

## INTRACELLULAR ASPECTS OF STIMULATING MOTONEURONES BY MUSCLE STRETCH

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Eminently useful as synchronous stimulation of bared afferents has proved in the elucidation of synaptic function (see e.g. Eccles, 1964), natural stimuli still pose their own problems which perhaps only exceptionally lend themselves to a straightforward quantitative interpretation in terms of slow shifts of membrane potential to indicate excitation, E, or inhibition, I (e.g. Granit & Renkin, 1961). This is because synchronous activation is always arranged to mobilize one dominant set of synapses, E or I as the case may be, the purpose being to analyse properties of nervous transmission. Clearly, therefore, the general effects of membrane potential on ionic currents must also dominate interpretation. When, as in the present work, a muscle is pulled upon, there will often arise a barrage of impulses of opposite sign, E plus I. The precise relation between excitatory membrane current and average membrane potential may not then be predictable from the records, accepting present notions that the brief subsynaptic currents represent differential and parallel permeabilities with electromotive force delivered from ionic batteries (see e.g. Katz, 1962; Eccles, 1964; Hodgkin, 1964). Difficulties may also arise from the variable location of the synapses and the limited 'field of vision' of the micro-electrode tip.

Synaptic activation by stretch of a muscle is reflected intracellularly by the following indicators: (i) a drastic increase of synaptic noise immediately heard in the loudspeaker; (ii) a change in the amplitude, direction and duration of the wavelets making up the noise; (iii) a change in the amplitude of the monosynaptic post-synaptic potential used for testing excitability; (iv) a general rise or fall of membrane potential, negligible or large, as the case may be. It has proved important to pay attention to all these changes and the results to be presented are a first attempt to sort out the complications encountered when the ultimate aim is to study the motoneurone as an executant of commands from naturally activated

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stretch receptors rather than as a transmitter of purposely synchronized volleys. This paper will be devoted to some general observations on synaptic noise elicited by stretch, to repetitive firing and to the accompanying variations of membrane potential. A second paper (Granit, Kellerth & Williams, 1964) deals with monosynaptic testing during stretch as an index of excitation and inhibition.

#### METHODS

Forty-three cats were used in these experiments. Eleven were decerebrated and twenty-one anaesthetized with pentobarbitone 35–40 mg/kg. In the rest the main carotid artery and all its branches up to and including the external and internal carotid arteries were tied under ether, as in preparation for anaemic decerebration. The animals were then given sufficient pentobarbitone intravenously (11–28 mg/kg) to maintain adequate anaesthesia throughout the experiment. They will be briefly referred to as anaemically narcotized cats.

The left hind limb was used for all experiments. The muscles tibialis anterior, extensor digitorum longus, triceps surae and semitendinosus were freed as much as possible from surrounding structures without damaging the nerves or blood vessels supplying them; the insertions of the muscles were cut and strings tied around the tendons to enable the muscles to be stretched. Tibialis anterior and extensor digitorum longus were tied together at their tendons. Stimulating electrodes were placed around the uncut sciatic, hamstring, peroneal and popliteal nerves. The motoneurons were identified by the monosynaptic response as popliteal, hamstring and peroneal. In all but eight preparations the ventral roots L7 and S1 were cut and placed on stimulating electrodes. To prevent movement of the spinal cord when the muscles were stretched it was found necessary to clamp the dorsal spines of the thoracic vertebrae, the lumbar vertebral bodies, the iliac crests and the ankles; the greater trochanter and the lateral condyles of the femurs were screwed to supports. With this method of fixation it was found possible to apply strong pulls to the muscles without movement of the micro-electrode within the motoneurons. When the preparation of the animal was completed, it was given gallamine triethiodide (Flaxedil) 20 mg/kg intravenously and respired on a pump with its chest opened.

Micro-electrodes filled with 2 M-potassium citrate (5–15 M $\Omega$ ) were used for intracellular recording. The strings tied to the muscle tendons were run over pulleys and the muscles stretched by attaching weights to the strings. The slight downward movement of the common axle of the pulleys caused by the pull was detected by a strain gauge device and displayed on a cathode ray oscillograph.

*The input in stretch.* The known average effects of various muscle nerves and fibre sizes upon different neurone pools may not always be identical with those obtained below when selected neurones are tested by stretch but the nature of the afferent input from large and small spindle afferents and static Golgi tendon organs can be reasonably well defined (see e.g. for summaries Granit, 1955, and Barker, 1962). There is a low-threshold initial barrage for which the phasic component of the large spindle afferents is responsible. At a slightly higher threshold the spindle secondaries are statically activated. In the de-efferented animals to be used below the spindle primaries adapt rapidly to a level of firing-frequency which does not differ much for different weights applied (Matthews, 1933) and in any case, for a first survey in animals lacking spindle efferents, it is hardly feasible to begin with threshold weights. With heavier weights static Golgi tendon organs are mobilized and many of them do not adapt significantly (see e.g. Matthews, 1933). For strong activation of tendon organs contraction is necessary (Granit, 1950; Hagbarth & Naess, 1950).

There is a general consensus of opinion that the large spindle afferents in all muscles cause autogenetic excitation. Similarly the tendon organs cause autogenetic inhibition in both

extensors and flexors but these effects are facultative, depending upon the setting of the interneurons, whereas the strong monosynaptic component of the large spindle afferents makes a measure of initial autogenetic (including synergic) excitation to stretch inevitable. The spindle secondaries cause autogenetic inhibition in extensors and autogenetic excitation in the flexors. These, too, are polysynaptic and the effects therefore facultative. In sum, the average autogenetic effects in extensors are likely to be  $E + I + I$ , those in flexors  $E + E + I$ . The inhibitory impulses should dominate in maintained stretch of de-efferented extensors unless suppressed by internuncial activity which will never be very strong in anaesthetized animals. In the present experiments autogenetic effects are represented in popliteal motoneurons by triceps pull, in hamstring cells by semitendinosus pull, in peroneal cells by tibialis anterior + extensor digitorum longus pull.

