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'ADJACENT' AND 'REMOTE' POST-SYNAPTIC INHIBITION IN MOTONEURONES STIMULATED BY MUSCLE STRETCH

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Owing to the difficulties encountered in measuring the increased electrical conductance and the inhibitory currents of inhibited cells within the central nervous system, many inferences concerning the presence and potency of inhibition in motoneurones have been based on the diminution of a post-synaptic test response (the EPSP in the terminology of Eccles, 1957, 1964). The present paper is partly devoted to a critical evaluation of the usefulness and significance of this criterion for inhibition (criterion (i) below) when the conditioning stimulus is a barrage of impulses of plus and minus sign arriving from a muscle loaded by a weight. Recording is intracellular throughout. Other signs for the presence of inhibition are drawn on for comparison, namely criterion (ii) which is a reduction in the firing rate of a motoneurone stimulated by transmembrane currents through the tip of the recording micro-electrode, (iii) the presence of synaptic activation noise during stretch, and (iv) a simultaneous shift in the hyperpolarizing direction of the average membrane potential. Thus the general theme is a study of the intracellular manifestations of inhibition and of their assessment when muscle stretch is used as conditioning stimulus.

In the previous paper (Granit, Kellerth & Williams, 1964) we demonstrate, by pulling on muscles, that the criteria (iii) and (iv) are perfectly valid whenever they can be applied. Unfortunately, during stretch either one or both may fail to give the desired information. The criterion (ii) is for the first time applied to the effect of stretch in the present work. It is the most useful of them all because, when the cell is fired artificially from the inside, a reduction in its rate of discharge caused by muscle stretch is bound to signify that the discharging membrane itself has been influenced. Thus the inhibition must have been what is customarily called post-synaptic, even if all other criteria had shown it to be non-existent. 'Dis-facilitations', too, would fall under this general definition.

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Frank (1959) introduced the concept 'remote inhibition' on the basis of experiments in which Frank & Fuortes (1957) had found that a test response consisting of an EPSP diminished as a consequence of a muscular afferent volley with no other demonstrable effects on the motoneurone. In several papers Eccles and his co-workers (summarized, Eccles, 1964) showed that these observations could be explained by presynaptic inhibition causing depolarization of the excitatory terminals (Wall, 1958; Eccles & Krnjević, 1959). The notion of remote inhibition was then abandoned, there being no definite evidence in favour of it. This concept will be re-instated below and used in a wider context justified by the comparisons among the four criteria enumerated above (cf. Discussion). The common denominator in the work of Frank & Fuortes and that of ourselves is the idea of spatial 'remoteness' of post-synaptic events within one and the same motoneurone for which the comparison of the four criteria provides some fresh evidence.

In the previous paper (Granit *et al.*, 1964) a review is given of extracellular results dealing with the average effects of muscle stretch from autogenetic and antagonistic sources with especial reference to the present types of experiment.

METHODS

The experimental material used in this paper derives largely from the assembly of motoneurones analysed from other points of view in the previous paper (Granit *et al.*, 1964). Ten new experiments have been carried out. The additional cats were all anaemically narcotized, which entails tying the main carotid artery and all its branches up to and including the external and internal carotid arteries under ether anaesthesia and then giving enough pentobarbitone intravenously (in the range of 10–28 mg/kg) to keep the animal fully anaesthetized throughout the experiment. Stimulation and recording were as in the earlier experiments except that a bridge arrangement similar to that of Araki & Otani (1955) was used to stimulate the motoneurone through the recording electrode and in six experiments the ventral roots were not cut.

RESULTS

The results, which refer to ninety-three motoneurones of spike size 45–105 mV will be presented in two sections. Part I deals with a number of individual experiments chosen to illustrate the difficulties and limitations encountered in the application of our four criteria for inhibition during muscle stretch. Part II summarizes in two graphs the relation between average membrane potential and percentage effect on the post-synaptic response for fifty motoneurones subjected to conditioning by stretch.

PART I

The standard experiment to be discussed is illustrated in Fig. 1 for a popliteal motoneurone. Its post-synaptic potential was sampled at high

sensitivity on the sweep (a.c.) while at the same time membrane potential (d.c.) and stretch were recorded on the standing spot. Evaluation of the average size of the post-synaptic potential, elicited by the monosynaptic route, was mostly based on ten responses before, ten during and ten after pull on the muscle, thus taking up more film than in Fig. 1.

In Fig. 1 triceps pull caused a reduction of some 25% in the post-synaptic response. Activation noise was feeble and there was a very small shift of the average membrane potential in the depolarizing direction.

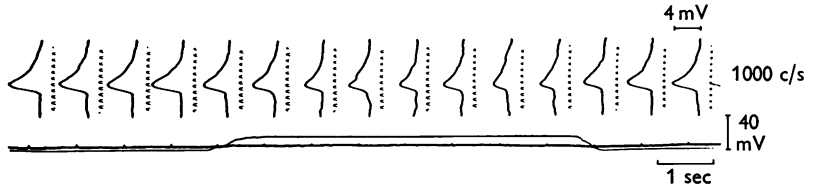

Fig. 1. Anaemically narcotized cat; pentobarbitone 10 mg/kg. Ventral roots L 7 and S 1 cut. Popliteal motoneurone (antidromic spike height over 65 mV). Popliteal post-synaptic response on sweep (a.c.) and standing (d.c.). Stretch of triceps by 500 g weight illustrated by movement of myograph. *Note.* The records on the sweep show some activation noise when EPSP diminished during triceps pull.

