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Tonic and Phasic Ventral Horn Cells Differentiated by Post-Tetanic Potentiation in Cat Extensors.

By

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Recently it was shown (GRANIT 1956 a) that, after tetanization of intact muscular afferents in decerebrate cats, the single-fibre stretch reflexes activated through the same afferents were potentiated. Instead of one or a few reflex spikes from the isolated motoneurones, there now followed a whole series of them, sometimes for as long as stretch was maintained. A state of spasticity — by definition an exaggerated stretch reflex — was thus created by tetanization, and further tests showed that a sequence of natural or adequate impulses had the same potentiating effect, provided that its frequency and duration were great enough for this purpose.

Now, since the gamma system by its activity causes the muscle spindles to discharge long-lasting series of impulses at high frequency, the motor neurones can be "labelled" by such spindle impulses and singled out for spastic behaviour or moderate facilitation, as the case may be. These facts raise a number of problems one of which will be dealt with in this paper.

When ventral horn cells have been made 'spastic' in this fashion, will they all behave in the same way or will some of them fail to respond iteratively? It was clear from the earlier experiments (GRANIT 1956 a) that potentiated motoneurones differed a great deal in their responses to stretch but more data seemed necessary for settling this question unequivocally. Therefore it was decided to explore in a preliminary survey the behaviour of 100 potentiated ventral horn cells (in ten animals) in order to establish whether fundamental or accidental differences accounted for the observations made. It is, of course, well known that in the non-potentiated state individual motoneurones respond very differently to stretch (GRANIT and STRÖM 1951, confirmed *e. g.* by ALVORD and FUORTES 1953).

This question may be restated in the following way: are the potentiated motoneurones which subserve long-lasting 'tonic' or postural motor functions (as determined by their stretch reflex to muscular afferents) different, within limits, from those that initiate phasic responses? We found this to be the case and proceed to present the evidence for this conclusion.

Methods.

The methods were set out in detail in the preceding paper (GRANIT 1956 a) and will now be briefly summarized.

Cats, decerebrated by precollicular suction and de-efferented up to L5, were used. The gastrocnemius-soleus muscle was isolated and one pair of stimulating electrodes placed on the adjacent nerves *gastroc. med.* and *lat.* The other nerves to the leg were severed. The myograph was fixed on a sledge which, for testing by stretch, was pulled up by hand 10 mm against a stopper. Initial tension was near zero, in fact, adjusted so as to make the myograph barely respond to light touch of the string joining muscle and myograph. This amount of stretch will activate a large proportion of the cells capable of responding with a stretch reflex. Single efferent fibres were isolated by dividing ventral roots. Sometimes two or three active motoneurones remained, but in most cases individual fibres were obtained. For reasons to become clear below it is of considerable interest also to have some preparations with more than one active fibre.

Stretch and the reflex spikes were recorded vertically on running paper while a rapid horizontal sweep served for separation of spikes on a fast time base in order to make close inspection possible.

Every experiment began with three control stretches. Then followed tetanization of the muscular afferents at around 500/sec. for 10 sec. During tetanization the muscle was disconnected from the myograph and thus contracted against zero load. In this arrangement the muscle spindles are tetanized antidromically. It will last not less than 15—20 sec. before they are capable of responding normally (GRANIT 1956 a). Therefore the first test by stretch followed 20 sec. after cessation of