Statistically speaking, with antagonistic combinations, flexors tend to be inhibitory on extensors at the same joint. The large spindle afferents in extensors are inhibitory on flexors but both tendon organs and small spindle afferents are excitatory. It is impossible here to quote the very extensive literature on which these general statements are based. Several of the most important papers will be found referred to in two recent studies by Bianconi, Granit & Reis (1964*a, b*).

The general distribution pattern of presynaptic inhibition (Eccles, Eccles & Magni, 1961; Eccles, 1964) need only be considered in relation to the actual muscles isolated. The upper flexor, semitendinosus, should give presynaptic inhibition on popliteal motoneurons and one of our problems is to find out by using a sensitive index such as synaptic noise whether or not with stretch as a stimulus it is necessary to invoke this concept.

## RESULTS

*Observations on noise.* Synaptic noise has been seen by most of the workers applying intracellular techniques to motoneurons ever since it was first noted by Brock, Coombs & Eccles (1952). Knowing as we do now from the work of Katz & Miledi (1963, with references) that the spontaneous noise in blocked motoneurons is caused by miniature potentials of the kind discovered in the motor end plate (Fatt & Katz, 1952; Katz, 1962), we shall use the term 'activation noise' for the response to stretch of muscles.

Noise caused by pulling on muscles is generally to be found in good motoneurons, provided that the muscles themselves are in good condition. When in such experiments the activation noise in response to stretch diminishes, the effect, excitatory or inhibitory as the case may be, likewise diminishes. The excitatory activation noise should be depolarizing, the inhibitory noise hyperpolarizing but natural stimulation by stretch, as we have seen, activates an assembly of mixed receptors and so it may not always be possible to identify the effect on the cell from the direction of the miniature wavelets. An instance is shown in Fig. 1.

In this experiment a popliteal motoneuron (75 mV spike height) for a brief while after penetration fired spontaneously, making it possible to demonstrate that triceps pull (500 g) accelerated the rate of firing, that tibialis anterior pull (500 g) diminished it a little and semitendinosus pull (200 g) a great deal. These tests served to characterize the input. Appar-

ently sealing of the membrane then took place and so activation noise alone was recorded during stretch as shown in Fig. 1. Now 500 g was used for semitendinosus also. With triceps pull there was more upward (depolarizing) noise of activation than with the flexors. The autogenetic effect should be  $E + I + I$ . The antagonists, especially semitendinosus, produced more downward noise. Their effect should be inhibitory. Of especial interest is that semitendinosus pull gave enough downwards noise to support our conclusion that its effect on the popliteal motoneurone was post-synaptic. Both flexors gave a slight shift of base line in the hyperpolarizing direction (2–4 mV) which likewise indicated that the effect was post-synaptic. With triceps pull there was a similar shift of base line in the depolarizing direction.

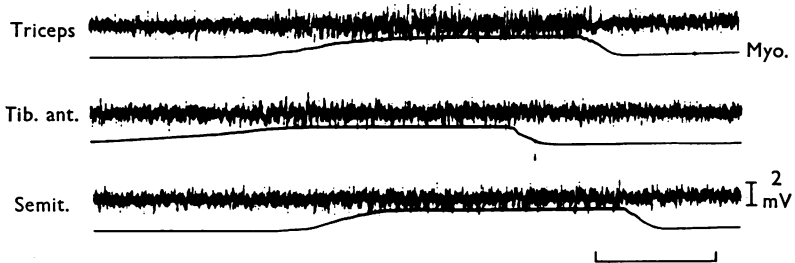


Fig. 1. Cat anaesthetized with pentobarbitone. a.c.-recording of activation noise in popliteal motoneurone (75 mV). *Myo.*, myograph record of stretch of triceps (autogenetic), tibialis anterior and semitendinosus (antagonistic). Weight of 500 g in all cases. d.c. controls showed that the cell membrane was depolarized by about 2 mV with triceps pull and hyperpolarized by much the same amount during pull on the flexors. Time marker 1 sec, in this and subsequent figures. See text.

In this case semitendinosus had the expected antagonistic effect on a popliteal motoneurone but we have had several cases of synergism between the two (see below) as seems reasonable enough when one considers that semitendinosus inserts on the tibia and may be used for fixation of knee position in extension. Yet, whenever semitendinosus exerted inhibitory effects on popliteal motoneurones, clear indications of post-synaptic behaviour were present and so it has never been necessary to invoke pre-synaptic events, invisible to the micro-electrode, in order to explain our findings. When nothing is seen to alter in the general level of membrane potential during pull on a muscle (Fig. 2), the hyperpolarizing noise is particularly useful as an index of subliminal activity. When such noise is present, the notion that an inhibition is presynaptic, if the general level of potential is uninfluenced by it, cannot be defended.

It should be mentioned in this connexion that we have had inhibitory effects, as judged by monosynaptic testing, without much activation noise. In such cases it has been possible

by firing the motoneurone from the micro-electrode to differentiate between pre- and post-synaptic effects and these will be dealt with in our second paper (Granit *et al.* 1964). Suffice it here to state that inhibition of popliteal motoneurons by semitendinosus pull may be post-synaptic despite lack of noise, signifying that the micro-electrode tip occasionally may be too far away from the source of noise to record it. Apparently, therefore, the miniature potentials need not always be conducted to the electrode tip.

