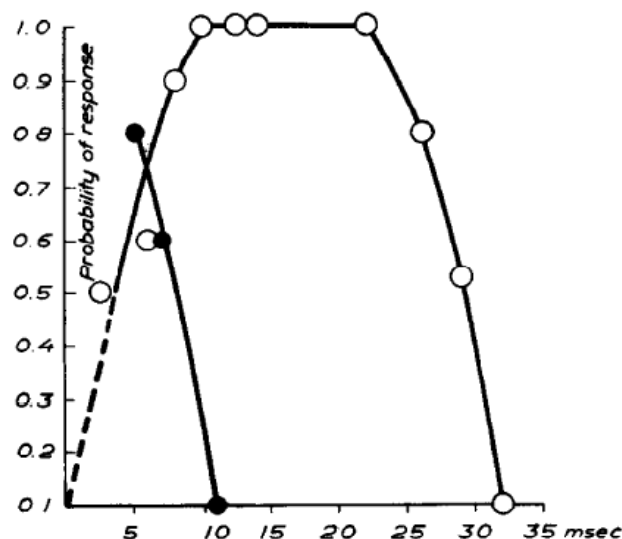


FIG. 8. F-neurone. Circles: light initial tension and stretch. Dots: same after great increase of tension. Supramaximal test shock for both curves.

Another F-neurone (Fig. 9) required more tension to rise at all above zero Pr. Then in curve 1 (dots) it showed one of the common two-phasic ex-

citability curves. Initial tension was further increased for curves 2 and 3. Despite the logarithmic time scale, curve 3, at the highest tension, is seen to have dropped to zero Pr faster than the other ones. This finding—that the drop in excitability sets in earlier for higher tensions—has been repeatedly verified though, from case to case, the time of onset of inhibition has varied. The secondary rise has sometimes been absent, sometimes present. Whether this is due to polysynaptic excitation or to a swing in the membrane potential of the neurone is at present a matter of conjecture.

