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FUNCTIONAL SIGNIFICANCE OF CELL SIZE IN SPINAL MOTONEURONS¹

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INTRODUCTION

SINCE THE BEGINNINGS OF NEUROHISTOLOGY it has been recognized that neurons within the central nervous system vary widely in size, but the functional significance of this basic observation has never emerged from the realm of speculation. The largest cells have surface areas which are at least 100, perhaps 1,000, times greater than those of the smallest cells. Correspondingly, the diameters of axons in the central and peripheral portions of the nervous system range from less than .25 μ . to more than 20 μ . This broad spectrum of physical dimensions invites a search for functional correlates.

This is one of a series of studies on the problem of size as it relates to spinal motoneurons. The preceding papers (21, 25) were concerned chiefly with the peripheral part of the motoneuron and the muscle fibers it innervates. They provided experimental evidence that the diameter of a motor nerve fiber is related to the number of muscle fibers it supplies. This finding seemed to make good sense: if a motor fiber innervates many muscle fibers and forms a large motor unit, it must have sufficient axonal substance to give off a large number of terminals.

The present paper is concerned with the central part of the motoneuron and the significance of its size in synaptic transmission. It asks whether the cell bodies (and dendrites) connected with large and small motor fibers have different functional properties which can be recognized by their discharge characteristics. In order to investigate this problem one must be able to distinguish the signals of a large motoneuron from those of a small one. This may be done by recording their action potentials from thin filaments of lumbar ventral roots. As Gasser (8) demonstrated, the amplitudes of nerve impulses recorded externally from peripheral nerves are directly related to the diameters of their fibers. If it may be assumed that the diameters of axons are also related to the sizes of their cell bodies, as scattered histo-

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logical evidence (see DISCUSSION) indicates, we may conclude that a large impulse recorded from a ventral root filament signifies the firing of a large motoneuron and a smaller impulse recorded from the same filament denotes the discharge of a smaller cell.

Using these criteria in an earlier study, Henneman (13) demonstrated that the excitability of flexor motoneurons varied inversely with their size, the reflex threshold of individual cells increasing in parallel with the amplitude of the impulses recorded from their axons. In the more extensive series of experiments to be reported here the stretch reflex of the triceps surae muscle in the decerebrate cat was selected for study. The advantage of this preparation is that a steady, well-maintained sensory input, variable in intensity and completely physiological in nature, can be applied by stretching the triceps muscles. In an excitable preparation this elicits a sustained rhythmic firing of motoneurons, which can be sampled by recording from ventral root filaments. Responses to other stimuli and their effect on the stretch reflex can easily be studied by slight modifications of this technique.

METHODS

Thirty-five adult cats with an average weight of 3.2 kg. (range 1.8–5.0 kg.) were used in these experiments. Under ether anesthesia a tracheal cannula was inserted and the cat was decerebrated by making a small opening in the skull, removing enough cortex by suction so as to expose the colliculi and making a spatula section between the colliculi. Ether was then discontinued.

With the pelvis rigidly fixed a laminectomy was performed from the fourth to seventh lumbar vertebrae. A drill was passed through the distal end of the left femur to provide fixation. All of the nerves innervating the left hind limb were cut distally except those to the triceps surae muscles, and these were dissected back far enough from the muscle to allow placement of stimulating electrodes. Skin flaps were pulled up to form pools for warmed mineral oil over the spinal cord and exposed tissues in the hind leg.

In order to measure the tension of the triceps surae with stretch, the Achilles tendon with part of its bony attachment was freed and attached to a myograph mounted on a rack and pinion. The myograph consisted of a mechanoelectric transducer (Grass model FT 10) which fed into a Tektronix type Q unit. This transducer gave a linear rise of signal up to 10 kg. tension. The tensions which were measured ranged from 0–8 kg.; they corresponded to stretch from resting length to about 27.5 mm. in de-efferented muscle.

The L_7 and S_1 ventral roots were exposed but not cut in all experiments except as noted. Ventral root filaments were obtained for recording by cutting small strands distally and pulling them free with fine watchmaker's forceps. The filaments were mounted on silver electrodes under mineral oil and the discharges of triceps units were recorded oscillographically. In most experiments all filaments that contained two to five axons with no evidence of conduction blocks were studied. Filaments with too many units for easy analysis were subdivided with the aid of watchmaker's forceps and sharpened needles over a blackened glass plate. Occasionally all of the S_1 and part of the L_7 roots would be used in one experiment. In the experiments in which the ventral roots were transected at the beginning, both roots were systematically subdivided into filaments containing two to four units, and all unblocked filaments were studied.

During the course of an experiment the extensor tone would occasionally decrease to a point where a maintained stretch discharge could not be obtained. When this occurred the anterior lobe of the cerebellum was removed by suction in order to increase the stretch reflex.

Recordings were made using two Grass P5 preamplifiers and a Tektronix type RM565 dual-beam oscilloscope with split beams. One beam displayed the filament discharge, one the tension measurement, and one functioned as a base line for the tension measurement. Tension calibrations were taken regularly by hanging weights in a pan from the transducer.