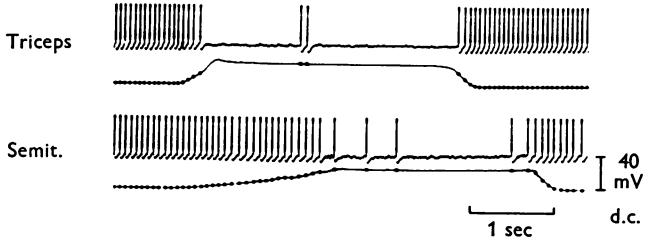

Fig. 2. Anaemically narcotized cat; pentobarbitone 15 mg/kg. Ventral roots L 7 and S 1 cut. Peroneal motoneurone (antidromic spike height over 70 mV). Motoneurone fired by injected current and discharge inhibited by muscle stretch. Weight 500 g in both cases.

During transmembrane stimulation stretch of triceps fired the neurone tonically. The diminution of the post-synaptic response was the most common effect of stretch with the autogenetic combinations: popliteal EPSP-triceps pull, peroneal EPSP-tibialis anterior pull and hamstring EPSP-semitendinosus pull. Some slight depolarization was also quite common. (The relation between size of EPSP and the average membrane potential will be dealt with in Part II.) Whenever transmembrane stimulation could be carried out, a subliminal depolarization sufficed to make the neurone fire repetitively when autogenetic stretch was added. Activation noise varied a great deal from neurone to neurone suggesting that the

tip of the micro-electrode had to be near a source of noise to record it well.

Figure 2 shows a peroneal motoneurone whose post-synaptic response had been studied during stretch of the semitendinosus (500 g). This could not be shown to be influenced (failure of criterion (i)) in spite of a slight hyperpolarization of the order of 1·5 mV during stretch (criterion (iv)). Activation noise had been feeble (criterion (iii)). We shall see in Part II that the average membrane potential may undergo small shifts which may even be contrary in direction to the effect indicated by the change in the EPSP, and so the question arose: did stretch cause excitation or inhibition or was its action too feeble to be measurable? The cell was next fired by transmembrane stimulation and both records shown in Fig. 2 begin with the repetitive activity that then ensued. The lower record refers to semitendinosus pull which finally silenced the peroneal cell in spite of the fact that hamstring and peroneal motoneurones generally have been synergistic. The true antagonist, triceps (upper record), did the same. (The effect of triceps pull on the EPSP had not been tried.) There is seen to be some hyperpolarization during the slow loading of semitendinosus. The powerful inhibitory effect of semitendinosus which was shown by criterion (ii) was not found when the cell was tested by criterion (i) suggesting that it was 'remote' with respect to the site at which the EPSP was recorded. The alternative explanation that depolarization as such revealed an otherwise non-existent inhibition would also have been applicable had not in the absence of transmembrane stimulation the inhibition already been present by criterion (iv).

During inhibition, noise was recorded (Fig. 2). When cells are depolarized by transmembrane currents there is always an increase of noise (cf. Sasaki & Oka, 1963) as well as an increased activation noise during stretch. This question will not be dealt with in the present paper.

Figure 3 illustrates a popliteal motoneurone whose post-synaptic response is shown in the records *A* during a 500 g stretch of tibialis anterior. From the 30 % reduction in the size of the post-synaptic response and the negligibly small shift of average membrane potential one might have been tempted to conclude that the inhibition was largely, if not wholly, pre-synaptic. In the records *B* the test with transmembrane stimulation at strength just above the rhythmic threshold (6 nA) is shown. This discharge was completely blocked by stretching tibialis anterior. Since recovery was incomplete, the same experiment (10 nA) was repeated somewhat later (records *C*), this time in the absence of the post-synaptic testing potential. The outcome was the same. The smaller spikes are due to less successful compensation of the depolarizing current. In both cases there was slight hyperpolarization during stretch of the antagonist muscle.

Figure 4 refers to a popliteal motoneurone (100 mV spike height) the post-synaptic potential of which had been analysed during pull on triceps, semitendinosus and tibialis anterior. The size of the control response was 6·2 mV. Triceps and semitendinosus pull reduced it to 5·4 mV, pull on

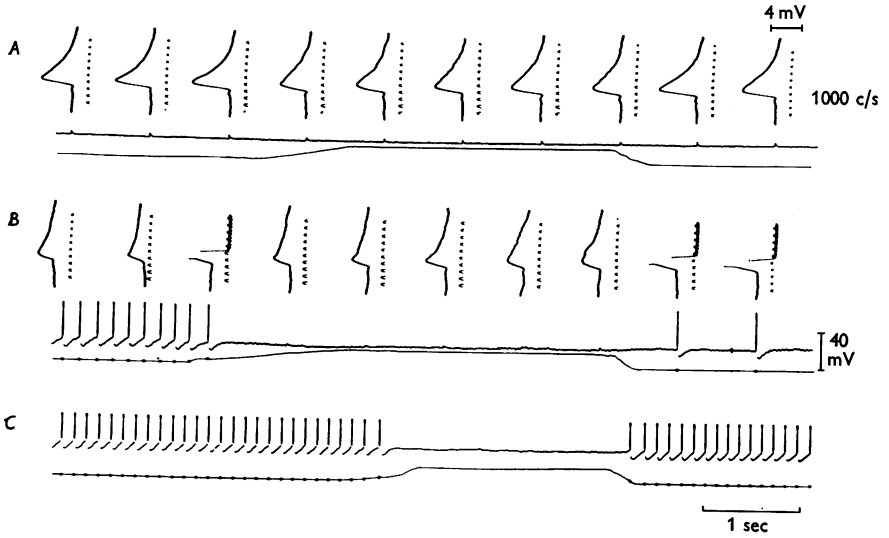

Fig. 3. Anaemically narcotized cat; pentobarbitone 10 mg/kg. Ventral roots L7 and S1 cut. Popliteal spike (65 mV spike height). *A*, on sweep (a.c.) and standing spot (d.c.), effect of stretching tibialis anterior (500 g) on popliteal post-synaptic potential. *B*, same experiment while neurone fired by injected current. *C*, repetition of *B* later on, without testing EPSP simultaneously.

tibialis anterior to only 6·1 mV which could hardly by itself be regarded as significant. Stretch of semitendinosus and tibialis anterior did not influence the average membrane potential—suggesting presynaptic inhibition—but the autogenetic triceps pull caused slight depolarization. There was good activation noise to pull on all of the three muscles, illustrated for triceps stretch in Fig. 5. Thus the synapses were engaged (criterion (iii)).