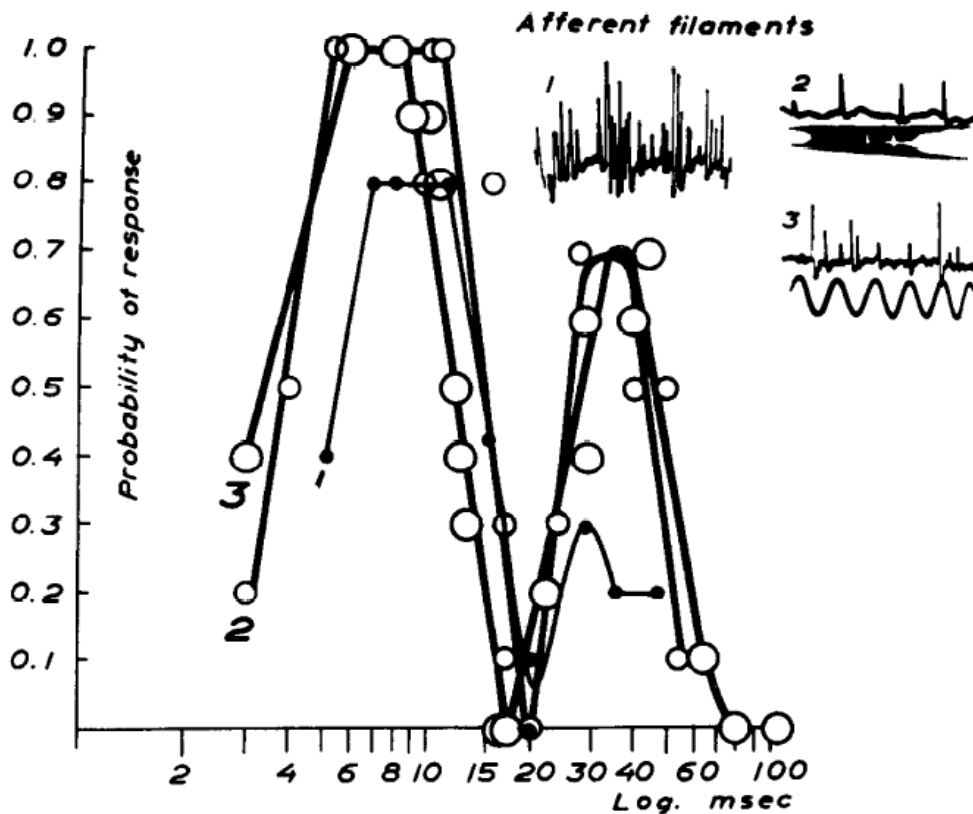


FIG. 9. F-neurone, tested with supramaximal test shock during 3 mm. stretch. Curves 1-3 show effect of increasing tension. *Inset*: Isolation of afferent filament 1, during stretch; 3, spontaneous discharge at rest and time in 100/sec; 2, filament cut down, record during stretch, as shown below spike record. 1 and 2 at maximum initial tension, same as used for curve 3 on left.

In this particular experiment an afferent filament was picked up also. Its spontaneous level of activity is shown in curve 3 (*inset*) together with the time scale (100/sec.). The same filament is seen discharging in response to stretch in curve 1 (*inset*). In curve 2 (*inset*) it was cut down to a single fibre and is reproduced together with a record of the stretch. The grouping, seen in record 1, varied from sweep to sweep. These records were taken at the high tension used for curve 3 (main Fig.) and thus demonstrate that impulses are being discharged up the afferent supply throughout the whole time of the sweep. Figures 8 and 9 leave little doubt that the neurones in the subliminal fringe reproduce the behaviour of those in the active zone.

5. *Inhibition, refractoriness, subnormality*

Having studied the effects of autogenetic inhibition on single ventral horn cells under conditions in which stretch in itself failed to elicit a discharge, we can now proceed to experiments in which the motoneurone has fired because of the natural or adequate stimulus. The difficulty is, of course, to know whether a silent period after the "natural" impulse is the result of the firing, the autogenetic inhibition, or both. This problem is further complicated by the necessity of knowing whether the ventral root fibre isolated belongs to an F-neurone or an A-neurone. These concepts now assume physiological significance of a different order.



FIG. 10. A-neurone. In 1 during light stretch, in 2 with muscle at rest. Test shock just subliminal at relative strength 0.8. Light initial tension. Time 1,000 sec. In 3-8, same after increase of stretch so as to produce in most cases an initial natural impulse (not in 3). Strength of test shock increased to 1.2, thus supraliminal with Pr 10/10 for muscle at rest. Shock artefact clearly visible. Below, right, record of stretch and time 100/sec.

If one has isolated an F-neurone which (after a sufficient increase in initial tension or degree of stretch) has succeeded in firing an impulse, it behaves as if by this exertion the cell, so to speak, had been overtaxing its strength. The test shock cannot reactivate it within 50 msec. or more. Occasionally it happens that the ventral horn cell refuses to set up a "natural" impulse every time the muscle is stretched. One can then demonstrate that the test impulse and the "natural" impulse always are alternative events; either one or the other turns up but each is succeeded by long silent periods which prevent the other from getting across. This is true for many A-neurones also but the intervals of depression are then shorter. A case in point is shown by Figure 10.

In record 2 (Fig. 10) (an A-neurone) is shown the control without stretch

and the subliminal test shock at relative strength 0.8; above it, in record 1, is the impulse set up by the same test shock during stretch. A slight increase of stretch sufficed to set up a "natural" impulse in records 4-8 (slower sweep). Not until the interval between "natural" impulse and test shock (increased in strength to 1.2) had reached the values shown in records 7 and 8 did the latter succeed in eliciting a discharge. At these intervals the discharges did not always occur. The test impulse also had a very much lower probability in position 7 than in 8. Record 3 shows an instance in which, in