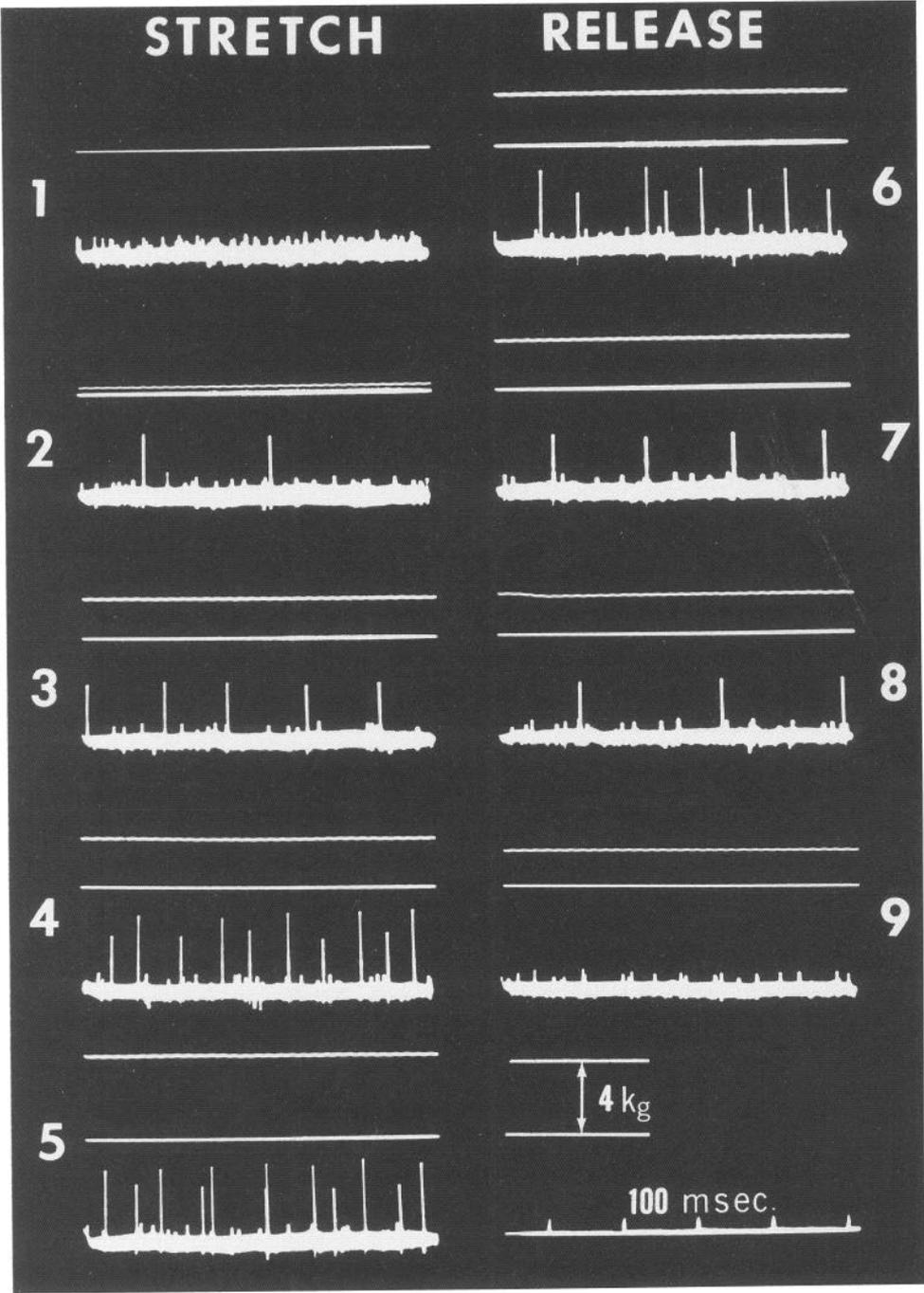


FIG. 1. Stretch-evoked responses of two alpha motoneurons recorded from a filament of the seventh lumbar ventral root. The amount of tension applied and developed reflexly is indicated by the separation of the two upper beams in each frame. Several seconds elapsed between successive frames while muscle was stretched (1-5) and released (6-9).

RESULTS

Sizes of motoneurons and their thresholds

From the outset of these experiments it was observed that there was a well-defined relationship between the sizes of motoneurons as judged by the amplitude of their action potentials and their thresholds in the stretch reflex. In a decerebrate preparation with a well-maintained stretch reflex it is not difficult to isolate from an otherwise intact L₇ or S₁ ventral root a filament which contains the axons of a few alpha motoneurons which respond with rhythmic firing to the stretching of the triceps surae muscle. Some oscillographic tracings recorded from a filament which contained only two triceps axons are reproduced in Fig. 1. With the muscle completely relaxed (line 1), the only activity recorded was a steady stream of impulses of very low amplitude. These small impulses were taken to be spontaneous discharges of gamma motoneurons, whose axons were of smaller diameter than those of the alpha fibers also present in the filament. As the triceps was elongated, the elastic and reflex tension produced by the stretch was recorded as an increasing separation of the upper two beams in lines 2-5. At a stretch tension of approximately 650 g. the smaller of the two alpha motoneurons in the filament began to discharge (line 2). With further stretch, the discharges of this unit increased in rate and regularity. It was obvious that they represented the action potentials of a single cell, responding rhythmically to the steady drive of stretch-evoked impulses. At a tension of about 2,770 g. a second motor unit, generating larger action potentials, was recruited into the stretch reflex (line 4). The firing rate of this unit was accelerated by further stretch, but no further units were recruited. Upon release of stretch (lines 6-9), the larger unit was the first to cease firing, followed later by the smaller unit.

In Fig. 2 a similar series of tracings is reproduced to illustrate recruitment in a filament containing at least five triceps axons. Prior to stretch only gamma activity was recorded (line A, left). With very slight stretch the smallest alpha unit, labeled 1, commenced to discharge at about 8/sec. Increasing stretch resulted in the recruitment of progressively larger spikes, labeled 2, 3, 4, and 5. The order of recruitment was the same as the order of spike amplitudes, but the unit with the largest spike (no. 5) had a slightly lower threshold for maintained stretch than did unit 4. Thus, the order of recruitment recorded in this case was 1, 2, 3, 5, 4. Upon release of stretch (lines C and D) the order of dropout was 5, 4, 3, 2, 1, i.e., the reverse order of the sizes.

These examples illustrate the first and the major finding of this study, namely, that in general the stretch threshold of motor units is directly related to the relative size of the action potentials recorded from their axons. As discussed in the INTRODUCTION, larger spikes are interpreted as impulses in axons of larger diameter and hence signify the discharge of larger motoneurons.

Statistical analysis of correlation between the order of recruitment of individual motor units and relative amplitudes of their action potentials

The association between spike amplitude and order of recruitment described above was not invariable. Occasionally a unit was recruited "out of order" as in the case of unit 5 in Fig. 2. It was therefore important to establish statistically the correlation between these two variables. For this purpose experiments were performed with the object of recording the recruit-