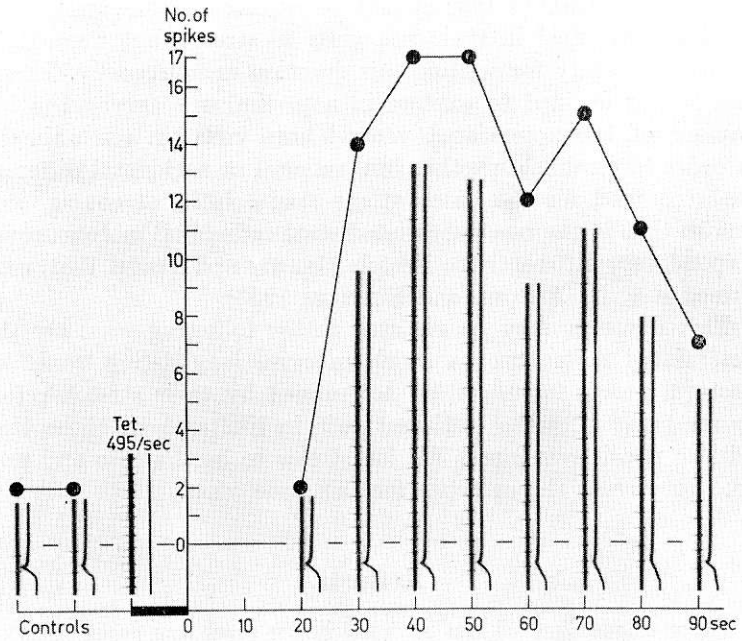

Fig. 1. Single ventral root fibre. Tonic type. Samples of original records of stretch reflexes inserted within graph in which ordinates show number of impulses delivered in response to stretch and abscissae time after tetanization period (black oblong), preceded by two observations on number of impulses in control stretch. Record taken during tetanization period inserted above black oblong. In this and successive figures the record during tetanization is cut to indicate time of 1 sec. Myograph record below stretch reflexes.

the tetanus, the following ones at intervals of 10 sec. In this manner the rise and fall of the post-tetanic potentiation was followed to the end by photographic recording. When no potentiation occurred the experiment could be carried out by inspection.

Results.

1. *Two types of ventral horn cells.* Fig. 1 illustrates a ventral horn cell that during control stretch responded with two impulses. Tetanization brought out another ventral root spike, not activated by stretch. It is seen that after the tetanus the number of reflex spikes rose to 17, stretch being discontinued when no more impulses were heard in the loudspeaker. Thus the index of the effect is total number of spikes. Frequency is less adequate

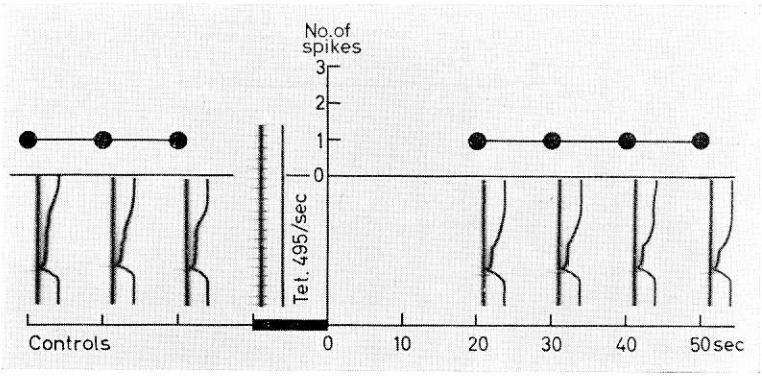

Fig. 2. Same as Fig. 1. Ventral horn cell of phasic type.

for the following reasons: (i) when there is one spike or a brief phasic discharge in the rising phase of control stretch, frequency is not an adequate basis of comparison of results before and after tetanization; (ii) frequency varied much less than the duration of the discharge which therefore is a better index when tonic effects are aimed at; (iii) it is well known from the work of ADRIAN and BRONK (1929), since repeatedly confirmed, that the frequency spectrum of ventral horn cells is exceedingly limited. Total number of spikes, however, may vary from zero to infinity. It is thus a good measure of the amount and duration of the post-tetanic effect. The variations of frequency may be studied in Figs. 1 and 4.

Another type of response, the phasic one, is illustrated in Fig. 2. The results are presented in the same manner as in Fig. 1. Clearly tetanization had no measurable effect whatsoever on this ventral horn cell. It was phasic and remained so.

The 100 spikes examined could be divided into three groups comprising phasic, moderately tonic and highly tonic cells but for the present purpose it suffices to divide them into the two main categories of phasic and tonic cells. Into the first group fell 53 % with very little or no potentiation. A reasonably good estimate of the type could be made already by inspecting the response to control stretch (before tetanization) because 88 % of the phasic cells only gave one spike or two closely spaced spikes on the rising phase of the pull, some of them none, while most of the tonic cells also responded to plateau stretch by a few impulses.