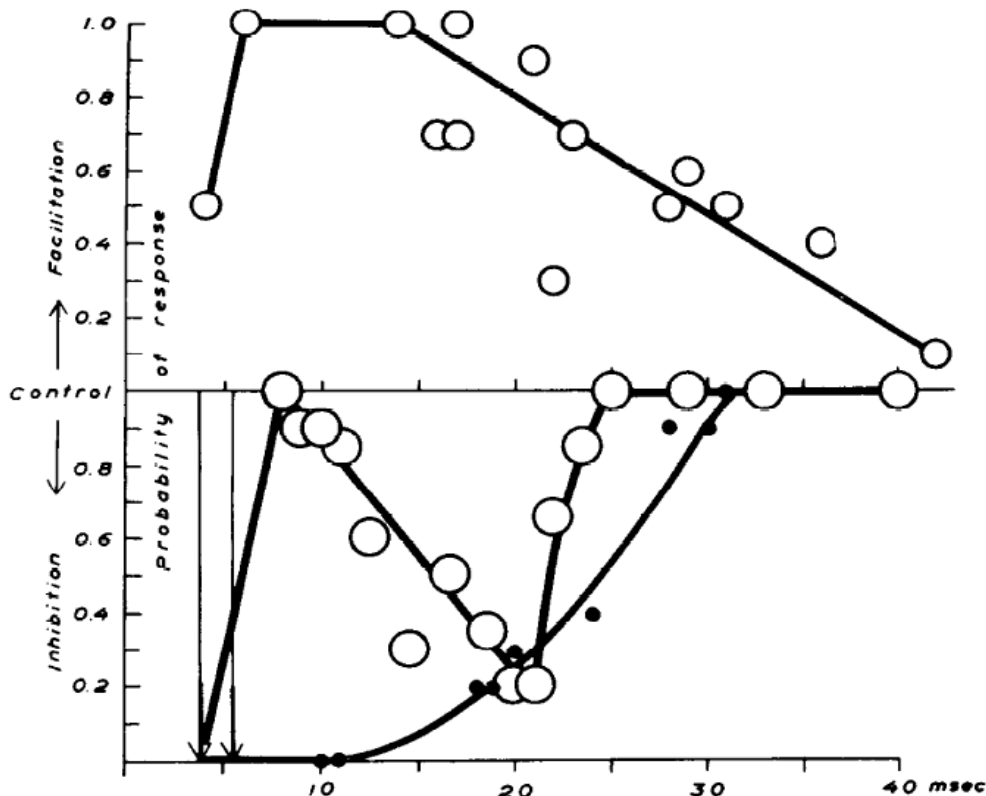


FIG. 11. A-neurone. In *upper curve* tested with test shock at strength 1.3, subliminal without stretch, in order to measure course of excitability during light 1.5 mm. stretch at light initial tension. *Lower curves*: test shock increased in strength to 3.9 in order to obtain a regular  $Pr = 10/10$ . *Dots*: high tension and 1.5 mm. stretch. *Circles*: tension diminished and stretch increased to 3 mm. Both lower curves obtained while one initial natural impulse arose in response to stretch. "Stretch responses" inside region marked by arrows on abscissa.

the same experiment, the "natural" impulse failed to appear. Immediately the test shock produced its customary effect. Whenever the "natural" impulse was absent the test shock elicited a discharge.

How are such experiments to be interpreted? Do they mean that an early test shock or an early "natural" impulse in all circumstances are followed by such long periods of unresponsiveness, subnormal states or whatever one decides to call them?

In order to reply to this question let us consider Figure 11. The upper half of the Figure illustrates an A-neurone for which the test shock was made just subliminal to give a  $Pr = 0/10$  for the control with the resting

muscle. Stretch was applied and the curve shows a long-lasting rise of excitability. The initial tension was low and the stretch around 1.5 mm. This particular curve was measured merely to demonstrate that the sense organs were capable of delivering facilitatory stretch impulses. The test shock was next made supramaximal to obtain a regular  $Pr = 10/10$  and very high initial tension used. The amount of stretch was left unaltered. There was now an initial "natural" discharge (marked in Fig. 11) to stretch just as in Figure 10. And, as in Figure 10, a long pause followed before the ventral horn cell began to reply in response to the test shock, as illustrated by the curve for which the readings are marked by dots.  $Pr$  began to rise for an interval around 6–8 msec., counted from the "natural" impulse to stretch, and reached 50 per cent around 20 msec., but it lasted as much as 25–30 msec. before a regular response at  $Pr = 10/10$  to the test shock could be expected. Finally tension again was decreased to a modest value but the degree of stretch was augmented to 3 mm., these adjustments being made to reduce the inhibition caused by high tension and to increase the facilitatory component from the muscle afferents.  $Pr$  was kept at 10/10 for the controls. Tension was kept just high enough to make the neurone still discharge a "natural" stretch impulse, as marked in the Figure; the location of the "natural" impulse varied within the area shown by the arrows. This time, however, the neurone was capable of delivering a response to the test shock with a  $Pr$  of 10/10 at 3.5 msec. after the "natural" spike. The state of excitability then fell gradually but never down to zero and rose again quickly between 11 and 12 msec. to its full value with  $Pr = 10/10$ . The shortest interval between "natural" spike and test spike ever seen in such experiments was 2.5 msec. We did not attempt to measure the refractory period systematically.