In Fig. 4 the neurone is shown firing to transmembrane stimulation. Triceps pull (autogenetic) accelerated its rate of discharge, pull on tibialis anterior elicited a strong phasic inhibition gradually petering out. Stretch of semitendinosus caused a barely detectable reduction in the firing frequency. There was some general hyperpolarization with the flexors, always a little doubtful when seen during the passage of current.

In this case one might have expected that the inhibitions during stretch of the antagonist flexors, semitendinosus and tibialis anterior, would have reflected the corresponding effects of pull on the size of the post-synaptic

response. This was considerably reduced for semitendinosus pull and hardly at all influenced by stretch of tibialis anterior which now by criterion (ii) proved to have the stronger inhibitory effect. Criterion (ii) therefore revealed an inhibition by stretch of tibialis anterior which by criteria (i) and (iv) was non-existent or, at the most, barely perceptible if the doubtful reduction by 0·1 mV of the post-synaptic response be accepted as relevant.

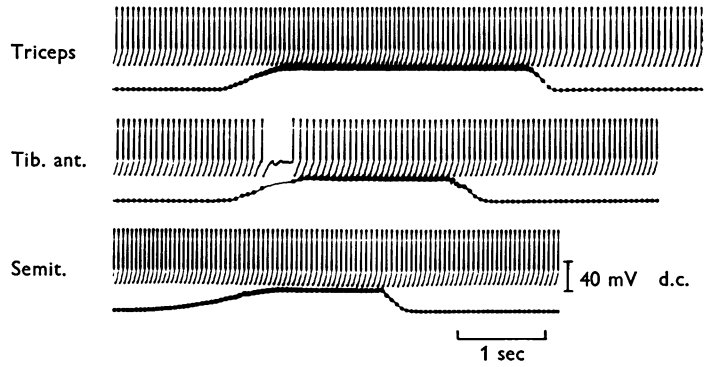

Fig. 4. Anaemically narcotized cat; pentobarbitone 20 mg/kg. Popliteal spike (100 mV spike height). Motoneurone fired by injected current. Influence on this discharge of loading muscles as indicated along records with weight of 500 g.

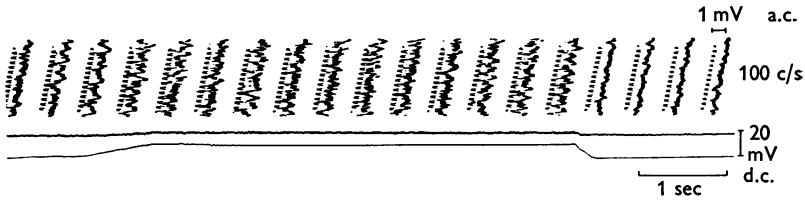

Fig. 5. Motoneurone of Fig. 4. Activation noise to pull on triceps (500 g).

Figure 6 shows a popliteal motoneurone whose post-synaptic response to autogenetic stretch of the triceps muscle underwent the characteristic diminution (to be considered in detail in Part II). However, pull on the tibialis anterior gave no effect on the popliteal EPSP. Subliminal transmembrane stimulation made the cell respond repetitively to the triceps pull by which Fig. 6 begins. While triceps stretch was being maintained throughout the record, tibialis anterior was pulled upon thrice in succession. Every time the frequency of discharge was reduced. The phasic component of the inhibition was stronger than its tonic counterpart but the latter improved when stretch was repeated.

So far the inhibitory effects demonstrated have been highly potent

against a background of firing (criterion (ii)) and small or even absent by criteria (i) and (iv). The reverse situation is illustrated by the records shown in Fig. 7 which refer to a peroneal motoneurone of 105 mV, the largest spike encountered among the 93 motoneurones that could be held long enough to make at least a few valid observations. The post-synaptic potential (EPSP) of this motoneurone was almost completely suppressed by pull on the antagonist triceps muscle. In Fig. 7 the cell is kept firing

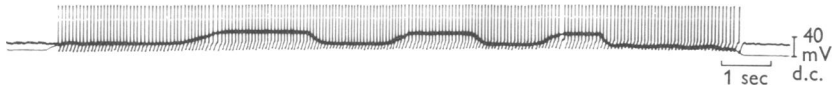

Fig. 6. Same cat as in Fig. 4. Popliteal spike (80 mV spike height). Motoneurone stimulated by injected current of subthreshold strength caused to discharge by maintained stretch of triceps (500 g). Three intercurrent stretches of tibialis anterior (400 g) shown.

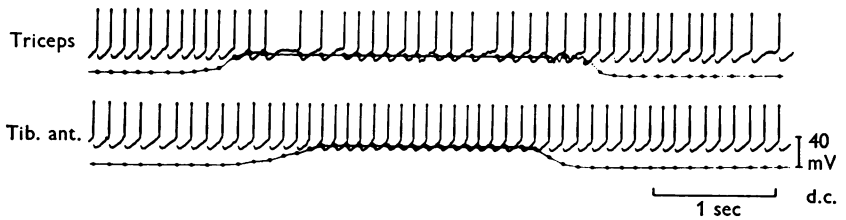

Fig. 7. Same cat as in Fig. 4. Popliteal motoneurone giving spike height of 105 mV. The cell is fired by transmembrane current. Stretch by muscles as indicated (500 g).

by transmembrane stimulation. Triceps pull reduced its rate of discharge, but very little by comparison with the strong effect that was expected from the application of criterion (i). Pull on the tibialis anterior accelerated the rate of firing of the cell. This was the expected autogenetic effect and, as usual in such cases when criterion (i) was applied, the post-synaptic potential was reduced (by 10 %) by stretching the tibialis anterior. In neither case was the average level of the membrane potential influenced by stretch. There was no activation noise. Nevertheless, the inhibition was post-synaptic.