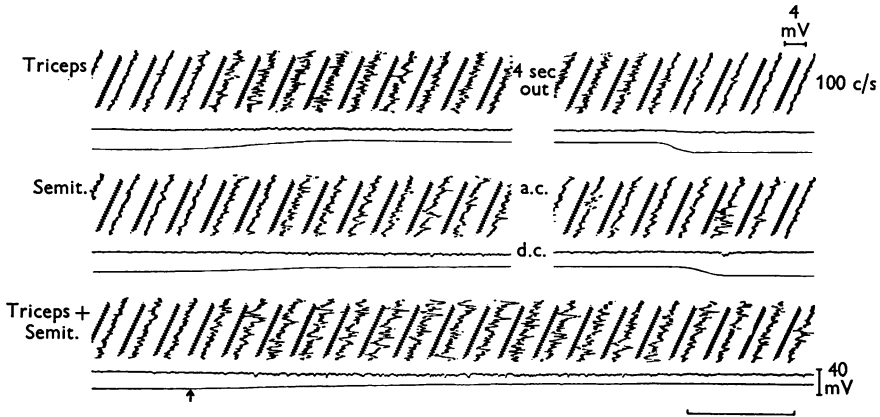


Fig. 2. Anaemically narcotized cat. Pentobarbitone 28 mg/kg. Popliteal motoneurone of about 75 mV spike height, membrane potential 65 mV. Activation noise recorded on sweep (a.c.) and standing spot (d.c.). The upper and middle records are controls with triceps (autogenetic) and semitendinosus (antagonistic). The lowermost record begins with the end of about 4 sec pull on triceps maintained throughout. At the arrow semitendinosus pull begins. Weight of 500 g used. *Note.* In this interference record hyperpolarizing activation noise of semitendinosus is more prominent than in the control. *Note* also 'spiky noise' to triceps pull as well as wavelets.

For marked activation noise one must count with an element of chance even when the muscles to all appearance are in excellent condition. We have, however, seen prominent activation noise in motoneurons which fired monosynaptic spikes only after post-tetanic potentiation, suggesting that the animal had been too deeply anaesthetized.

Inhibitory or hyperpolarizing noise was often very strong. This point is of some interest because apparently Katz & Miledi (1963) had some difficulties in finding it in their excised cords of the frog and, in the cat, Kolmodin & Skoglund (1958) state that hyperpolarizing miniatures were 'found only occasionally'. In our experiments with semitendinosus pull influencing popliteal motoneurons hyperpolarizing activation noise has been quite common. An example is shown in Fig. 2. This is a popliteal motoneurone which initially fired to gastrocnemius pull (Fig. 9C) and then was inhibited by semitendinosus pull. Noise was displayed on the sweep (a.c.) and level of potential on the standing spot (d.c.). The autogenetic noise to triceps pull is mixed but largely excitatory, the semitendinosus

pull is predominantly inhibitory (downwards). The direction of the noise is well seen also in the d.c. records. There is an inhibitory rebound of hyperpolarizing noise at cessation of semitendinosus pull. In the lower-most record triceps pull was maintained throughout and the portion

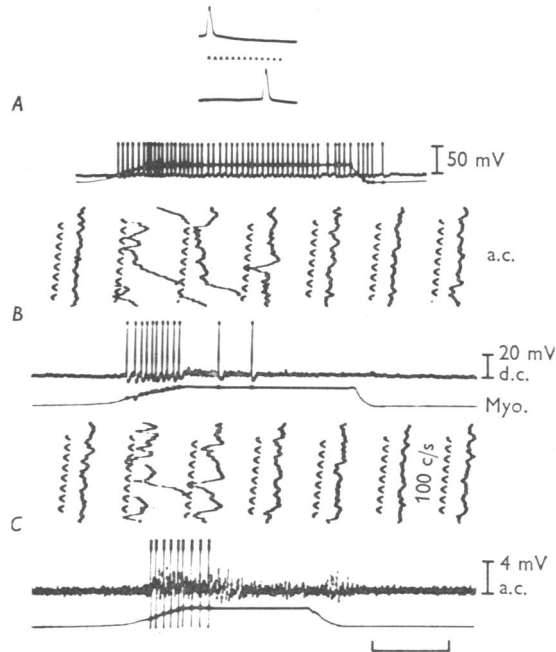


Fig. 3. Cat anaesthetized with pentobarbitone. Popliteal motoneurone which stayed at a spike height of 60 mV for a considerable time with same overshoot. It also fired to pull in tibialis anterior (insufficient mechanical isolation?), used in these records. Weight 500 g. Firing, initially tonic (A), then phasic (B, C). Activation noise (a.c.) recorded on sweep and also (d.c.) in B on standing spot at sensitivity cutting out upper part of spikes. Note. Off-discharge of activation noise in C and just visible in B also. Uppermost records, samples on sweep and time msec.

illustrated begins at the point at which 4 sec were cut out from the uppermost record. At the arrow semitendinosus was slowly stretched, and, against the background of permanent triceps noise, the hyperpolarizing wavelets became larger and more frequent than in the control of semitendinosus pull by itself. This is not always the case. Interference between de- and hyperpolarizing activation noise introduces complex problems which fall outside the scope of the present paper. It should be noted that in spite of considerable synaptic activation the membrane potential which was in excess of 60 mV did not change significantly with triceps pull but increased a little when semitendinosus pull was added.