In this case we have (upper curve, Fig. 11) obtained a general idea of the likely course of facilitation. Against this background we must evaluate the two lower curves, so different in outline, although both begin with a "natural" impulse. We know that the long depression (curve marked by dots) occurred when the inhibitory component from the muscles had been emphasized by the great increase in initial tension. We also know from Figure 7 that the autogenetic inhibition is fast enough to add itself to the "natural" refractoriness which must be a short-lasting process (see below). For this reason it is necessary to conclude that the fall in excitability in the curve marked by circles must have been due to an autogenetic inhibitory component that had been somewhat delayed by the decrease in tension and never had been strong enough fully to overcompensate excitation. Tension and stretch are within limits interchangeable, as far as autogenetic inhibition is concerned (13), and for this reason some inhibition occurred when the muscle at lower tension was subjected to slightly increased stretch.

The final conclusion, then, is that the length of the silent period is chiefly due to the balance of excitation against inhibition. This conclusion leads to the consequence, several times verified in our experiments, that F-neurones

which (by an increase of tension) have been made to discharge in response to stretch must, because of the deficient facilitatory component and the increased inhibition, be particularly prone to keep silent after a "natural" spike. In fact, it is very difficult to make F-neurones discharge a second time within the time allotted to stretch, once they have fired. The interval of unresponsiveness has therefore very little to do with the refractory period and can be ascribed to "subnormality" merely by extending, without experimental justification, this concept to cover a situation of balance be-