With this peroneal neurone, stretch of tibialis anterior against a background of firing revealed the excitatory component that under such circumstances was the most common effect of autogenetic stretch, previously illustrated with popliteal motoneurones. Similar results were obtained with hamstring motoneurones: diminution of the EPSP with semitendinosus pull but acceleration of a discharge, maintained by transmembrane depolarization.

It has been pointed out that, though activation noise, when present, is a reliable index of engagement on the part of the cell membrane, it may often be mixed, both de- and hyperpolarizing, and so it may be difficult to evaluate its significance (Granit *et al.*, 1964). Absence of activation noise need mean no more than that the tip of the micro-electrode happens to be 'remote' with regard to the critical sites of activated synapses. There is definite evidence in favour of this conclusion: often stretching several muscles had a definite post-synaptic effect on the motoneurone while only one of the muscles caused strong activation noise. Thus it alone had

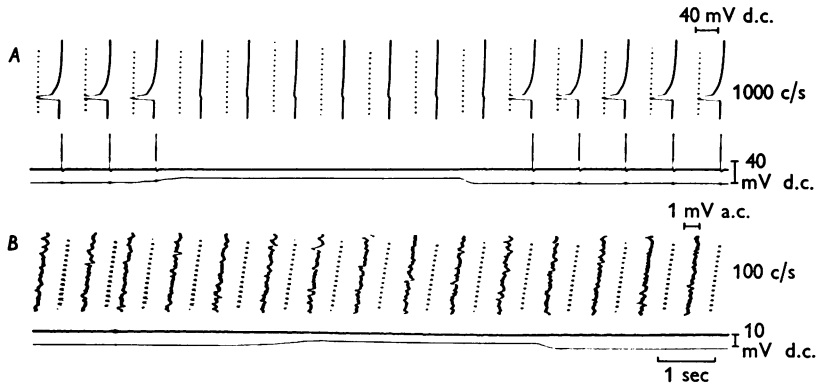

Fig. 8. Same cat as in Fig. 4. Popliteal motoneurone giving spike height of 75 mV. *A*, cell firing full spike to popliteal shock is inhibited by stretching semitendinosus (200 g). *B*, same experiment without shock to popliteal nerve to show on sweep (a.c.) that there is no activation noise to semitendinosus pull (cf. Fig. 5 for activation noise).

afferent projections on 'adjacent' synapses. In all, there were eighteen cases in which stretch, effective by some or all of the other criteria, failed to cause activation noise. These cases referred to fifteen motoneurones to eight of which transmembrane stimulation had been applied and revealed definite effects of stretch. A very striking case was a hamstring motoneurone in which pull on tibialis anterior (500 g) suppressed the full monosynaptic spike and inhibited firing to transmembrane current but had no measurable effect on the EPSP.

On the other hand, whenever activation noise was present, loss of effect of stretch on the motoneurone and general deterioration of it always went hand in hand with disappearance of activation noise. From this it is again concluded that non-existent activation noise in an otherwise responsive cell must mean that it is present but remote with respect to the tip of the micro-electrode. Strong activation noise consists of 'spiky' noise and of small wavelets of the order of 2–4 mV (Granit *et al.*, 1964). It seems

reasonable enough not to expect these wavelets to be recordable unless critical sites of activated synapses are adjacent to the electrode tip. Thus, with regard to activation noise as criterion for the presence of inhibition (or of excitation, for that matter), 'adjacent' and 'remote' effects of muscle stretch denote legitimate distinctions.

An example of a definite inhibitory effect with little activation noise and with no effect on the average membrane potential is shown in Fig. 8 (note the high sensitivity used for the samples on the sweep). This is a popliteal spike of 75 mV which during semitendinosus stretch became subthreshold (records *A*) and was replaced by its post-synaptic potential. Repeated in the records *B*, without the test response included, pull on the same muscle failed to increase the spontaneous noise sampled on the sweep. Increasing amplification and testing by the size of the EPSP gave 2·91 mV for the controls, 2·66 mV during stretch of semitendinosus. Unfortunately this neurone did not respond by maintained repetitive activity to transmembrane depolarization. If this had been a single experiment one might have been tempted to describe it as a case of presynaptic inhibition. Against our present background of experience, as developed in this and the previous paper (Granit *et al.*, 1964), it is more accurately described as a case in which it proved impossible for technical reasons to supply a crucial test of whether or not the cell membrane was engaged by the afferent barrage consequent upon stretch of the muscle.

PART II

This section presents a comparison of the effects of autogenetic and antagonistic muscle stretch on the post-synaptic potential (EPSP) and on the average membrane potential.

When popliteal, peroneal or hamstring motoneurones had been identified by their monosynaptic response, stretch—as stated—was regarded as autogenetic when triceps, tibialis anterior or semitendinosus respectively was the conditioning muscle. Within each group the individual muscles (as e.g. within the triceps group, gastrocnemius, soleus and plantaris) tend to be synergists with regard to the action of stretch impulses and thus each group represents a functional pattern. This synergism has also been demonstrated by the intracellular approach (Eccles, Eccles & Lundberg, 1957). Different motoneurones may be struck within the group but we shall see that there is an average uniformity of behaviour even though the synapses activated by autogenetic stretch represent a mixture of homo- and heterosynaptic impulses with respect to the monosynaptic response.