The activation noise is sometimes more, sometimes less synchronized into wavelets; sometimes it consists of small, very brief spikes of the kind seen before triceps pull begins in the uppermost record of Fig. 2. 'Spiky' activation noise varies but little in size and may be hyper- or depolarizing. It is clear that if depolarizing wavelets are to indicate firing, they must have appreciable duration or else be excessively large (cf. strength-duration curve of motoneurons, Frank & Fuortes, 1956; Coombs, Curtis & Eccles, 1959). 'Spiky noise' has not been seen with firing cells. This kind of noise has been both hyper- and depolarizing in character and may well represent the nearest approximation to unit miniature potentials. It has also been observed with definitely polysynaptic reflexes.

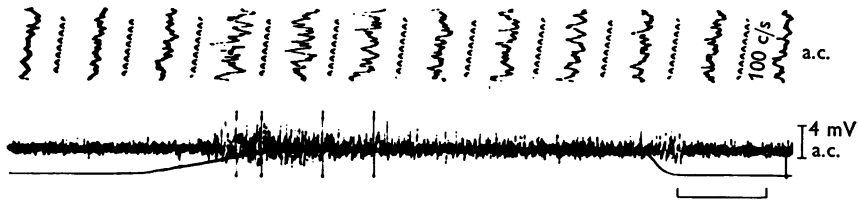


Fig. 4. Same motoneurone as in Fig. 3 responding to 1000 g pull on triceps. Again off-discharge of activation noise.

Unless the motoneurone is hyperpolarized, firing by depolarizing wavelets of activation noise is to be expected. A case in point is shown in Fig. 3 which also is of interest because the antagonists triceps and tibialis anterior were synergistic, the only case of its kind. Whether this was an aberration or due to insufficient mechanical isolation is impossible to tell. Soon after penetration this popliteal motoneurone responded repetitively to pull on the tibialis anterior (Fig. 3, *A*). In records *B* and *C* it gave a phasic response only. There is hardly any base line shift but large depolarizing wavelets occur and these must be the expression of an activity responsible for the firing. The off-noise should be noted. The response to triceps pull of this motoneurone is illustrated in Fig. 4. Again there are large depolarizing wavelets which indicate currents of long enough duration to fire a spike. An off-noise arose with triceps pull also. With both muscle groups it was in the depolarizing direction. Off-noise is seen every now and then but not with all motoneurons. So far we have had only one case of hyperpolarizing off-noise (see Fig. 2, after semitendinosus pull).

*Shift of membrane potential by stretch.* The prevalent view based largely on synchronous shocks as stimuli is that shifts of membrane potential, the so-called post-synaptic excitatory and inhibitory potentials, are likely to be the best indicators of post-synaptic excitation and inhibition respectively as, no doubt, they sometimes are. This led us to expect that

tonic firing and inhibition of a tonic discharge would illustrate unequivocally the validity of these indicators. We have already seen that the development of excitation and inhibition by means of activation noise suggests complications by interference of currents which as yet cannot be fully understood. There is clearly a repetitive firing to stretch which is based largely on synchronized wavelets of activation noise (Figs. 3-4) against an almost steady average membrane potential. It is not known whether the synchronizing factor resides in the discharging sense organs,

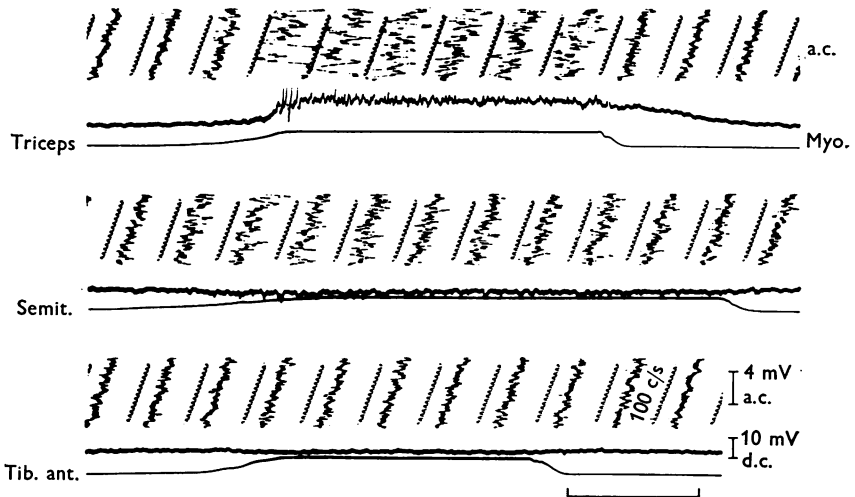


Fig. 5. Cat anaesthetized with pentobarbitone. Popliteal motoneurone (70 mV spike height). Activation noise (a.c.) and shift of membrane potential during stretch (d.c.), as marked. Weight 500 g. See text.

in a strategic disposition of synapses or in an internuncial control such as e.g. the pyramidal rhythm of Adrian & Moruzzi (1939). It will now be shown that large post-synaptic shifts of potential in response to stretch are likely to require internuncial triggers to appear at all.