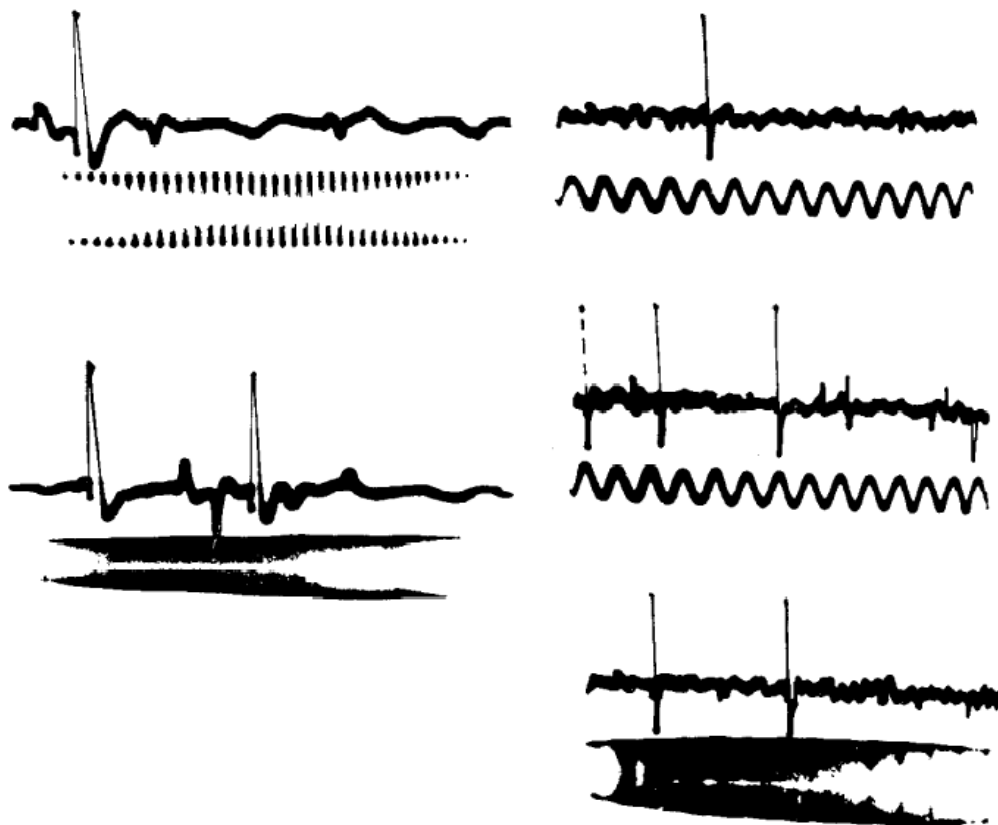


FIG. 12. Records from two different experiments on A-neurones. *Left, upper record* shows natural impulse alone and time 1,000/sec. during stretch. *Below*, natural impulse followed by supraliminal test shock eliciting second response. Record of stretch on lower beam of cathode ray. *Right*, A-neurone tending to fire repetitively during stretch alone. It fired irregularly one, two or three impulses. First impulse in middle record out of focus and filled in. Record of stretch below.

tween excitatory and inhibitory inflow plus remnant relative refractoriness. Figure 11 explains why under certain circumstances motoneurones deliver the double discharges, noted by so many workers (*e.g.*, 6, 9, 17, 11, 7, 12).

Figure 12 illustrates on the right some typical responses to stretch capable of eliciting two or three impulses, on the left a "natural" spike followed by a test spike at 13 msec. Eccles and his collaborators (3) have come to the conclusion from work on antidromic stimulation of motoneurones that the refractory period is of the order of 1.3 msec.

On the whole, it seems that one must be cautious in drawing conclusions as to the properties of motoneurones from work in which no steps have

been taken to ensure that pure excitatory effects are being measured. The gastrocnemius ventral horn cells may not as such differ from those of the ocular muscles in which Reid (25) recently has found firing frequencies as high as 175 per sec. We think it just as likely that the final common path is represented by cells of great plasticity capable of doing what the particular experiment chosen makes them do and that it may be well nigh impossible ever to find the anterior horn cells, which are converged upon by such a multitude of paths, exhibiting pure excitation and maximum excitability for more than a few msec.

6. *Two ventral horn cells in simultaneous action*

We need hardly doubt that a non-synaptic mechanism of electrical interaction between adjacent neurones plays a role with the single shock