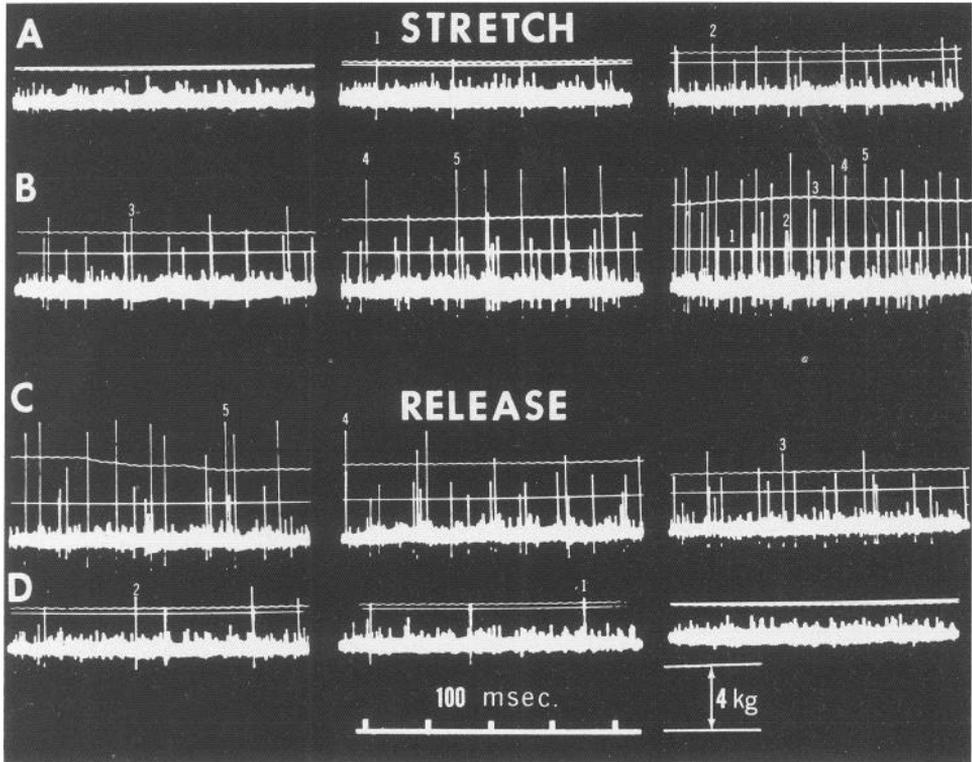


FIG. 2. Stretch-evoked responses of five alpha motoneurons recorded from a filament of the first sacral ventral root. Small numerals above action potentials indicate rank of units according to size.

ment pattern in as many unselected ventral root filaments as possible. In these experiments the entire seventh lumbar and first sacral ventral roots were cut near their exit from the spinal cord. Both severed roots were subdivided systematically into filaments which, upon viewing their electrical activity on the monitor oscilloscope, appeared to contain two to four individually recognizable motor units responsive to stretch of the triceps muscle. Filaments which showed evidence of a conduction block were excluded from the study because the amplitude of blocked impulses is not a reliable index

of fiber size. From each of the acceptable filaments records were taken on running film, with the beam stationary, while the muscle was stretched in small steps and held at each new length for a few seconds. The same procedure was followed during release of stretch. The whole sequence of recordings was repeated two to four times for each filament. The photographic records from each filament were examined separately. In each series the motor units were identified and ranked according to the size of their action potentials. The minimum level of tension at which each unit first responded with rhythmical firing of at least 1 sec. duration was also noted and defined as its threshold for tonic response. The sequence in which units started to give tonic responses while the muscle was being stretched was termed the "order of recruitment."

The relation between the order of recruitment and the size rank of spikes within each filament in three different experiments is shown in Table 1. The data from filaments containing two, three, and four motor units have been tabulated separately, for the weight of a "second place" is not the same in a two-rank as in a three-rank or four-rank series. Thus, the upper left entry in the first 2 x 2 table means that in experiment I we found the smaller of the two units in each of ten pairs to be the more sensitive to stimulation by stretch. The upper right entry means that in one pair the larger of the two units became active before the smaller one. A result of this type will henceforth be referred to as "reversed recruitment." The data pooled from all three experiments are shown for two-unit filaments in the upper right 2 x 2 table, and for the three-unit and four-unit filaments below. On five occasions "ties" were recorded because the thresholds of two units were indistinguishable. Tied ranks were assigned half scores distributed between the shared places.

It is obvious that the largest numbers in all the tables occur in the positions along the diagonal lines, where size ranks are identical with place numbers in the order of recruitment. The probability of random distribution, calculated from χ^2 , is less than 0.001 for each of the tables of pooled data.

As a numerical index of the association between size and order of recruitment the rank correlation for all results combined was calculated. Kendall's (17) method was used because his coefficient τ is based on a consideration of ranks compared in pairs, and thus may be applied to results pooled from series containing different numbers of members. For the purpose of this calculation each three-unit filament was regarded as representing three comparisons (the number of possible combinations of twos between three objects), and each four-unit filament was regarded as representing six comparisons. For example, if the spike amplitudes in a three-unit filament are ranked from smallest to largest, and during stretch these spikes appear in the order 3-1-2, then this filament would yield two negative and one positive scores. Recruitment in the sequence 3-2-1 would be scored as three reversed pairs. The number of possible combinations of two in the entire

Table 1. Relationship between size-rank and order of recruitment

		2-unit filaments				4-unit filaments			
		Exp. I		Exp. II		Exp. III		Exp. I + II + III	
		Size (rank): 2		Size (rank): 2		Size (rank): 2		Size (rank): 2	
		1	2	1	2	1	2	1	2
Recruitment:	1st	10	1	11	3	15	2	36	6
	2nd	1	10	3	11	2	15	6	36
				filaments: 42 units: 84 pairs: 42					
				$\chi^2 = 42.8$ $P < 0.001$					
		3-unit filaments			4-unit filaments				
		Exp. I + II + III			Exp. I + II + III				
		Size (rank): 2			Size (rank): 2				
		1	2	3	1	2	3	4	
Recruitment:	1st	18	4	1	7.5	1.5	1	0	
	2nd	5	14	4	1.5	5.5	2	0	
	3rd	0	5	18	0	3	4	2	
					0	0	2	7	
		filaments: 23 units: 69 pairs: 69			filaments: 9 units: 36 pairs: 54				
		$\chi^2 = 56.2$ $P < 0.001$			$\chi^2 = 42.1$ $P < 0.001$				

Total number of filaments: 74.
 Total number of units: 189.
 Total of possible combinations of twos (pairs compared): 165.
 Out of all combinations of twos, recruitment of smaller followed by larger: 142.5.
 Out of all combinations of twos, recruitment of larger followed by smaller: 22.5.
 Coefficient of rank-correlation (Kendall (17), $\tau = 0.73$).
 χ^2 for all combinations of twos = 87.3.
 $P < 0.001$.

material of Table 1 is 165. Out of these the order of recruitment followed the size ranking in 142.5, whereas in 22.5 the recruitment was reversed. Hence the coefficient τ is 120/165 or 0.73 (perfect correlation = 1.0). Correcting for tied ranks would slightly increase this ratio.

It is worth noting that the number of cases in which a smaller unit was recruited after a larger one was roughly the same for two-unit filaments in the three experiments (Table 1). Furthermore, the proportion of reversely recruited pairs in three- and four-unit filaments (13.4%) was similar to that for two-unit filaments (14.3% when the three experiments were pooled).

On the whole, the order of recruitment of units in any one filament was very consistent. In a few instances the observation of a filament extended over more than an hour, and the sequence in which units commenced discharging and ceased firing remained unchanged in 15–20 trials. On rare occasions, however, two units exchanged their places in the order of recruitment. Such inconsistencies were seen in 2 of the 165 comparisons detailed in Table 1, and these were included in the “tied” ranks.