We have actually had a number of cases in which the motoneurones responded with large depolarizations up to and in excess of 10 mV. An instance is the popliteal cell illustrated in Fig. 5. Some time before the records were taken the interneurones had been stirred up by a strong tetanus at 500/sec to the popliteal nerve. The autogenetic effect of triceps pull (500 g) was a large depolarization of the order of 14 mV and a few spikes at the onset of pull. For a while this response was repeatable. The noise was in the depolarizing direction. Pull on the semitendinosus (Fig. 5) caused a hyperpolarization of the order of a few millivolts with noise in the hyperpolarizing direction. The same effect on the general level of

potential was obtained with pull on the tibialis anterior. Amplification was next increased to give a clearer picture of the activation noise. Figure 6 emphasizes the characteristic difference between the effects of triceps and semitendinosus, the latter record being a good example of practically

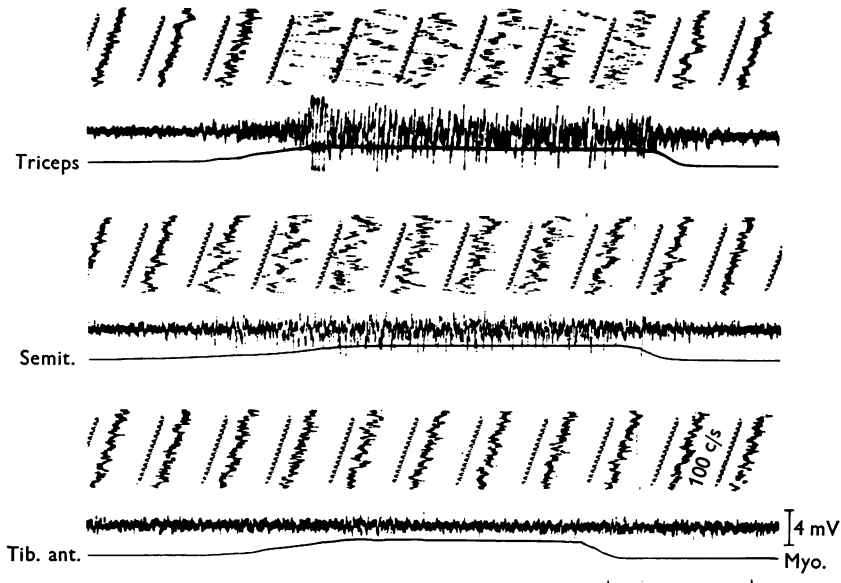


Fig. 6. Same motoneurone as in Fig. 5 of which this is a repetition with a.c.-recording throughout.

pure inhibitory or hyperpolarizing noise. Stretch of the tibialis anterior also caused inhibitory activation noise but much less of it than was elicited by semitendinosus pull. Since for the two flexors the general change in the level of membrane potential (Fig. 5) was of the same order, it is likely that the synapses responsible for noise and those responsible for the setting of the membrane potential were different, at least with respect to their location on the motoneurone. There was no off-effect proper after triceps pull (Fig. 6) but the noise outlasted stimulation and had hyperpolarizing components.

Now this same motoneurone had also been studied before tetanization of the popliteal nerve. It then responded in a different manner. As shown in Fig. 7, the triceps pull caused an inhibition with a considerable hyperpolarization instead of the large depolarization in Fig. 5. Semitendinosus pull had the same effect as in Fig. 5 but stretch of the tibialis anterior elicited a large depolarization with some firing. This motoneurone thus had two modes of functioning implying complete reversal between the effects

of triceps and tibialis anterior. The significance of this finding is clearly that some internuncial switch altering bias was responsible for the reversal and for the general effect on the membrane potential.

It has not proved possible to relate this internuncially triggered 'generator potential' definitely to the basic level of membrane potential or the size of the spike, as will be shown by a number of records sampling

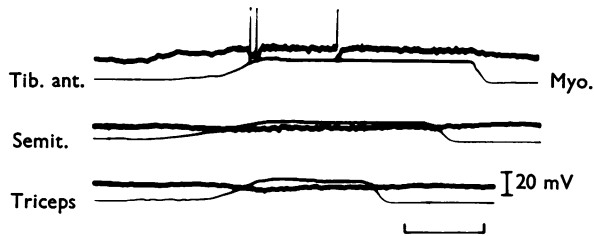


Fig. 7. Same motoneurone as in Fig. 5. Stretch as marked. Weight 500 g. See text.

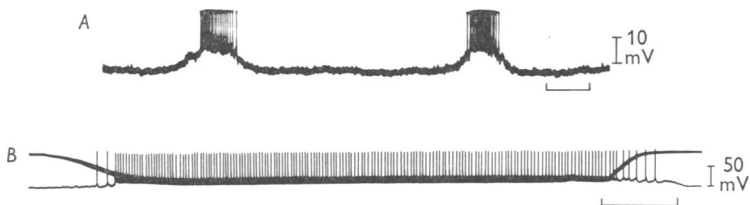


Fig. 8. *A*. Anaemically narcotized cat. Pentobarbitone 11 mg/kg. Popliteal motoneurone of about 30 mV spike height firing to spontaneously recurring post-synaptic potentials. Spikes cut off. *B*. Decerebrate cat. Popliteal motoneurone (95 mV spike height) firing to triceps pull by 500 g weight. Shift of membrane potential is around 17 mV. Spikes retouched.

repetitive responses. Thus the record of Fig. 8*A* is from an apparently highly depolarized popliteal motoneurone of only some 30 mV spike height which spontaneously delivered regularly recurring and large swings of depolarizing potential with a high-frequency discharge on top of them, behaving in this respect like the respiratory motoneurons of R. Eccles, Sears & Shealy (1962), as confirmed by Gill & Kuno (1963). The record (Fig. 8*B* below it) is from a decerebrate cat and shows a popliteal motoneurone triggered by triceps pull to a membrane shift of the order of 17 mV (exceptionally the myograph registers pull downwards). Its membrane potential was not measured but the size of the spike suggests that it was high. The initial rate of discharge was 28/sec and the cell fired with but small after-hyperpolarizations separating the individual spikes.