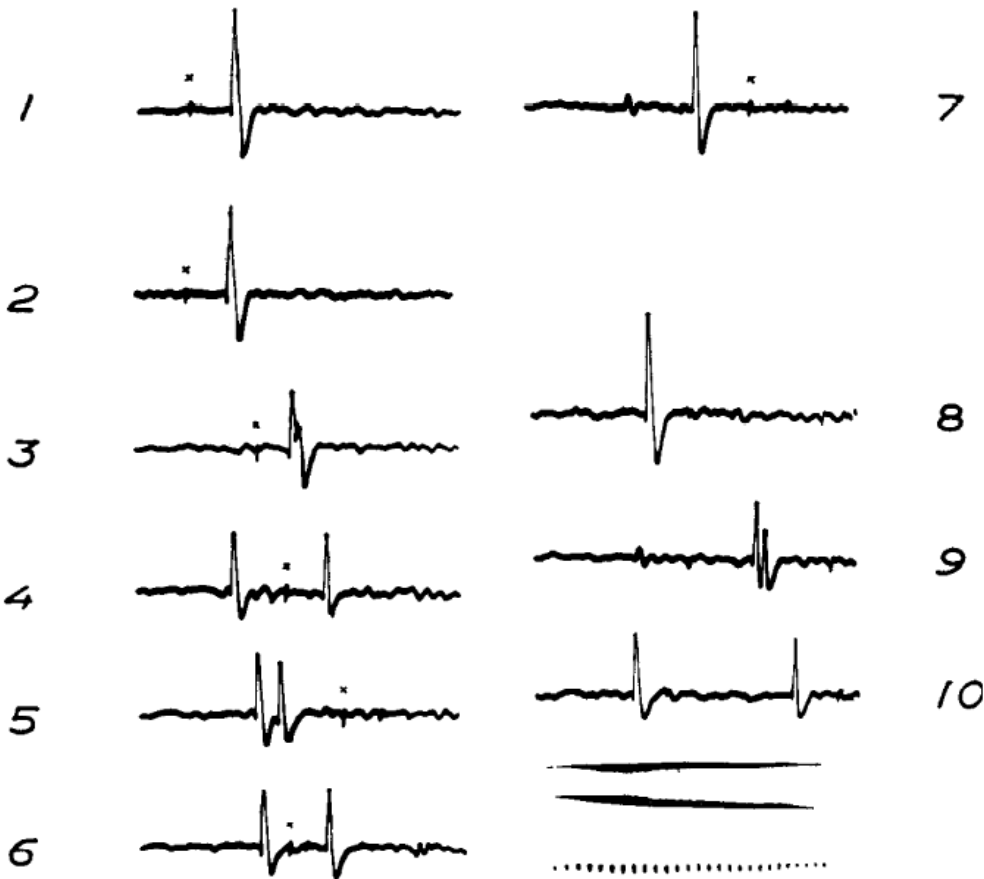


FIG. 13. Two A-neurones. All records during stretch at time 1,000/sec. Tension high enough to make both neurones fire in response to stretch alone, as shown by 8-10. In records 1-7 test shock introduced as shown by minute cross just above shock artefact. Explanation in text.

technique (26). Strong synchronous conditioning and test shocks may, however, involve the neurones in situations which for their normal mode of operation are of doubtful significance. Our present technique of single fibre isolation has obvious limitations in the study of such questions. Nevertheless,

a case in which we succeeded in isolating two active fibres in the same extremely thin filament is of sufficient interest to be put on record. It is illustrated in Figure 13. Records 1-7 have been taken during stretch which facilitated the response and often, in addition, activated either spike directly. Record 1 shows both spikes coinciding in response to the test shock, in 2 they can just be separated, in 3 they have gone further apart. In record 4, the slightly larger spike is fired by stretch alone. The shock then fired the other one, as can be concluded from record 5 in which both spikes had been fired at a brief interval from each other by stretch alone. There was then no response to the test shock. In record 6 the slightly smaller spike is fired by stretch alone. The test shock therefore succeeded in eliciting the larger one. Finally in 7 stretch has fired both impulses simultaneously and then the test shock again became incapable of firing either of the two. A very large number of records were taken from this experiment and many different positions for the test shock tried. Without exception it was found that each ventral horn cell gave refractoriness only for its own axon and not for the other one. Records 8-9 complete the experiment with some observations on stretch alone.

If it be permissible to assume that the two neurones were adjacent inside the cord, as they were in the root, the experiment may be held to demonstrate that there is a high degree of independence between such neurones. In this connection it serves the major purpose of showing how clearly a combination of stretch and test shock succeeds in discriminating between single fibre and multifibre preparations.

#### DISCUSSION

The experiments presented differ from current analytical work in using physiological stimulation of muscle afferents as conditioning effect and employing a synchronous electrical shock merely in order to test the variations in excitability set up in single ventral horn cells by excitatory and inhibitory afferents. They are therefore essentially studies of the general problem of the effect of "background." One aim of such experiments is to find out how the motoneurones deal with a specific operational task and to what extent current concepts, derived by the single shock technique, are helpful in understanding their behaviour. When using the single shock technique alone, *i.e.*, conditioning plus test shock, one has to assume that the events take place against a constant background of facilitatory and inhibitory impulses bombarding the motoneurones from different sources. The level of excitability itself, as determined by that background, remains unknown. In the present work, by using adequate conditioning stimuli, one is actually measuring a variation in background, and the isolation of single ventral horn cells ensures that levels of excitability are being gauged. In addition, such experiments provide an opportunity of following levels of excitability in A- and F-neurones separately.

The results presented have unmistakably shown that the mode of action

of the ventral horn cells is intimately tied up with their state of excitability. This is particularly clear when concepts such as inhibition, relative refractoriness and subnormality are being considered. We have conclusively proved that inhibition from autogenetic sources as such is independent of whether the motoneurone has fired or not. When well developed it may entirely stop the discharge in both A- and F-neurones and the consequent silent period is therefore quite independent of motoneurone refractoriness. Full conviction on this point actually required the single fibre preparations used. The autogenetic inhibition may then be regarded as one of the factors conditioning the background.