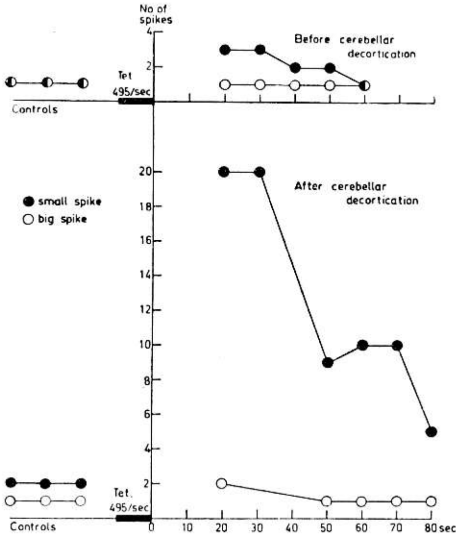

Fig. 3. Ventral root filament with two active fibres (small and large spike) showing their stretch reflexes (as in preceding figures) *before* (upper) and *after* (lower graph) decortication of cerebellar anterior lobe by suction.

Exceptions from this rule of thumb were found among the tonic cells some of which gave a brief phasic response in the controls (as *e. g.* the intensely tonic spike of Fig. 4). This is easily understood since, if for some reason or other excitability of the ventral horn cell is low or if autogenetic inhibition is present, post-tetanic potentiation will be needed to force the cell to sustained activity.

A few cells discharged permanently already to control stretch so that total number of spikes (our index) from the beginning was infinity. In these cases an effect of tetanization could be seen in the increased frequency of discharge. By our index these cells could not be classified.

Our material contained 3 % unclassified cells, 16 % cells intensely tonic after tetanization (a specimen in Fig. 4), 28 % tonic and, as stated, 53 % phasic cells.

This distribution cannot be held to be significant for an extensor population. To be sure, tonic cells were found in all cats but sometimes it was necessary to make a search for them. Phasic cells proved easier to find (see also below, section 3 on fibre size). The most important limiting condition would seem to be the state of the animal, *i. e.* amount of extensor tonus. Post-tetanic poten-

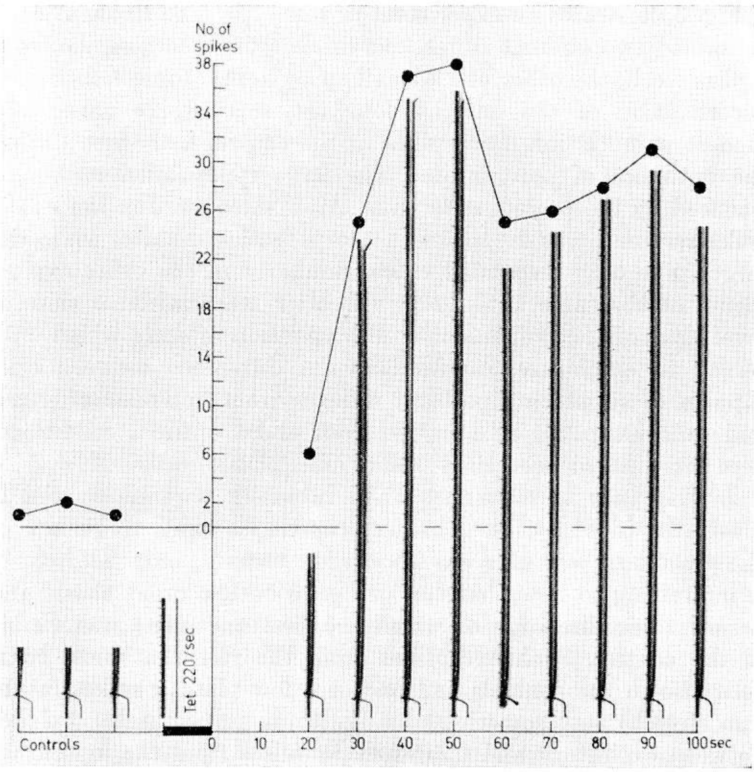

Fig. 4. As Fig. 1 but illustrating single-fibre stretch reflex in ventral horn cell showing intense potentiation. Initial phasic discharge not clearly visible at the reduced size of the records.

tiation is a presynaptic effect but it is tested with the aid of a post-synaptic index which requires a good level of excitability.

In order to throw light on this factor we had recourse to spinalization or decortication by suction of the anterior lobe of the cerebellum. In the spinalized state extensor tonus is well known to be low and accordingly it proved difficult to demonstrate the tonic response, despite potentiation. More important from the present point of view were the cerebellar ablations which create a state of high extensor tonus (Bremer 1922; Stella 1944 a, b; Moruzzi 1950, Granit, Holmgren and Merton 1955). Three such experiments were carried out. In them filaments tested before cerebellar decortication were set apart to be retested after the operation.