It was not necessary to tabulate separately the data on the order in which units ceased to fire upon release of stretch, because in the great majority of filaments this order was exactly the reverse of the order in which they were recruited. The rare exceptions may be termed “discordances” between the order of recruitment and the order of dropout. An example of a discordance of this type was seen in Fig. 2. In this case the two largest units were recruited in reversed order, but dropped out during release as expected from their relative sizes. Ten possible combinations of twos can be arranged with 5 units, so exceptional behavior was found in 1 out of 10 pairs in this filament. Data on both recruitment and dropout are available for 236 possible combinations of two units. Discordances were found in only 11 of them. (These figures are derived from the experiments used to compile Table 1, as well as from others.) The nature of discordances, a subject of considerable theoretical interest, will be discussed in a later paper.

Phasic and tonic responses of units

The data of Table 1 pertain only to motoneurons which could be classified as “tonic” units by the operational definition of Granit, Henatsch, and Steg (9), for they were all capable of rhythmic firing of more than 1 sec. duration in the absence of gamma drive, i.e., after the ventral roots were cut. At a low level of stretch these units often responded phasically with brief bursts of impulses. When the muscle was stretched further, they became tonically active.

Many of the filaments contained, in addition to these tonic units, others which responded phasically with all degrees of stretch. The spike amplitude of the purely phasic units was usually, but not invariably, greater than that of the largest tonic unit in the filament. Furthermore, initially phasic responses were more easily elicited from smaller units than permanently phasic responses were from larger units. In our experiments the tendon of

the muscle was pulled by a hand-operated device and therefore the rate of extension was not uniform. Since the velocity of stretch is an important parameter influencing the threshold of phasic responses, we did not attempt to work out a statistical table for phasic responses similar to that for tonic responses.

The response of a unit to stretch is a function of the general level of extensor tone in the preparation and the sensitivity of the stretch reflex as well as the size of the neuron. With a change in the decerebrate state, the response of a unit may alter from tonic to phasic, or vice versa. On a number of occasions when the majority of motor units were responding purely phasically, a lower transection of the brain stem or a removal of the anterior lobe of the cerebellum transformed the phasic responses into well-maintained tonic discharges. On the other hand, ether or barbiturates in small amounts readily turned tonic responses into phasic ones. Accordingly, we believe that the motoneurons of a pool do not fall into separate phasic and tonic classes, but instead form a continuous spectrum of sizes and excitabilities.

Distribution of stretch thresholds of motoneurons

The distribution of thresholds to stretch is shown for 208 motoneurons in Fig. 3. Only the preparations in which ventral roots were cut at the beginning of the experiment were suitable for this purpose, because when filaments were successively separated from undivided roots the amount of gamma outflow was continually diminishing as the experiment progressed.

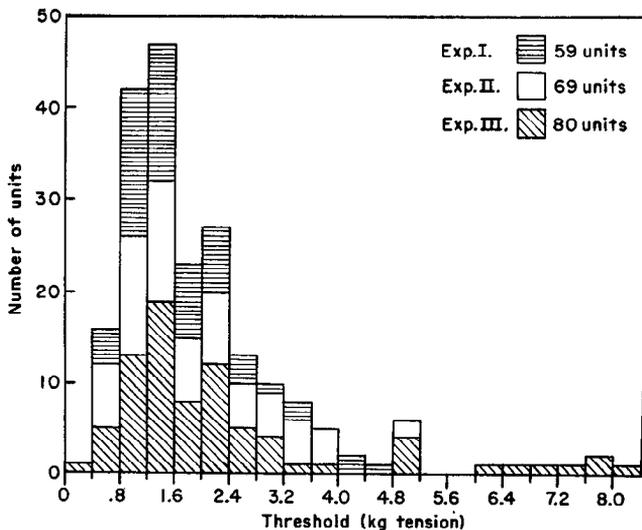


FIG. 3. Frequency distribution of thresholds of tonic responses in the three experiments of Table 1. *Abscissa*: threshold tension of de-afferented triceps muscle in kilograms. *Ordinate*: number of units whose thresholds fell between the values indicated on the *abscissa*.

Even so, it must be emphasized that the level of excitability of a decerebrate preparation varies considerably. The thresholds shown in the histogram of Fig. 3 must, therefore, be regarded as representative of the average condition of the preparation during the period of data collection. Figure 3 was compiled from the same experiment as Table 1, but a larger number of units was included because filaments containing only one tonic unit could also be used. The data collected from each of the three experiments are distinguishable by their crosshatching. This was done to show how similar the distribution of thresholds was in the three experiments.

As Fig. 3 reveals, the thresholds of motoneurons range from less than 0.4 kg. to more than 8.0 kg. with a distribution peak between 0.8 and 1.6 kg. Although the thresholds represented in Fig. 3 are those of tonically responding units only, their distribution suggests comparison with Fig. 6 of McPhedran, Wuerker, and Henneman (21) and Fig. 4 of Wuerker, McPhedran, and Henneman (25). The comparison indicates that the size of a motor unit and the threshold of its motoneuron are closely interrelated.

Tonic stretch reflex of triceps pool of motoneurons

Figure 4 is the result of an attempt to build up a "synthetic stretch reflex" by combining the data acquired in this study and previous studies on motor units (21, 25). To

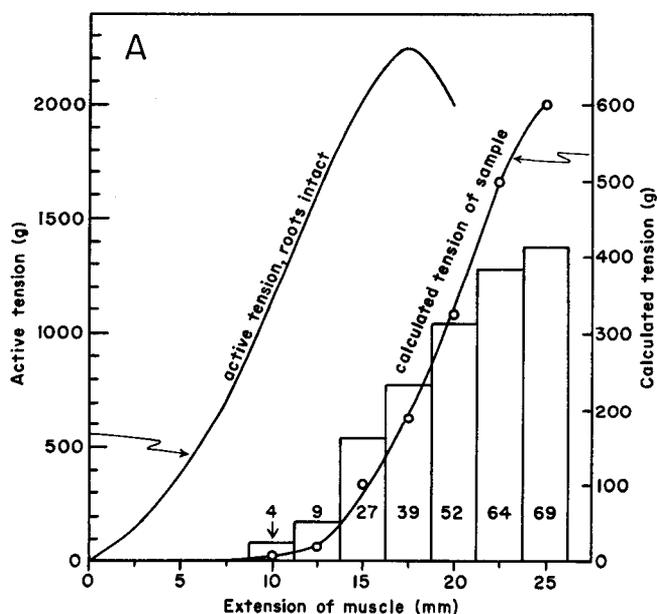


FIG. 4. Synthetic stretch reflex of de-efferented muscle compared with actual tensions developed by the whole muscle at various degrees of stretch. Left-hand ordinate and curve labeled "active tension" show development of tension in the whole muscle. Vertical columns form a cumulative frequency histogram showing the tonically active fraction of the sample of motoneurons plotted against the length of the muscle. Numerals show the actual numbers of units recruited at the lengths indicated on the abscissa. Right-hand ordinate and curve show calculated tensions exerted by these units. For method of calculation see text. Data from exp. II of Fig. 3 and Table 1.

construct it, the fraction of the triceps motoneuron population active when the muscle was extended to a known length was calculated, and then an estimate was made of the tension generated by each fraction. Details of the computation are given below. When length rather than tension is used as a measure of threshold, as on the abscissa of Fig. 4, the distribution of thresholds is altered accordingly. Since our sample of the triceps population was made up entirely of units which gave tonic responses to stretching of de-efferented muscle, it was assumed that all of these motoneurons innervated red or slow motor units, regardless of which head of the muscle the axon supplied (cf. 10, 11, 4). For this reason the data used to calculate the contractile tensions in Fig. 4 were taken from the study of soleus motor units (21) rather than from the m. gastrocnemius results (25). Figure 4 is based on a single experiment (exp. II of Table 1 and Fig. 3).