Refractoriness and subnormality are concepts that should be used only in connection with firing, in the original sense of Gasser (10). Our results imply that perfectly physiological variations in the excitability of the ventral horn cells determine the duration of the ensuing phase of inexcitability, be it called by one term or another. The F-neurones in general refuse to fire a second time during the period in which they had shown increased excitability before being allowed to fire at all. The A-neurones behave in a similar fashion if the inhibitory depression (from autogenetic sources) of their level of excitability has been given free play, say, by a sufficient increase in tension. Again, if steps are taken to counteract this inhibition, they show relatively refractory states of the order of magnitude found in motor axons. The heightened early excitability is often followed by a shorter period of depression, extremely variable in duration and depth from case to case. At the moment it is impossible to tell whether this is due to a subnormality and positive after-potential or to a simple lowering of the state of excitability by inhibitory afferent impulses or to both these factors. There are, of course, in a motoneurone pool all gradations of excitability between extreme A- and F-neurones. Nevertheless this distinction (Sherrington) conveys something important in terms of physiological function. The F-neurones will, in the operational task selected for analysis, of necessity be limited to very low firing frequencies and a more sporadic form of intervention whilst the A-neurones are carrying the main burden of the work. These are, as it were, leading neurones, but the others on the subliminal level follow their excitability variations like a shadow. They are called in or thrown out according to emergency. A-neurones for one facilitatory system may be F-neurones for another or *vice versa*.

One of the main conclusions would therefore seem to be that, in a particular physiological situation, little meaning can be attached to explanations of functional inactivity in terms of relative refractoriness and subnormality unless one knows the level of excitability of the neurones concerned. On the other hand, these concepts may be applicable in the sense of the "théorie interneuronique" if for some reason the cells concerned are at a low level of excitability. Another essential result is the definite evidence for autogenetic inhibition, in perfect accord with the conditions previously defined (13).

So much remains to be done with this type of preparation that we feel

that, at this stage, little is gained by elaborating comparisons with the important results obtained by the single-shock technique.

#### SUMMARY

The monosynaptic responses of single ventral horn cells have been isolated in the ventral roots (de-efferented cats) and their excitability tested by a shock to the gastroc. nerve, using stretch to condition the background of excitability. A-neurones from the active zone fire in response to the test shock alone, F-neurones from the subliminal fringe only when the muscle is stretched. Their level of excitability during stretch can be assessed by a method based on the probability of response in ten trials with the conditioning stretch (see Introduction).

The medial and lateral gastroc. nerves may fire the same horn cell—generally, however, with great difference in relative stimulus strength.

With light initial tension the level of excitability is raised for the whole duration of light stretch (1–100 msec.).

With a slight increase of muscle tension this rise of excitability is often interrupted by an abrupt drop at around 10–30 msec. so that the excitability curve during stretch turns up with two maxima.

This drop occurs earlier and is deeper the greater the initial tension of the muscle. In F-neurones this drop often may imply zero excitability in terms of a supramaximal test shock.

Autogenetic inhibition increases with initial tension, and by merely pressing on the string joining tendon and myograph it is often possible to stop every response to the test shock.

Suppression of excitability due to high tension receptors (autogenetic inhibition) need not be preceded by any firing whatever in response to stretch or high tension as such.

Since it is possible by varying muscle tension to condition the excitability either way and in addition make the isolated neurone fire a stretch impulse, one can study how firing influences the level of excitability of ventral horn cells at different levels of excitability.

F-neurones suffer great protracted depressions after firing. A-neurones may do the same if their level of excitability has been lowered by an increase of initial tension in the gastroc. muscle, but if properly facilitated by adjustments of stretch and tension, the A-neurones can be made to fire a second time a few msec. after the impulse set up by stretch alone.

Concepts such as relative refractoriness and subnormality cannot therefore be used to explain "inhibitions" without further specifications because their quantitative significance is wholly dependent upon the level of excitability of the neurone tested. A neurone, physiologically speaking, is a strategic point for shifting the level of excitability and its integrative behaviour is intimately tied up with this property.

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