The three motoneurons of Fig. 9 had average membrane potentials of the order of 50, 55 and 65 mV respectively. *A* is a popliteal moto-

neurone tested by its monosynaptic EPSP and fired by triceps pull from a steady level of some 10 mV. It was recorded at high sensitivity to show noise. The hamstring neurone *B* was 'noisy' and fired an occasional spontaneous spike. Hence it must have been near its discharge level. Repetitive firing to semitendinosus pull took place without a potential of the 'generator' type. *C*, finally, was tested antidromically at regular

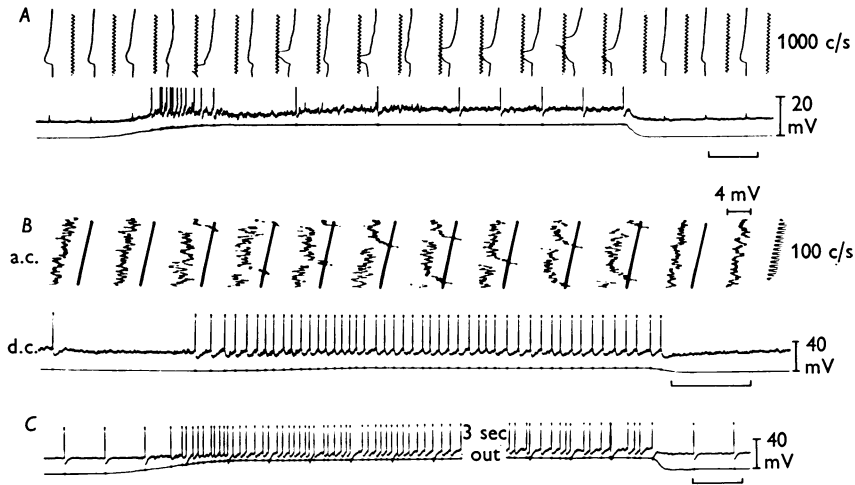


Fig. 9. *A*. Anaemically narcotized cat. Pentobarbitone 28 mg/kg. Popliteal motoneurone (85 mV spike height) tested by monosynaptic EPSP before and during pull on triceps by weight of 500 g. Membrane potential of the order of 50 mV spike height. Spikes cut off. *B*. Similar preparation. Pentobarbitone 11 mg/kg. Hamstring motoneurone (70 mV spike height), membrane potential of the order of 55 mV. Cell fires an occasional spontaneous spike and the repetitive discharge is to 200 g pull on semitendinosus. Activation noise on sweep circuit (a.c.). *C*. Same animal as in *A*. Popliteal motoneurone (75 mV spike height) tested by popliteal shock at regular intervals. Spikes cut off. Membrane potential around 65 mV. Pull on triceps. Weight 500 g. Activation noise of this motoneurone shown in Fig. 2.

intervals and responded repetitively to triceps pull without an appreciable shift of level of membrane potential but autogenetic activation noise (illustrated in Fig. 2) was present.

Figure 10 shows one of the popliteal motoneurons (85 mV spike height) on which semitendinosus and triceps acted as synergists. It had a membrane potential of the order of 70 mV. A shock to the popliteal nerve was elicited at regular intervals. To semitendinosus pull of 200 g the cell started firing with no shift of base line, but suddenly, as if some trigger had been released, the firing rate rose to about 60/sec and the base line shifted with an amount corresponding to the size of the after-hyperpolarization which

at that rate vanished. To triceps pull of 200 g it responded without an appreciable shift of base line.

From the recent work with intracellular stimulation (Granit, Kernell & Shortess, 1963) we know that tonic firing is firing with after-hyperpolarization and always takes place at a definite current strength. When an increased rate of discharge is caused by increased trans-membrane

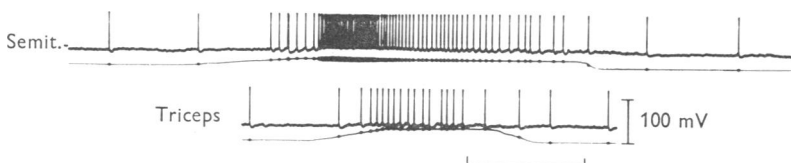


Fig. 10. Anaemically narcotized cat. Pentobarbitone 11 mg/kg. Popliteal motoneurone on which semitendinosus acts synergistically. Membrane potential around 70 mV. Weight 200 g in both cases shown on the lower myograph trace. Running monosynaptic test of spike. *Note.* Sudden increase of firing rate with rise of base line and diminution of after-hyperpolarization with semitendinosus pull.

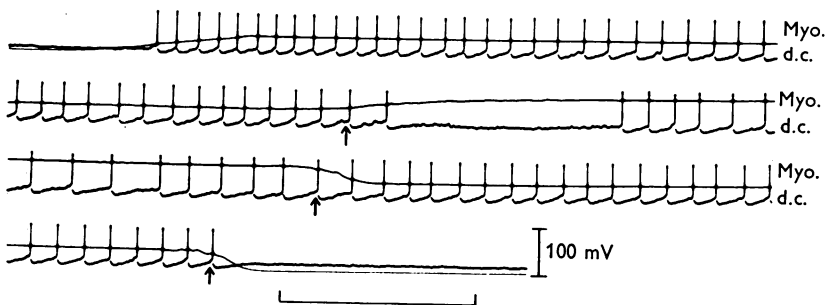


Fig. 11. Anaemically narcotized cat. Pentobarbitone 11 mg/kg. Popliteal motoneurone (75 mV spike height). Membrane potential around 65 mV. Pull on triceps (500 g) maintained till last arrow; between first and second arrow pull on tibialis anterior (400 g). *Note.* Excitatory and inhibitory stretch reflexes of the kind shown were present for a long time in this cell. Semitendinosus inhibition was more powerful than the tibialis anterior inhibition illustrated, otherwise similar.