Fig. 3 illustrates an experiment of this type. The filament used contained two efferent fibres, one discharging a large spike from a phasic cell, the other one a small spike from a tonic cell. Before decortication of the anterior lobe the small spike gave very modest potentiation, two spikes in the control and three during the optimum of potentiation. The large spike failed to be influenced by the preceding tetanus. After the operation the small-spike response was 20 spikes in the potentiated state, while the large spike only responded twice. Similarly in the other experiments of the same kind there was after tetanization a shift of level upwards accompanied by an expansion of scale which only served to emphasize the fundamental difference between cells capable of supporting postural reflexes from muscular afferents and cells incapable of doing so even under optimal conditions, here for convenience called 'tonic' and 'phasic' cells.

2. *Frequency of tetanization.* An intensely potentiated cell is illustrated in Fig. 4 from an experiment in which frequency of the tetanizing stimulus was lower than usually, only 220/sec. It is interesting to note that this cell gave both a rapid phasic and a slow tonic discharge of which the first one alone was visible in the control. Such exceptions from the rule that most tonic cells also in the controls had one or a few plateau spikes (at 10 mm stretch) may sometimes be due to an unfavourable balance between excitatory and inhibitory impulses from the muscle receptors redressed by the facilitation in the potentiated state. By our criteria this cell is highly tonic.

Some systematic experiments on frequency of the tetanizing stimulus proved that beyond 200/sec. this factor was of little importance. The following figures are from a typical case: a cell which responded with two spikes to control stretch began to show definite potentiation with 5 spikes at stimulus rate 115/sec. for 10 sec.; at rate 130/sec. it gave 22 spikes, at 200/sec. 50 spikes, at 320/sec. the same number, which was maintained up to tetanizing frequencies around 500/sec. The agreement between our figure of 200/sec. and LLOYD's (1949) value of the same order with the monosynaptic response as index of potentiation may well be fortuitous. The monosynaptic index measures number of subliminal-fringe neurones added by potentiation, as tested by synchronous shocks. We are measuring total number of impulses of a single active neurone stimulated adequately by stretch.

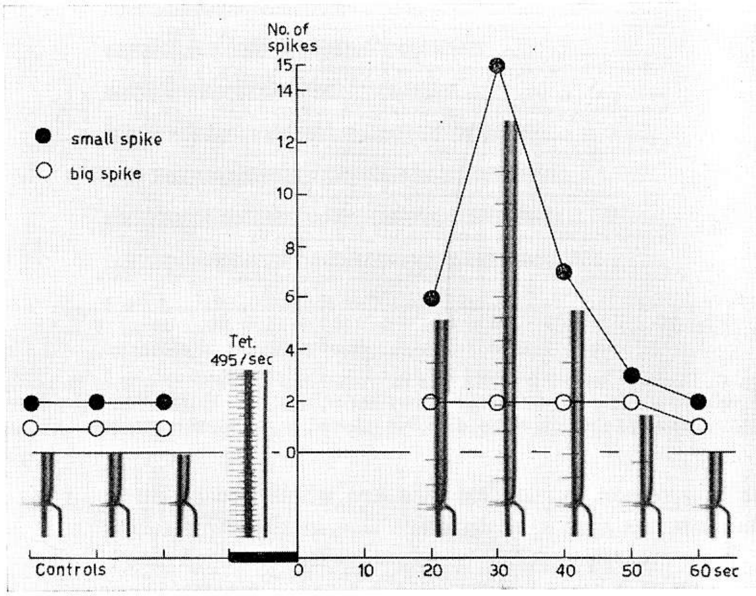

Fig. 5. As Fig. 1 with the difference only that the ventral root filament contained one large and one small spike.

3. *Significance of fibre size.* A common experience in these experiments was that when large and small spikes were present in the same filament, the large ones gave phasic and the small ones tonic types of response. This is shown in Fig. 5 (cf. also Fig. 3). While dissection of the filaments proceeded, we often at intervals tested their properties by tetanization and stretch in order to find out what kinds of spikes were left. Many such filaments were discarded because of unsuccessful isolation in the final stage, others became part of our collection. Discarded filaments did, however, enrich our material of observations on spike size and supported the generalization that tonic spikes tended to be among the small ones. In particular it was found that the largest spikes practically always were phasic.