To obtain the mean contractile power of motor units recruited at any particular length of the muscle the following procedure was adopted. First, the number of units recruited at each length was computed as a fraction of the entire sample. The actual numbers of the units whose thresholds were at a given muscle length or less are shown on the histogram of Fig. 4. Next, the nearest corresponding fractions in the histogram of maximum tensions for soleus units (21) were identified. Since the percentile divisions in the two frequency distributions were not exactly equal, deviations were corrected by appropriate interpolations. The average of the maximum tensions for units recruited at each of the various lengths was taken as being the mean between the upper and lower limits of tension. Since the units responding tonically in the stretch reflex were firing at only 10–15/sec., it was estimated that they developed only 60% of their maximum capability, and the tensions were corrected accordingly. The values so found were plotted in cumulative fashion and are shown as circles in Fig. 4.

For example: at 10 mm. extension there were 4 units tonically active, representing 6% of the entire sample of 69 units in experiment II. Figure 6 of McPhedran, Wuerker, and Henneman (21) shows 8 units falling into the 0- to 5-g. group, forming 8% of the total of 97 units. Now, $6/8$ of 5 g. is 3.8 g., giving the upper limiting value of the most excitable fraction of motoneurons. The lower limit is the maximum tension of the smallest motor unit, taken to be 2 g. (actually, the maximum tetanic tension of the smallest unit in the soleus population was 3.5 g. and in the m. gastrocnemius 0.5 g. (21, 25). The mean between the upper and lower limit is 2.9 g. Since there were 4 units in this category, the maximum capability of this group of motor units is 4×2.9 g. or 11.5 g. Sixty per cent of 11.5 g. is 7 g., being the calculated output of these 4 motor units.

Although the assumptions on which the construction of Fig. 4 depends are rather speculative, the results appear reasonable. The two continuous curves of Fig. 4 may be compared with Figs. 1, 2, and 3 of Matthews (20). Matthews measured the tension of the soleus muscle as a function of extension before and after the application of a weak solution of procaine to the muscle nerve, a procedure believed to block gamma fibers only. The two tension-extension curves which he obtained are very similar to the two continuous curves in Fig. 4 (one showing the actual tension before cutting the ventral roots, the other being the synthetic curve calculated for interrupted gamma outflow).

Effect of interrupting the gamma loop upon the recruitment and firing pattern of motoneurons

Since, as noted, some of the experiments were performed with the seventh lumbar and first sacral ventral roots cut and others with a varying fraction of these roots still intact, it was important to establish what influence the presence of gamma drive has on the behavior of motor units in the stretch reflex. In five decerebrate cats six filaments were studied before and after cutting the L₇ and S₁ ventral roots. In each of these the first set of records was obtained at the beginning of the experiment, with almost the entire outflow through the ventral roots still intact.

In no case did the cutting of ventral roots alter the sequence of recruit-

ment of the motor units in the filament studied. As expected, the responsiveness of each motoneuron declined markedly after interrupting the gamma outflow from the cord. Not only was a greater extension of the muscle needed to evoke reflex firing, but also some of the units which readily gave tonic responses before, became incapable of maintained rhythmic firing after cutting the roots, i.e., they responded phasically or not at all.

Figure 5 illustrates such an experiment with an L_7 filament containing two triceps motor fibers. "Phasic response" means trains of spikes less than 1 sec. in duration; for phasic responses the total number of spikes fired is represented on the ordinate scale. For tonic responses the frequency of firing per second was taken, as measured in the second half of the first second after the muscle had been extended to the length indicated on the abscissa. Each point on the graphs represents the mean value from four successive runs. The smooth curves were drawn to indicate the trend, to give the least deviation of points as judged by inspection. It may be seen that the smaller of these two units showed purely tonic behavior as long as the ventral roots were intact (Fig. 5B1). The larger unit gave a phasic response to moderate stretch (Fig. 5B2), but a persistent tonic response when the tendon was pulled out further (Fig. 5B1). After removal of the gamma drive partially (Fig. 5C) and then completely (Fig. 5D) the smaller unit behaved phasically at moderate and tonically at strong stretch, while the larger of the two failed entirely to generate maintained rhythmic firing (Fig. 5D). Using a different experimental approach, Henatsch, Schulte, and Busch (12) have already noted that motor cells which respond tonically under one set of conditions may become purely phasic when the amount of background excitation is reduced; a change in the opposite direction may also occur when extensor tone increases.

It may be noted in Fig. 5 that the threshold level of extension needed to start the firing of a unit was less than the length of the muscle at which firing ceased while the muscle was allowed to retract. This phenomenon appears to be the basis of the hysteresis of the stretch reflex studied in detail by Matthews (19). It is evident in the tension measurements of the whole soleus represented in Fig. 5A. It may be relevant to point out that in filaments which contained one or two gamma fibers along with the alpha fibers, it was usually found that the discharge of the gamma units decreased or stopped entirely when the muscle was extended as far as necessary to obtain autogenetic inhibition. It may be that inhibition of the discharge of gamma motoneurons is responsible, in part at least, for autogenetic inhibition of the stretch reflex. The peripheral destination of gamma fibers contained in divided ventral root filaments cannot be ascertained, but Eldred, Granit, and Merton (6) have demonstrated similar autogenetic inhibition of fusimotor fibers by the indirect method of recording the afferent spindle discharge. Hunt (15) had previously made similar observations by recording from a small branch of a muscle nerve.