currents, this merely implies that the rate of rise of depolarization is faster thereby counteracting the negative feed-backs which in the records appear as after-hyperpolarization. In order to imitate this mode of firing by a stretch reflex, the miniature synaptic currents must be frequent enough over a large enough area of the membrane to produce by temporal and spatial summation the sum total needed, but evidently great changes of the average membrane potential are not required. The typical result is well illustrated by Fig. 11 in which during triceps pull a firing popliteal motoneurone is inhibited by pull on the tibialis anterior. The

excitatory portion of the rhythmic response cannot be distinguished from records obtained by Granit *et al.* (1963) of maintained intracellular stimulation nor from the one published by Granit, Phillips, Skoglund & Steg (1957) of a soleus motoneurone reacting to soleus stretch. The inhibitory shift of base line during pull on tibialis anterior is very small; characteristically there is a slow rise (depolarization) before a slight fall (hyperpolarization) sets in. We have several records of antagonist inhibition of stretch reflexes which are practically identical with that of Fig. 11.

#### DISCUSSION

The concepts of Katz and his co-workers (Fatt & Katz, 1952; Katz, 1962), derived from a study of the miniature end-plate potentials of amphibian muscles, generally accepted though they be, have not been much in the foreground in work on motoneurons. This is because the intracellular work developed in a natural way from monosynaptic and antidromic testing which provided a solid background of reflexological facts (cf. Eccles, 1957, 1964). The asynchronous barrage of mixed impulses, elicited by muscle stretch, is a more complex stimulus and here the activation noise may be the sole indication of the E and I currents. These interfere in a complex manner not necessarily leading to a definite change of average membrane potential during stretch. We shall see more of the difficulties in equating changes of potential with changes of excitability in our second paper dealing with monosynaptic testing (Granit *et al.*, 1964). Enough has been presented, however, to substantiate our conclusion that with natural stimuli the activation noise provides us with valuable clues as to whether and to what an extent the cell membrane has been subliminally engaged.

In the present work we have found that motoneurons respond to stretch in the following ways: (i) by autogenetic activation noise alone, de- or hyperpolarizing, but more often than not both processes going on simultaneously and apparently interfering in such a manner as to prevent changes of membrane potential from taking place; (ii) with large wavelets of autogenetic depolarizing noise accompanied by some firing but without significant changes in the general level of depolarization during stretch; (iii) with prominent wavelets of hyperpolarizing noise signifying post-synaptic inhibition and more often than not accompanied by modest shifts of base line in the same direction; (iv) with activation noise and large changes of base line in the depolarizing direction (type of response reminiscent of generator potential of sense organs) with or without firing, the discharge when present often being at a high frequency and with little after-hyperpolarization following individual spikes; (v) the typical organ-

ized firing of the autogenetic stretch reflex which takes place at slow rates, each spike followed by a large after-hyperpolarization.

The post-synaptic signs of inhibition and excitation, independently of whether they have consisted of activation noise alone, such noise plus changes of membrane potential, or chiefly of changes of membrane potential, have in general been in accordance with what one is entitled to expect from the known effects of the muscle receptors in autogenetic and antagonistic action.

(i) The autogenetic activation noise of type E + I is often difficult to interpret and we shall have more to say about it in our second paper dealing with monosynaptic testing (Granit *et al.*, 1964). Unless largely unidirectional it may be no more than the random effect of meaningless stimulation, meaningless because in the absence of changes in excitability, determined by other parts of the central nervous system, which control the sign and frequency of the miniature synaptic currents, the motoneurone is not able to act on the E + I interference picture presented to it.

(ii) This already represents a stage of excitation that could lead to action. The motoneurone responds with spikes when the activation wavelets are large. The level of membrane potential is not significantly influenced but a certain amount of basic depolarization is likely to be required. This kind of response has been more commonly found in flexor motoneurons. It is assumed that for the large wavelets of depolarization some synchronizing process may be required; possibly it is derived from the upper level of the neuraxis. This mode of functioning may well represent a degree of freedom available for normal control. It is a common type of response to stretch among our motoneurons.

(iii) When the activation noise is characterized by prominent hyperpolarizing wavelets, there is more often than not a corresponding, small hyperpolarizing shift of base line, suggesting that the underlying conductance changes have released inhibitory currents. This is the post-synaptic inhibition by stretch of muscles, antagonistic to the one represented by the motoneurone isolated. It is thus definitely an organized response in the sense that it illustrates a sensible command obeyed by the motoneurone. The presynaptic inhibition expected from semitendinosus pull upon triceps motoneurons has never been observed (cf. above p. 438). Our next paper (Granit *et al.*, 1964) will deal fully with the problems of inhibition by stretch.

(iv) In some cases it has been possible to prove that the large shifts in the depolarizing direction looking like generator potentials derive from internuncial cells capable of producing the strong excitatory currents needed to fire the cell repetitively. When under such circumstances high

rates of discharge are obtained, the cell fires with little or no signs of after-hyperpolarization. Interpretation of this mode of firing will be considered below.