When flexors have been operated against extensor motoneurones or the other way round we have called the stimuli antagonistic and this, too, proved to be justified by the average effects of which several examples

already have been given in the figures. It is known (Eccles *et al.*, 1957) that a number of 'aberrant' effects occur in intracellular work on motoneurones. Synergisms which previous methods have failed to detect are also revealed by the intracellular approach. When in our experiments stretch of semitendinosus sometimes excites and sometimes inhibits a popliteal motoneurone, though inhibition alone may be the expected antagonistic effect, the excited neurone can hardly be called aberrant. The

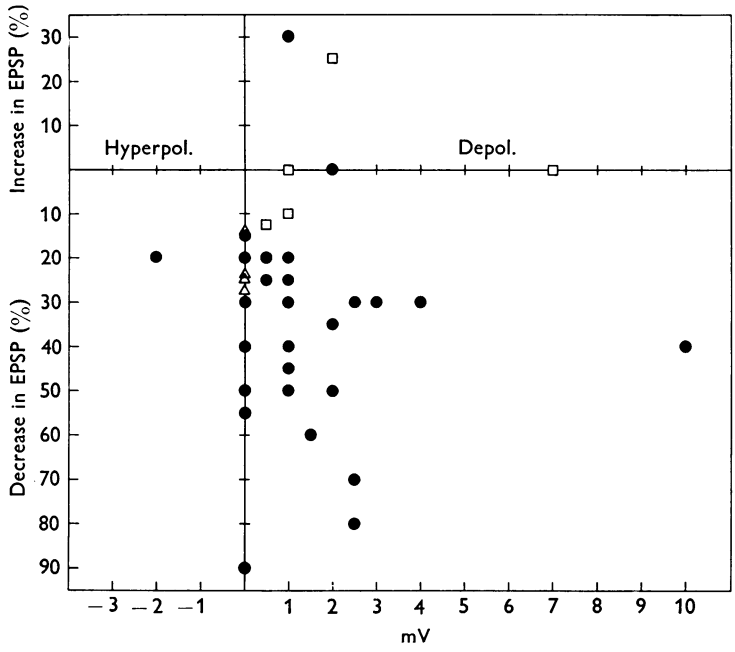

Fig. 9. Relation between percentage increase or decrease in the post-synaptic test response and the shift of average membrane potential during autogenetic stretch of triceps (circles), semitendinosus (squares) and tibialis anterior (triangles).

result merely expresses the fact that semitendinosus may co-operate functionally with triceps to fix the position of the leg (cf. Granit *et al.*, 1964). In the graphs autogenetic effects are treated separately.

Autogenetic stretch. The graph shown in Fig. 9 correlates autogenetic combination shifts of average membrane potential during stretch with the percentage change in the post-synaptic test response, increase upwards, decrease downwards. The three groups of motoneurones are separately marked.

The graph shown in Fig. 9 shows that the average effect of autogenetic impulses during muscle stretch in terms of size of the post-synaptic test response (criterion (i)) is inhibitory. Twice only has the test response in

stretch undergone an increase. The large majority of the inhibitory effects were marginal. If shock strength was increased so that a spike was elicited instead of a post-synaptic potential alone, then it was often impossible to demonstrate the autogenetic inhibition. It is seen that there is also a number of cases in which the post-synaptic test response has been very much depressed, say, by 50 % or more to take an arbitrary boundary.

A striking fact is the finding that for eleven cells there has been inhibition by criterion (i) without any definite shift of the average membrane potential by stretch (criterion (iv)). In one case only is there a hyperpolarization; all the others show no effect or some depolarization. Three cells gave depolarization to stretch without an effect on the post-synaptic potential. Especially interesting is the case in which pull on triceps caused a depolarization of 10 mV, initially with some firing. The post-synaptic test response was reduced by 38 % when taken as soon as the cell had ceased to respond by discharging spikes. These cases with large shifts of membrane potential in response to muscle stretch are rare with our type of preparation (cf. Granit *et al.*, 1964).

The number of motoneurones in which the post-synaptic potential failed to be influenced by autogenetic stretch is very much greater than is shown in the graph because in the beginning of our work most cells of this kind were rejected as uninteresting from the point of view of our problem. At the time it was held to be of greater importance to go on with the work while the muscles were in good condition and to try to locate fresh cells sensitive to stretch rather than to spend time on motoneurones which for some reason or other failed to display a reduction or an increase in the EPSP. Later on it was realized that many of them actually would respond with synaptic activation noise to stretch and some during transmembrane stimulation (introduced at a later stage of our work) and that for this reason they were of some interest.

There is no obvious correlation in Fig. 9 between average shift of membrane potential and the degree of inhibition by criterion (i). In fact, there is in one case a 90 % suppression of the test response without a shift of membrane potential. This cell is illustrated in Fig. 7. In other cells with strong inhibition by criterion (i) the shifts are seen to be small and in the depolarizing direction.

Modest depolarization is not uncommon with autogenetic stretch, it is in fact more common in the graph than absence of a change by this criterion (iv), and several of those motoneurones could actually be fired by adding transmembrane stimulation, occasionally even by stretch alone. In such cases the monosynaptic test response was studied without application of transmembrane stimulation, or else when 'natural' repetitive firing was reduced to a few initial spikes, or no more obtainable. In nearly all these cases there was a substantial reduction of the post-synaptic test response. Examples are the motoneurones in Figs. 4, 6 and 7. In one popliteal and in one hamstring neurone (shown in the graph) there was an increase of

the EPSP. It is quite clearly demonstrated in the graph that a barrage of impulses from stretch afferents whose average effect is excitatory on the motoneurone, but insufficient to fire it, causes a marked depression of the post-synaptic test response (the EPSP), often together with slight depolarization.