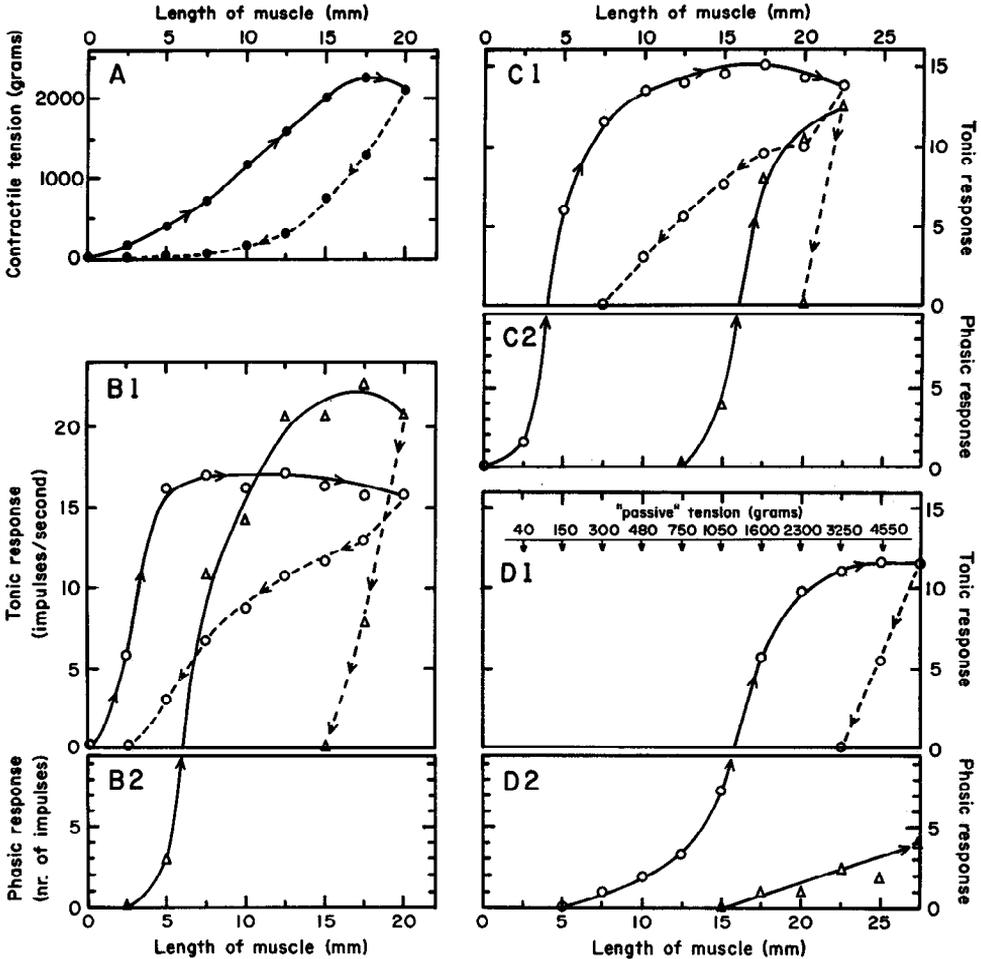


FIG. 5. Effect of cutting L_7 and S_1 ventral roots on responses of motoneurons in the stretch reflex. *A*: contractile tension developed by triceps surae muscles (*ordinate*) plotted against length of muscle (*abscissa*). Continuous line: stepwise stretch of muscle; broken line: release. *B*: responses of two motoneurons recorded from L_7 VR filament with remainder of root intact. Circles: small, low-threshold unit; triangles: larger unit. *B1*: tonic responses, with impulses/sec. plotted against muscle length. *B2*: phasic responses, with the number of impulses in each response on the *ordinate*. Data for *A* and *B* recorded simultaneously on moving film. *C*: as in *B* after cutting L_7 ventral root. *D*: as in *B* and *C* after L_7 and S_1 ventral roots were cut. Passive or "elastic" tension of muscle shown on inset scale corresponds to length of muscle on *abscissa*. "Contractile tensions" in *A* were obtained by subtracting the passive tensions (*D*) for each length of the muscle from the total measured tensions. Each point on all the graphs is the mean of four successive runs.

DISCUSSION

The size-principle and the apparent exceptions to it

The results described above reveal a highly significant correlation between the threshold or excitability of individual neurons and the size of the

impulses recorded from their axons. Translating impulse size into fiber diameter (7, 8) and fiber diameter into cell size (see following section), we may conclude that there is a general rule or principle applying specifically to motoneurons and perhaps to all neurons, according to which the size of a cell determines its threshold. This size principle dictates the order of recruitment in the stretch reflex and, as previously shown, in the flexor reflex (13). Before we discuss the biophysical basis of the size principle or its functional implications, it is appropriate to consider the apparent exceptions to the rule, which constituted about 15% of the pairs of units. In particular we would like to distinguish between true or real exceptions and those attributable to technical artifacts.

It is not difficult to surmise how true exceptions might occur. The stretch reflex in a decerebrate preparation is a mechanism of some complexity. Stretch elicits afferent discharges from three types of receptors whose reflex effects differ qualitatively and quantitatively. Two of these receptors, tendon organs and secondary flower-spray endings, supply input to spinal circuits which are inhibitory to extensor motoneurons such as the triceps group; one type, the annulospiral ending, supplies excitatory impulses. We may assume that the triceps motoneurons in these experiments were subjected to mixed excitatory and inhibitory input from these sources, possibly from some undenervated receptors in the hind limb and from higher levels in the spinal cord and brain stem. The response of the motoneurons therefore represents the net effect of a complex process of integration. In view of the many factors influencing motoneurons, some exceptions to the size principle might be expected to occur due to slight variations in the precise mixture of excitatory and inhibitory impulses impinging on different cells in a pool. There is, however, no evidence that any physiologically explainable exceptions actually occur and no necessity for assuming that they do, because a more likely explanation for reversed recruitment is available.

There are good reasons for explaining the majority of the reversals as artifacts arising from the experimental procedure. Despite all precautions, the separation and subdivision of ventral root filaments is frequently damaging to some of the fibers in them. The occurrence of conduction blocks, revealed by inversion of negative action potentials, indicates serious injury. Blocking is easy to recognize, but the incidence of lesser damage, which may result in reduced amplitude of action potentials, is hard to estimate. Fibers on the outside of filaments where the shearing forces are greatest during the separation probably suffer most. In one experiment (III of Table 1) records were taken deliberately from filaments showing some evidence of damage, i.e., prodromal positivity of more than 10% of the total amplitude of the spike. These filaments contained 15 pairs of units, of which 27% showed reversed recruitment. The apparently undamaged pairs of units in the same experiment had 15% reversed recruitment.

In view of the higher percentage of exceptions in damaged filaments and the lack of evidence regarding the occurrence of real or physiologically ex-

plainable reversal of recruitment, we believe that the incidence of true exceptions to the size principle is considerably lower than the total of 13–15% shown by our statistical analysis.