(v) This case represents the typical stretch reflex, so well imitated by transmembrane stimulation: slow regular firing, each spike being succeeded by a large after-hyperpolarization. A fairly extensive area of the cell must then have been stimulated by a unidirectional depolarizing current from 6 to 10 nA upwards which is what the rhythmic threshold requires (Granit *et al.*, 1963). It is a well-organized response to autogenetic stretch, the motoneurone making use of the feed-back mechanisms consisting of a basic membrane response supported by recurrent inhibition together recordable as after-hyperpolarization. Though a well-organized mode of functioning, it is not necessarily an automatic response to stretch because the motoneurone requires a background of excitation derived from other centres for it. This may be artificially produced by decerebration or apparently also in light anaesthesia if the carotid supply to the vessels of the brain has been carefully closed (anaemically narcotized cats). For a recent discussion of these central settings, see e.g. Holmqvist & Lundberg (1959, 1961).

Transmembrane stimulation has shown that firing frequency is proportional to current intensity (Granit *et al.*, 1963) within a range of current strengths of around 3 to maximally 4 times the value required for the rhythmic threshold. Firing will therefore always take place when a constant threshold intensity of current has been reached and its frequency will depend upon current strength. The findings enumerated under (iv) show that this mode of firing also may occur as a consequence of stretch which then has mobilized an internuncial apparatus supplying the necessary intensity of current. As a matter of fact spinal internuncial cells firing at high rates to single shocks are well known (see e.g. Eccles, Eccles & Lundberg, 1960, with references; Lebedev, 1963). Granit & Kernell (unpublished) have studied such interneurons in cats by transmembrane currents and found their slope constants 10–20 times larger than those of tonically firing motoneurons. Thus they could serve as amplifiers discharging at very high rates on slight provocation. It is assumed that such cells are capable of acting as triggers supplying by summation of their post-synaptic effects the amount of current, which motoneurons will need in order to be able to fire at fast rates. As a matter of fact many of the interneurons encountered by an advancing micro-electrode, aimed for the motoneurons, discharge high-frequency bursts to stretch. Kolmodin (1957) found 90% of his interneurons in the lumbar region stretch-sensitive.

In their study of pyramidal control of forelimb motoneurons in the

baboon, Phillips and his co-workers (Landgren, Phillips & Porter, 1962) have shown that tetani from the large muscular afferents fail to fuse and thereby do not raise the depolarizing potential to a steady level, whereas similar monosynaptic activation by the pyramidal route is capable of building up remarkably large steady levels of depolarization (cf. also Corazza, Fadiga & Parmeggiani, 1963), larger in some motoneurons than in others. To muscular Ia afferents the forelimb and hind-limb motoneurons behave in the same manner, the latter having been studied with repetitive stimuli by Curtis & Eccles (1960). Respiratory motoneurons, however, discharge at high rates on top of large slow waves of depolarization (R. Eccles, Sears & Shealy, 1962; Gill & Kuno, 1963). In the cells of the ventral spinocerebellar tract, the post-synaptic excitatory potentials increase during repetitive stimulation of Ib afferents (Eccles, Hubbard & Oscarsson, 1961). Again, with recurrent stimulation, very small changes of potential occur with large changes of excitability (Wilson & Burgess, 1962).

The possibility of special synapses with large reserves of transmitter substance has been mentioned (Eccles *et al.*, 1961) as a possible explanation of the large depolarizing potentials we have been discussing. Our explanation that such responses are engendered by special 'amplifier' interneurons firing at fast frequencies to slight depolarization is offered as a more likely alternative, at least in the case of motoneurons. The prevailing emphasis on changes of membrane potential derives in a natural manner from the use of synchronous stimuli and the micro-electrode technique itself which fails to display the basic fact that motoneurons discharge when a depolarizing current has reached threshold value at some firing site. The shifts of potential emerge as the products of unknown currents and conductances and hence need not accurately reflect the asynchronous current fluxes across the membrane of a motoneuron, one small portion of which is 'viewed' by the tip of the micro-electrode.

#### SUMMARY

1. Intracellular observations have been made on popliteal, hamstring and peroneal motoneurons during slow stretch of the *triceps surae*, of the *tibialis anterior*, tied up together with the *extensor digitorum longus*, and of the *semitendinosus* muscles. Weights have been used to stretch the muscles.

2. This paper concentrates on activation noise caused by the muscle afferents and on the various stretch reflexes and their relation to accompanying shifts of membrane potential.

3. Activation noise of autogenetic (synergistic) muscles was generally

mixed, hyper- and depolarizing miniature potentials interfering. Antagonist muscles produced activation noise which was largely hyperpolarizing in character.

4. Off-noise after release of weight was observed from time to time, both de- and hyperpolarizing in direction.

5. Diminution of noise during intracellular recording was always a sign of deterioration of the cell and was regularly accompanied by diminution of the excitatory (or inhibitory) effect on the motoneurone.

6. Repetitive firing to stretch occurred in motoneurons in which the depolarizing activation noise was characterized by synchronized wavelets. Under the circumstances there may then be little if any general depolarization produced by the muscle afferents.

7. Inhibitory effects on a cell were fairly regularly accompanied by slight general hyperpolarization of the cell membrane.

8. Presynaptic inhibition was not observed although pull on semitendinosus with popliteal motoneurons isolated should have provided a favourable condition for it.

9. Large post-synaptic (generator) potentials with or without repetitive firing could sometimes be elicited by stretch and the evidence obtained shows them to require specific internuncial activity.

10. The typical organized tonic stretch reflex in extensor cells requires only a small shift of average membrane potential, its inhibition a likewise slight shift in the opposite direction.

11. The discussion is chiefly devoted to the various types of synergistic and antagonistic activation initiated by muscle receptors, their functional significance and relation to results obtained by stimulating motoneurons from the tip of the micro-electrode.

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