Spikes will, of course, become small also when their fibres are killed below the point of recording but there is nothing to suggest that large tonic fibres would be more brittle than large phasic ones. Caution should be exercised in evaluating the size of spikes studied at different times of the day or in different animals and

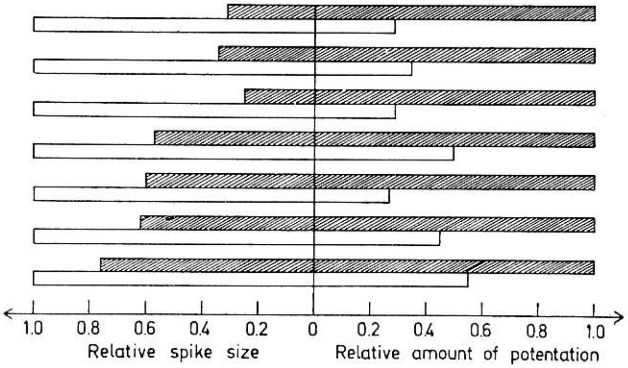

Fig. 6. Diagram referring to six ventral root filaments with a pair of spikes in response to stretch, one large, the other smaller, the latter hatched. Relative spike size and relative amount of potentiation plotted in terms of a maximum of 1.0.

for this reason our conclusions are wholly based on comparing simultaneous spikes of different size in the same filament.

There were a number of experiments in which good isolation of a pair of spikes of different size was the successful net result of dissection. These have been collected in the diagram of Fig. 6. The larger spike arbitrarily has been given size 1.0 and the maximum potentiation of the smaller spike similarly a potentiated value of 1.0. Relative size and relative amounts of potentiation have been plotted in terms of these maxima. It is seen that there is less potentiation in the larger spike of a pair.

Finally Fig. 7 is a plot of results with a filament in which three clearly discernible spikes were obtained. Their relative size is given in the inset, the main figure illustrating the amounts of potentiation obtained, which is seen to be less, the greater the spike size.

It is concluded that there is a definite tendency of the tonic cells to discharge spikes in smaller fibres than the phasic ones. The interpretation is that large cells are more likely to issue large fibres and hence that fibre size is an index of cell size. Thus the majority of the tonic cells would be grouped among the smaller alpha motor cells of the ventral horn.

4. *Spike frequency during tetanus.* If the failure on the part of the large cells to fire repetitively in response to stretch following tetanization were due to inhibition from muscular afferents or tendon organs, one would expect them to be more easily suppressed by tetanization, at least if Golgi tendon organs with

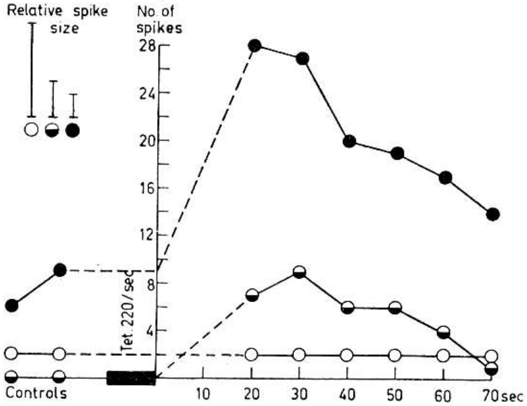

Fig. 7. As Fig. 5, but with three spikes in the filament. Their relative size shown.

their large afferents were responsible for the postulated effect (cf. discussion by GRANIT 1955). There was, however, nothing to indicate such a state of affairs. On the contrary, it was our impression that the large spikes ran up to higher frequencies during tetanization than the small ones. Samples of firing during tetanization are given in Figs. 1, 2, 4 and 5. Our results cannot very well be used to settle this point because other spikes than the ones responding to stretch turned up so often that evaluation of the whole material from this point of view proved difficult.

Discussion.

The idea in this paper has been to use post-tetanic potentiation as a means for detecting fundamental categories of motoneurones (in their relationship to muscular afferents). Some indications of the two categories described may often be found without recourse to potentiation but a preceding tetanus, as it were, tears them apart by favouring repetitive firing and revealing latent tonic behaviour. Differences on a small scale are thus enormously magnified by potentiation and thereby the experiment becomes far less dependent on the state of the preparation. (Anaesthesia must be avoided in experiments on tonic cells because under the influence of almost any depressant drug all cells tend to become phasic (cf. ALVORD and FUORTES 1953). Chlorpromazine (HENATSCH and INGVAR 1956) and myanesin (GRANIT 1956 b) have the same effect as anaesthetics.)