Relation between size of a cell and the diameter of its axon

Since the dimensions of neurons vary so widely, it is curious that studies of this aspect of morphology are scarce. So far as we can learn no systematic investigation has been made of the relation between the size of a cell and the diameter of its axon. The most reasonable assumption is that, since they are parts of the same cell, they are scaled in proportion to each other. Cajal discusses this problem and concludes (1, pp. 52, 53) that the size of a cell is related to the diameter of its axis cylinder and especially to the number and thickness of its collaterals and terminals. As examples of large cells with large axons he cites spinal motoneurons, giant "corpuscles" of the torpedo, Golgi cells of the cerebellum, horizontal cells of the retina and, as a case of special interest, the Mauthner cell. As examples of small cells with thin axons which give off few collaterals, he cites granular cells of the cerebellum, bipolar cells of the retina, and the granular cells of the fascia dentata. In chapter XV (ref. 1), which deals with dorsal root ganglia, Cajal points out that ganglion cells of large volume have thick axons and those of small volume have thin axons (see ref. 1; Fig. 157, p. 428; Fig. 158, p. 430; Fig. 159, p. 432). Many other examples could be cited. The giant cells which have been described in various species invariably have large axons. Small cells never do. In the mammalian nervous system there seem to be no striking exceptions to this general trend. In the absence of any contrary evidence we may, therefore, conclude that the diameter of an axon is a reliable index to the size of its cell.

A tentative explanation of the relation between size and excitability

The biophysical basis for the relation between the size and excitability of motoneurons is of great interest because it may indicate how general this relation is throughout the nervous system. We have no definitive explanation to offer at present although various possibilities have been considered. Among them we may mention the following. 1) The input to small cells may be more potent than to large cells due to the location, arrangement, or greater relative size of the presynaptic terminals. There is no evidence, however, to substantiate any of these possibilities. 2) Small cells may receive relatively more excitatory input from stretch receptors and cells connected with them. This suggestion will be disposed of effectively in a paper which will appear later. 3) The liminal depolarization needed to fire a cell may be related to its size. Kuno has shown (18) that the resting membrane potential is not correlated with cell size. The firing threshold of small cells, however, may be lower than that of large cells. 4) An equal degree of presynaptic excitation may generate a larger synaptic potential in small cells due simply to their dimensions and geometry. As indicated below, there is pertinent, though not conclusive, evidence to support this last hypothesis.

In a study of miniature end-plate potentials (EPPs) in muscle fibers of various sizes in the frog, Katz and Thesleff (16) reported that their mean amplitude varied over a range of more than 10 to 1. They showed that the input resistance⁴ of muscle fibers, which varies inversely with their diameter, is correlated with the amplitude of their miniature EPPs: the smaller the fiber, the larger the input resistance and the bigger the miniature EPP. Since miniature EPPs of different amplitudes are due to the release of equal quantities of transmitter, it is clear that the shape and size of a cell may determine the effectiveness of current passing through the membrane in bringing about depolarization.

Does the size of a motoneuron influence the amplitude of its synaptic responses in the same way and thereby determine its excitability? We cannot assume that observations on simple, cylindrical cells with localized junctions apply to neurons with their complex geometry and widespread synapses. The information required to answer this question by means of a theoretical analysis is not available. What is available, however, is a piece of experimental evidence indicating that synaptic responses are larger in small than large cells. The "aggregate EPSP," i.e., the intracellularly recorded response of a motoneuron to maximal stimulation of its group IA fibers, were investigated by Eccles, Eccles, and Lundberg (3). Responses recorded from the motoneurons of red muscles such as soleus were significantly larger on the average than those from the motoneurons of pale muscles such as m. gastrocnemius. It is known (5, 21, 25) that the motor nerve fibers to soleus as a group are smaller and more slowly conducting than those of m. gastrocnemius and that soleus motoneurons are, in general, more readily discharged (2). The aggregate EPSP may not be the ideal criterion for our purposes since the IA inputs to different motoneurons may not be comparable. The observations, however, offer a tentative explanation for our results, which is consistent with the observations on muscle fibers.

Electrical excitability and physiological excitability

The true measure of excitability should be the ease with which a neuron may be discharged via a normal physiological mechanism. Unfortunately, "excitability" is frequently regarded as synonymous with electrical excitability, expressed as the current or voltage required to discharge a neuron. This is usually not justified. For example, the classical findings of Erlanger and Gasser (7) on the electrical excitability of peripheral axons showed that fibers of small diameter are relatively inexcitable compared with larger ones. These findings are perfectly valid, but they are not relevant in considering synaptic events. In stimulating nerves with external electrodes only the portion of the current which flows inside the fiber and across the membrane is effective. The relation between fiber diameter and electrical threshold is determined by properties of the nerve fiber (such as the internal resistance per unit length) which have little to do with its normal response to synaptic

⁴ The resistance offered by the cell to the flow of current between an electrode inside the cell and one outside the cell.

excitation. There is no reason, therefore, to regard electrical excitability as an index of physiological excitability.

As we have shown, the excitability of a motoneuron varies inversely with the diameter of its axon. The fact that small cells are easier to discharge synaptically seems to make sense in terms of energetics. The impulse of a small cell represents a small quantum of dischargeable energy. That it requires less input (synaptic energy) to discharge a small quantum than a large one is not surprising. Viewed in this way, neurons of different sizes represent different quanta of dischargeable energy available for use through the application of different amounts of input energy.

The size-principle and the organization of the motoneuron pool

The motor fibers supplying a muscle group such as the triceps surae range in diameter from about 1 to 20 μ . The cells with which these fibers are connected centrally exhibit properties which vary systematically with their sizes. At the lower end of the size scale are the cells whose axons range in diameter from about 1 to 8 μ . These gamma motoneurons innervate muscle spindles. Their tendency to fire continuously, which is evident in Figs. 1 and 2, is apparently a functional expression of their small size. Presumably they have a higher input resistance than the larger alpha cells and as a consequence are more susceptible to discharge. Ordinarily their greater excitability is manifest in recordings from ventral root filaments or fine muscle nerves as continual firing, which goes on even in resting or anesthetized animals when no alpha discharges are occurring. The afferent inflow from receptors in the resting limb is evidently sufficient to maintain activity in a considerable number of gamma neurons at all times. It is, in fact, difficult to find filaments without some spontaneous gamma activity. When such filaments are found and various types of graded reflex discharges (except stretch reflexes) are elicited, they appear at lower threshold in gamma fibers. From a control standpoint it is appropriate that the gamma system which is an essential part of the servoloop controlling muscle, should be fashioned of smaller and therefore more sensitive elements (nerve cells, nerve fibers, and intrafusal muscle fibers) than the alpha system.

In the middle and upper end of the size scale are the cells whose fibers range from about 9 to 20 μ . in diameter. Except in spastic or rigid preparations these alpha cells have a sufficiently high threshold so that they do not discharge when the limb is at rest. We have shown that they are recruited into the stretch reflex in an order dictated by their relative sizes. It is, however, unlikely that the tonically responding units which we studied statistically are a fair sample of all the triceps motoneurons.