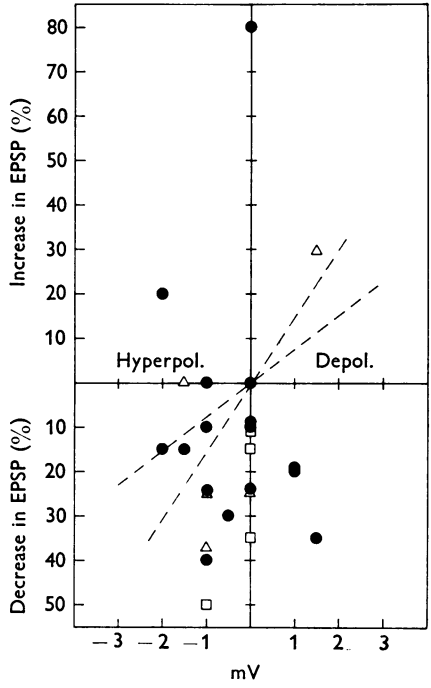

Fig. 10. Relation between percentage increase or decrease in the post-synaptic test response and the shift of average membrane potential during stretch of muscles antagonistic to popliteal (circles), hamstring (squares) and peroneal (triangles) motoneurones.

Antagonistic stretch. In the graph shown in Fig. 10 the combinations have been antagonistic and hence also wholly heterosynaptic. If now during stretch there were some quantitative relation between the average shifts of membrane potential and the size of the post-synaptic test response, one would expect the corresponding curve to follow roughly some alternative of the kind indicated by the two broken lines of different slopes. But nothing of the kind is found in the data. Slight hyperpolarizations, to be sure, are quite common in this graph but two of them are without an influence on the test response, in one it is increased. In eight there is a definite depression of the post-synaptic response. Three show inhibition with slight depolarization and of the nine cells without a shift of average membrane potential during stretch, one has a greatly increased EPSP, the

others, just as with the autogenetic combinations, fall in various places along the negative ordinates.

The profound inhibitions by criterion (i) seen with some autogenetic combinations in the graph shown in Fig. 9 do not occur here, yet, of course, whenever antagonistic stretch was applied to a repetitively firing cell the discharge with very few exceptions was reduced in frequency or wholly stopped. Examples are given in Figs. 2, 3, 4 and 6. Of the 25 motoneurones tested by a post-synaptic response, three only showed an excitatory effect by this criterion. However, antagonistic combinations were often tried without adding a test by the post-synaptic potential and as a rule the effect was an inhibition of the firing rate.

Neglecting for the moment the difference between autogenetic and antagonistic combinations, the asynchronous barrage of stretch impulses which is known to mobilize both excitatory and inhibitory synapses, as briefly summarized in our previous paper (Granit *et al.*, 1964), may be said to do something which on the whole expresses itself as a diminution in the size of a monosynaptic test response. This it may do independently of whether the average membrane potential rises, falls or fails to shift in the least during stretch. It is more than likely that when the average membrane potential shifts in the depolarizing direction, excitatory currents dominate, inhibitory currents doing the same when it shifts in the hyperpolarizing direction. The ensuing increase of conductance will serve as a general leak for the test response. This provides one likely explanation of the fact that reduction of an EPSP is the most common effect in non-firing motoneurones influenced by asynchronously-arriving impulses from stretch afferents, independently of their source of origin. In addition, with homosynaptic testing, there is direct interference by engagement of identical synapses, unpredictably complex because of the mixed input but on the whole leading to a suppression of the post-synaptic test response.

Heterosynaptic autogenetic stretch. The terms homo- and heterosynaptic with respect to monosynaptic testing were introduced by Brock, Eccles & Rall (1951) to indicate conditioning by the same or synergistic afferents respectively. Their work was concerned with the after-effect of one monosynaptic volley tested by another. Conditioning by stretch is a far more complex affair (cf. above Granit *et al.* 1964). The afferents are of mixed character, E + I, and there is a poly-synaptic component variable with type and state of the preparation used. Homo- and heterosynaptic testing may give similar or different results depending on circumstances (Granit, 1950; Granit & Ström, 1951). Granit & Job (1952) and Job (1953) compared the two methods systematically with stretch as conditioning stimulus and on the whole saw more depression of excitability in homosynaptic testing, at least initially in stretch. Comparing this test with

reflex firing (electromyography), they found that the latter method would indicate 'very little changed or even increased reflex activity at a time when the monosynaptic test indicated diminished excitability of the motoneurones' (Granit & Job, 1952, p. 419), which agrees with the findings in Part I above (criterion (ii) compared with criterion (i)).

'Central settings' of excitability are decisive for the outcome in many cases (e.g. for presence or absence of stretch reflex). In animals anaesthetized with pentobarbitone the effect of autogenetic stretch on non-firing cells tends to be depression in monosynaptic terms (Bianconi, Granit & Reis, 1964*a*, *b*). The homosynaptic pathway may be eliminated by cutting, say, the lateral gastrocnemius nerve and comparing the effects of a test shock

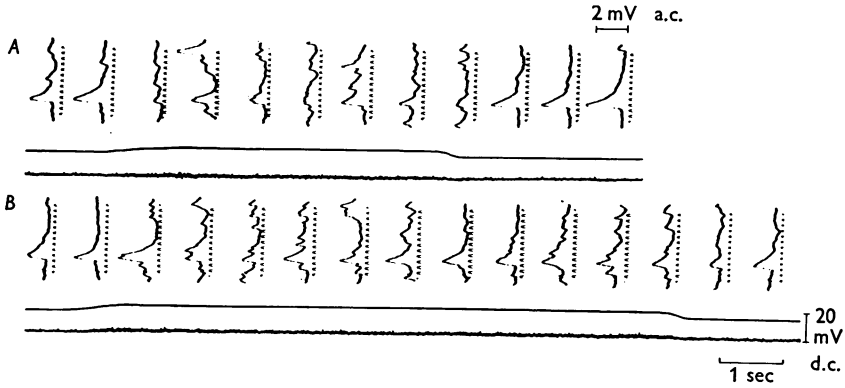

Fig. 11. Popliteal motoneurone. Comparison between homosynaptic (*A*) and heterosynaptic (*B*) EPSP during stretch of triceps (500 g). In *A* test shock from popliteal nerve minus cut lateral gastrocnemius nerve, in *B* from cut lateral gastrocnemius nerve. A great deal of activation noise but depression of EPSP definite only in *A*.