One further advantage of potentiation is that this state by no means is unnatural. It was recently shown (GRANIT 1956 a) that the effect which here has been obtained by means of an electrical tetanus also occurs after natural stretch of sufficient intensity. This means that the gamma system will be of paramount importance for tonic cells because, activated by the brain stem (GRANIT and KAADA 1952, ELDRED, GRANIT and MERTON 1953, GRANIT and HOLMGREN 1955) this system may set up long-lasting states of intense activity from the muscle spindles succeeded by post-tetanic potentiation. The present work shows that the spindle loop acquires a different order of significance depending upon whether its influence is directed towards tonic or phasic cells. Only in the former case can it exercise its full effect on postural reflexes. The tonic cells, as it were, provide the *raison d'être* for the tonic gamma control from higher levels. At the same time the gamma control, on account of the presynaptic nature of potentiation, will label ventral horn cells over the spindle loop, as explained in the introduction. It is well known that spastic patients may appear normal, unless tested by stretch, when a potentiated state of their ventral horn cells is revealed.

The amount of stretch is a factor of considerable importance in that the tonic motoneurones on the whole seem to require less tension (elongation of muscle spindles) than, at least, the largest phasic ones. In the previous paper (GRANIT 1956 a) low tension (= small amounts of stretch) was used in an attempt to avoid reflex spikes in the controls and demonstrate potentiation by the sudden appearance of a tonic discharge to stretch after tetanization. It is, however, a problem in its own right, as yet wholly unsolved, to elucidate the difference between tonic and phasic ventral horn cells from the point of view of the variations in afferent input.

It is not necessary to overemphasize the correlations between, on the one hand, phasic and tonic motoneurones, on the other, their relative spike sizes from which we have inferred that the tonic neurones tend to group themselves among the smaller alpha ventral horn cells. Such biological classifications generally tend to go with a considerable region of overlap and transitional forms. This, however, does not detract from their significance.

Though not found in mammals, subdivision of ventral horn cells into motoneurones for phasic and tonic reflexes may well explain some amphibian results (BREMER and MOLDAVER 1934).

In their work a fast phasic reflex contraction was followed by a slow tonic one but no steps were taken to prove by dissection that different motoneurones were concerned.

In a recent paper NORRIS and GASTEIGER (1955) used fine microelectrodes for discrete responses from human muscles in voluntary contraction. There were four classes of spike amplitudes. The extremes were spikes of amplitude 0.63 and 2.47 mV. The small spike turned up first with weak voluntary contractions and increased its rate of firing a great deal as strength of contraction increased. The big spike required very strong contractions and fired less than 5 spikes in occasional bursts. The amplitudes of spikes, intermediate in size, represented motoneurones with properties transitional between the extremes. Since it is unknown how these muscle spikes are related to fibre size in the motor nerves, the results at the moment are difficult to compare with ours, though suggestive as far as they go. The work of NORRIS and GASTEIGER should be repeated in animals using post-tetanic potentiation to test the stability of the discharge properties of the muscle fibres isolated by such means. Ordinary myography would seem to be too coarse a method for this purpose. Also there is a considerable amount of overlapping or multiple innervation (HUNT and KUFFLER 1954) of individual muscle fibres which means that tonic and phasic fibres may run to the same motor unit. In such cases resolution by electromyography would be extremely difficult.

Summary.

In decerebrate cats stretch reflexes from the ankle extensors have been studied in 100 fibres from dissected ventral root filaments. These reflexes have been maximally facilitated by post-tetanic potentiation, as described by GRANIT (1956 a).

In the post-tetanic or potentiated state the reflexes to a constant 10 mm stretch have been found to fall into two main categories: (i) tonic reflexes which go on firing for a long duration in maintained stretch and (ii) phasic ones which only fire one or two spikes on the rising phase of stretch, in spite of any amount of post-tetanic potentiation.

Analysis of spike size showed that the tonic ventral horn cells tended to fire smaller spikes than the phasic ones. From this it was concluded that the tonic motoneurones tend to group themselves among the smaller ventral horn cells.

The difference between tonic and phasic ventral horn cells was maintained when the extensor tonus was raised by decortication of the anterior lobe of the cerebellum.

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