The rates of tonic discharge we observed were usually in the range from 5–30/sec. and seldom exceeded 35/sec. Such rates would be relatively ineffective in developing tetanic tension in most pale motor units (25). The tonically responding units which formed the main basis of our study were probably motoneurons innervating “slow” or “red” muscle fibers. They

were presumably made up, in large part, of soleus cells and, to a lesser extent, of small cells in the gastrocnemius pools. The phasic responses which were encountered were probably those of larger cells supplying "pale" motor units. The rates of discharge required to produce maximum tension in pale units, however, were not seen in this study.

In attempting to organize and systematize his observations on spinal reflexes, Sherrington evolved the concept of the motoneuron "pool." He conceived of the pool as a population of cells which was "fractionated" by an excitatory input into a "discharge zone" and a "subliminal fringe" of cells excited but not fired. "Facilitation" occurred when the subliminal fringes associated with different inputs overlapped and "occlusion" occurred when the discharge zones overlapped (22, 23).

In the light of our results the concept of the motoneuron pool may be redefined as follows. "Fractionation" occurs because of the differences in excitability or threshold of cells of different size. Whatever the extent of the discharge zone may be at any time, it consists of all the alpha motoneurons whose thresholds are below a certain level, or, according to our size principle, of all the cells below a certain size. The larger cells with higher thresholds constitute the subliminal fringe. Recruitment and "decrutment" involve the group of cells which form the shifting boundary between discharge zone and subliminal fringe. Facilitation simply represents an expansion of the discharge zone due to additional excitation. Occlusion occurs because this expansion is not necessarily proportional to the sum of the inputs.

In the case of the stretch reflex which we investigated, the cells within the discharge zone were, according to our view, those with sufficiently low thresholds to fire "tonically." Cells of slightly larger size which responded with brief bursts of discharge comprised a phasic group within the subliminal fringe. The remainder of cells in the pool were those with such high thresholds that our slowly applied stretches did not suffice to discharge them. It appears that cells should not be divided into fixed tonic and phasic types because, depending upon the relation between their size and the existing level of excitatory input, they may behave in either mode.

The essence of the motoneuron pool, as redefined, is the spectrum of sizes represented in it. So far as one can judge at present, the input to the pool is distributed equally to all the alpha cells in the pool in accordance with their relative sizes. The distribution of sizes and thresholds in the pool makes of it an anatomically "built-in" grading mechanism, one which responds automatically to any input and emits an appropriately "sized" output. The spectrum of sizes is also a spectrum of energy levels, for it represents the varying amounts of contractile energy which can be triggered off peripherally (14, 21, 25) and the corresponding amounts of synaptic energy required to do so.

The functional significance of the size of motoneurons and the distribution of sizes in the grading of muscular tension is taken up in the following paper (14) and need not be discussed here. It may be noted, however, that

this grading of output is reminiscent of the Weber fractions known in the field of sensory discrimination. We are not proposing a precise mathematical relationship for the motor system comparable to Weber's rule of the "... just noticeable difference" of sensation but the analogy is clear: the smallest increment that can be added to the force exerted by a muscle becomes greater as the force of contraction itself increases.

Usage of motoneurons

One consequence of the size principle as it applies to motoneurons is that the various cells in a pool are fired rarely, moderately, or frequently according to their sizes. The over-all frequency of discharge or "usage" of a cell, i.e., is in inverse proportion to its size. We have no data from which to estimate how widely usage varies for different alpha motoneurons participating in a normal range of motor activities. The rates of discharge observed in stretch reflexes were generally well below those required to produce fused tetanic contractions in large motor units, which suggests that large cells are seldom fired in the course of postural activities.

Although the metabolic consequences of usage are not insignificant for motoneurons, the cost of activity is necessarily far less than for the muscle fibers they innervate. The fact that all large motor units in the triceps are composed of pale muscle fibers and that small units tend to be red is perhaps related as much to the size of their motoneurons and the usage they undergo as to other factors (14).

In this connection Vrbová (24) has recently reported that tenotomy causes the soleus of the rabbit to alter from a slow to a fast muscle. She believes that the decrease in efferent impulses to the soleus, which is observed after its tendon is cut, is responsible for the change, for the concomitant increase in activity of the contralateral soleus results in a significant slowing of its contraction speed.

SUMMARY

1. In decerebrate cats the order of recruitment of individual motoneurons discharging reflexly in response to stretch of the triceps muscles was compared with the amplitude of the impulses recorded from their axons in ventral root filaments.

2. In general, the smallest motoneurons, i.e., those whose axons yielded the smallest spikes, had the lowest threshold to stretch, and the largest cells had the highest thresholds. By comparing each unit with all of the other units in the same filament, 165 combinations of twos were assembled. In 142.5 of them the smaller unit was recruited first, followed by the larger. In 22.5 combinations the order was reversed. The coefficient of rank correlation (Kendall) for all results combined was 0.73 and the probability of a random distribution of this kind was less than 0.001. In only 2 of the 165 comparisons did the order of recruitment change during the period of observation.

3. When stretch was gradually released, units "dropped out" of the dis-

charge in the reverse order of their recruitment. Exceptions to this rule (discordances) were found in only 11 of 236 possible pairs.

4. Only units capable of rhythmic firing for more than 1 sec. in the absence of gamma drive were used for statistical studies. At low levels of stretch they often responded phasically with brief bursts of impulses. Units which were incapable of sustained firing, but responded phasically, were often encountered and their spike amplitudes were generally greater than that of the largest tonically responding unit in the filament. Phasic and tonic behavior, however, are not fixed characteristics of motoneurons. A cell may respond in either mode according to the prevalent level of excitation.

5. The distribution of stretch thresholds for motoneurons was compiled in a histogram (Fig. 3), which resembles the distribution of maximum tensions of motor units. A "synthetic stretch reflex" was constructed (Fig. 4) for comparison with experimentally obtained data on whole muscle.

6. In five cats recruitment was studied before and after sectioning the L_7 and S_1 ventral roots. Interrupting the gamma outflow decreased the responsiveness of all the motoneurons to stretch, but did not alter the sequence of recruitment in any filament.

7. The exceptions to the size principle are discussed and an explanation for most of them is proposed. Some of the histological evidence that the diameter of an axon is a reliable index to the size of the cell is reviewed.

8. A tentative explanation of the relation between size and excitability is advanced, which is based on evidence that the shape and size of a cell may determine the effectiveness of current passing through the membrane in bringing about depolarization.

9. The size principle is discussed in relation to the organization of motoneuron pools. "Fractionation," "discharge zone," "subliminal fringe," "facilitation," and "occlusion" are redefined briefly in terms of the sizes and excitabilities of the cells in a pool.

10. It is pointed out that cell size determines the over-all frequency of discharge or "usage" of a motoneuron, and some implications of this are discussed.

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