to the cut end with that of one to the whole popliteal nerve (then containing from the triceps group only the medial gastrocnemius and plantaris stretch afferents). This comparison has been carried out in the experiment of Fig. 11 making use of a fairly 'noisy' popliteal motoneurone, showing also the common slight depolarization in response to autogenetic stretch of the triceps muscle.

Against the background of heavy activation noise the EPSP from the cut lateral gastrocnemius nerve (*B*) is seen to be less influenced by stretch than the EPSP elicited from the popliteal nerve (*A*). The former afferent supply (*B*) is purely heterosynaptic, the latter corresponds to our usual mode of mixed autogenetic conditioning. The outcome of this experiment confirms the view that engagement of partly identical synapses in con-

ditioning and testing is likely to favour depression of the test response when conditioning is done by muscle stretch. Only two such experiments were performed. They gave the same result.

DISCUSSION

Clearly with muscle stretch as conditioning stimulus the most elementary question one can raise is whether its effect on the motoneurone that has been penetrated by the micro-electrode is excitatory or inhibitory. The asynchronous barrage of stretch impulses consists of excitatory and inhibitory components and 'central settings' can never be neglected when spinal reflex action is concerned. Nevertheless, we shall neglect them to begin with because the main task of the present work has been to investigate the criteria by means of which the existence of post-synaptic inhibitions can be reliably assessed, a preliminary to every functional analysis. The question of 'settings' or bias will be considered at the end.

It has been shown that of the four criteria used only one is always unequivocal, namely the reduction during muscle stretch of the frequency of a discharge produced by stimulating the cell by transmembrane currents. Vice versa, an acceleration by stretch of this discharge is, of course, an equally reliable criterion of excitation.

Part II showed that evaluation of size of the post-synaptic potential (criterion (i)) in combination with an assessment of shifts in the average membrane potential (criterion (iv)) can lead to false conclusions. A specific case of considerable interest is stretch of an antagonist across one joint resulting in a diminution of the post-synaptic response without general hyperpolarization, a combination of effects suggesting presynaptic inhibition (Eccles, Eccles & Magni, 1961; Eccles, 1964). Whenever it has been possible in such cases to apply criterion (ii), this suggestion failed to be borne out by experiment. The inhibitions proved to be post-synaptic and so it was held to be unnecessary to burden our paper on asynchronous stimulation with a superfluous hypothetical explanation, taken over from different experiments with a group of synchronous volleys. Of considerable interest is the fact that strychnine in a dose of 0·2 mg/kg does not remove the antagonistic inhibitions to stretch in firing cells, as Kellerth (1964) has found in preliminary experiments.

Formulating our general findings in a different way, we define inhibitions which have been non-existent or weak by criteria (i), (iii) or (iv) but existent and even highly potent by criterion (ii), as remote with respect to the tip of the micro-electrode. 'Remoteness' may have different explanations and we shall begin by considering the spatial alternative.

Criterion (iii) which refers to synaptic activation noise allows of an

interpretation of 'remoteness' which in this particular case has everything to commend it. The explanation was given on p. 460 and implies that the critical site at which the major synaptic events take place is not adjacent enough with respect to the tip of the micro-electrode.

In a similar manner inhibitions (criterion (i)) defined by size of the post-synaptic potential, may be remote as, e.g. for the cell shown in Fig. 2. Its post-synaptic potential was not influenced by stretch but criterion (ii) revealed strong inhibitory action. We have reasons for assuming that transmembrane currents only fire a cell when a considerable portion of its surface is depolarized (Granit, Kernell & Shortess, 1963; Granit, Kernell & Smith, 1963). This mode of firing may well sensitize the cell to inhibitory synaptic currents produced by stretch impulses reaching critical sites which are spatially remote with respect to the tip of the intracellular electrode. Depolarization increases the effects of the inhibitory currents, as suggested by the increase of inhibitory post-synaptic potentials in depolarized cells (Coombs, Eccles & Fatt, 1955). An augmentation of hyperpolarizing noise from pull on semitendinosus during maintained depolarization of a popliteal motoneurone by triceps stretch has occasionally been seen (Granit *et al.*, 1964, their Fig. 2). By this process a motoneurone subjected to an asynchronous barrage from inhibitory and/or excitatory muscular afferents would be capable of staying 'neutral' unless biased for specific functions by internuncial 'amplifiers' in the excitatory or inhibitory direction. The idea has interesting implications because it can explain the normal absence of stretch reflexes. We shall return to its functional significance at the end of this discussion.

It is not being maintained that post-synaptic inhibitions remote by criteria (i), (iii) or (iv) but revealed by criterion (ii) always have to be remote in the spatial sense. The intracellular technique has limitations other than those deriving from the restricted 'field of vision' of the electrode tip. The results are, for instance, obtained in terms of change of potential only, while current and conductance also need be considered. These technical limitations of recording can be overcome in various ways with simple structures such as peripheral nerve and even to some extent with motoneurones when problems of transmission are analysed with the aid of synchronous shocks, as shown by all the work of Eccles and his colleagues (cf. also Araki & Terzuolo, 1962). Not so, when an asynchronous barrage of mixed impulses arrives at this protean structure most of whose synapses are forming critical sites on the dendrites. The average shift of membrane potential during stretch of a muscle, as 'seen' by the micro-electrode, need not then stand in fixed relation to excitability (cf. also Granit *et al.*, 1964). Some of the possible complications in cells provided with dendrites have been discussed by Frank & Fuortes (1957), Kuffler

(1960), and more recently by Bennett (1964). Attention should also be called to a paper by Coombs *et al.*, (1955) in which it was found that inhibitory and excitatory synaptic currents may interact directly when adjacent in time and space; furthermore to observations by Hubbard & Willis (1962*a*, *b*) according to which at the motor end-plate depolarization and hyperpolarization slowly influence the output of transmitter in the opposite direction. It is hardly necessary to pursue speculation further.

The term 'remote' inhibition has been used above in a much wider sense than originally implied by Frank (1959). Since the results of Frank & Fuortes (1957) were explained by the findings of Eccles and his colleagues (Eccles *et al.*, 1961; Eccles, 1964) on presynaptic inhibition, the term was available for re-interpretation to mean inhibitions which by criteria (i), (iii) or (iv) or by a combination of (i) and (iv) are non-existent or small, yet definitely present, or even strong, when checked by criterion (ii) making use of transmembrane current to fire the cell. By our definition we also have remote excitations.

The autogenetic inhibitions differ from the antagonistic ones in that as a rule they can be demonstrated merely by criterion (i), i.e. by a diminution of the EPSP in autogenetic stretch. If the average membrane potential shows any change at all, it is generally in the depolarizing direction thereby indicating excitation and in line with this is that criterion (ii), when applicable in those cases likewise indicated autogenetic excitation. The autogenetic inhibitions to stretch have, however, their exact functional counterpart in the corresponding depressions of the monosynaptic reflex, recorded from the ventral roots in similar preparations using ankle extensors and flexors (Bianconi *et al.*, 1964*a*, *b*, with references to earlier work).

We believe the autogenetic inhibitions in stretch to be a mixture of (i) direct interference between excitatory and inhibitory activation noise, (ii) engagement of synapses making them non-responsive to a testing volley hitting the same synapses and (iii) membrane leaks through the conductances created by the impulse barrage arriving at the excitatory synapses and reducing the current flow of the constant test response (the EPSP). The relative role of the various components is likely to vary a great deal from case to case. It is of but little interest to expand this analysis to a systematic comparison between homo- and heterosynaptic testing. This has already been done with the monosynaptic ventral root response, as pointed out at the end of Part II. The principal fact, EPSP-depression together with general depolarization, is well enough illustrated by the findings reported.

From a functional standpoint our results underline the importance of the 'setting' of a motoneurone for its performance. This crucial factor

emerges in the present work as depolarizing current while applying criterion (ii) and it alone regularly makes sense of the asynchronous afferent barrage from the muscles. Doubtful inhibitions then become true inhibitions, subthreshold excitations really excite. Without criterion (ii) available one might have felt tempted to state that the effect of an asynchronous barrage of mixed stretch impulses more often than not merely is destined to die out as meaningless synaptic activation noise. Most of the motoneurones in which this happens have actually been neglected in our presentation. Nevertheless, we think of this fact as illustrating a normal and sensible mechanism for neutralizing information from the muscle which is unwanted, yet unavoidable. The living organism stands, for instance, in no need of permanent stretch reflexes. Apparently no special inhibitory process is needed for blocking these reflexes until the moment they are wanted. Various internuncial controls are operated to produce the appropriate 'settings' whenever there arises some need for making the motoneurones act. Certain built-in patterns (which we call reflexes) then go off as scheduled. We have been able to imitate such 'settings' by making the cell fire in response to outward transmembrane currents. In the normal life of the organism internuncial control systems (including supraspinal and the γ-spindle loop) must take over this role or otherwise the animal would not be capable of using its reflexes.

SUMMARY

1. Intracellular records from cat popliteal, peroneal and hamstring motoneurones have been obtained during stretch of the triceps surae semitendinosus or tibialis anterior + extensor digitorum longus muscles (extending the work of Granit, Kellerth & Williams, 1964).

2. Inhibitions during stretch were studied by four criteria: (i) drop in size of the EPSP; (ii) reduction in rate of a discharge set up by transmembrane stimulation; (iii) synaptic activation noise; (iv) increase in the average membrane potential.

3. Criterion (ii) alone was found to be non-equivocal. Inhibitions to stretch which by this criterion were highly potent, often could not be demonstrated by the other criteria or were revealed only by one or two of them.

4. When an inhibition from antagonistic muscles, characterized by criterion (ii) as a reduction in the firing rate to injected current, had been established, it was termed 'remote' with respect to criteria which had failed to reveal it. Interpretations of 'remoteness' are being discussed.

5. Autogenetic inhibitions proved to be a special case. They were detectable only by criterion (i) as a diminution in the size of the

EPSP, Criterion (ii), whenever applicable, then indicated autogenetic excitation.

6. A systematic comparison was made (graphs shown in Figs. 9 and 10) between percentage change in the size of the EPSP and the amount by which the average membrane potential was altered by stretch. There was no generally valid simple relation between the two quantities.

7. When from pull on a muscle at another joint a diminution of the EPSP occurred without accompanying general hyperpolarization thereby indicating 'presynaptic inhibition', the discharge set up by transmembrane stimulation always underwent a reduction, proving the inhibitory effect to have been largely if not wholly post-synaptic.

8. Without some definite 'bias' on the motoneurone the effects of unwanted, yet unavoidable synaptic activation noise from muscle receptors is likely to be dissipated at the cell membrane and hence also to be functionally irrelevant.

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