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Postsynaptic Population Potentials Recorded From Ventral Roots Perfused With Isotonic Sucrose: Connections of Groups Ia and II Spindle Afferent Fibers With Large Populations of Motoneurons

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SUMMARY AND CONCLUSIONS

1. A method is described that makes it possible to record a composite of all the individual EPSPs elicited monosynaptically in a large population of motoneurons by impulses in single Ia or spindle group II fibers.

2. By infiltrating a ventral root with an isotonic sucrose solution so that its extracellular resistance is extremely high, the interiors of its axons can be used as wick electrodes to record voltage changes in all the motoneurons of that spinal segment. With an averaging computer, triggered by the action potentials from a single, functionally isolated but intact afferent, the synaptic effect of a Ia or group II impulse on the whole population of motoneurons can be extracted from the physiological and electrical noise. Potentials with the general waveform of EPSPs can be recorded under these conditions. These responses are designated postsynaptic population potentials (PSPPs).

3. The general appearance of 230 PSPPs, elicited by impulses in 176 Ia, 52 spindle group II fibers, and 6 Ib fibers from medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LG-S) muscles was studied. The PSPPs were recorded from the first sacral ventral root or a caudal part of the seventh lumbar ventral root in cats anesthetized with α-chloralose.

4. The latencies of PSPPs were widely distributed. The mean latencies were 0.85 $\pm$ 0.27 ms for Ia responses and 1.19 $\pm$ 0.41 ms for group II PSPPs. This difference was attributed chiefly to slower intraspinal conduction in group II fibers. Peripheral and intraspinal conduction velocities were positively correlated.

5. Latency measurements led to the conclusion that both Ia and group II PSPPs were evoked monosynaptically, but polysynaptic effects were not excluded.

6. The rate of rise of the PSPPs showed a weak, but significant positive correlation with the conduction velocities of the afferent fibers, the mean rise time for Ia PSPPs being 2.54 $\pm$ 1.05 ms, that for group II PSPPs being 3.1 $\pm$ 1.2 ms. This correlation was taken as evidence that the distribution of the active terminals of group Ia and group II spindle fibers on the surface of motoneurons is governed in part by the size of the afferent fiber. The more distally located synapses from the group II fibers, generating slower EPSPs, might be chiefly responsible for the maintenance and regulation of the general excitatory state of the motoneuron pool. They would be well suited to process input from secondary endings, which are most sensitive to the static length of the muscle. In contrast, the more proximally located input would be more apt to cause rapid triggering of reflex discharges. This would be an appropriate response to input from primary receptors, which are most sensitive to rapid changes in muscle length. The sustained activity in the more distal dendrites produced by input from secondary endings may, therefore, modify the effectiveness of the input from primary endings.

7. The amplitudes of the PSPPs were positively correlated with the conduction

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velocities of the afferent fibers. The mean amplitude of Ia PSPPs was 1.28 ± 0.71 μV, that of group II PSPPs was 0.37 ± 0.19 μV. These findings were taken as evidence that a fast-conducting afferent fiber gives off more terminals to a large population of motoneurons than a small, slowly conducting afferent fiber does.

8. This paper extends the application of the size principle to primary sensory neurons. The data indicate that neuronal size may have the same general implications for sensory cells as for motoneurons.

INTRODUCTION

In earlier studies it has been shown that the neural energy required to discharge a motoneuron, the energy it transmits and releases in its muscle, its excitability and inhibitability, its mean rate of firing, and even its rate of protein synthesis are all correlated with its size (12, 13, 30, 35, 42, 48). This set of correlations has been referred to as the size principle (12). The influence of cell size on the functional properties of neurons has been demonstrated to such an extent in only one class of cells, the motoneurons. Equivalent information on the significance of cell size in sensory neurons is not available. It has been shown (31), however, that the amplitude of an EPSP evoked in a motoneuron by impulses from a single Ia fiber is directly related to the diameter of that fiber. Accordingly, the hypothesis has been advanced that a large Ia fiber gives off more terminals to a motoneuron than a small one. It is also possible that larger Ia fibers give off terminals to a greater number of cells, thus producing larger and more widespread excitatory effects in motoneurons. Until now there has been no direct way of measuring the total effect of impulses from a single fiber on a large population of motoneurons. This paper describes a method for doing so. It will be shown how perfusion of a ventral root with isotonic sucrose, in conjunction with spike-triggered averaging techniques, makes it possible to record the intracellular response of all the motoneurons in that root that receive both excitatory and inhibitory input from a single afferent fiber.

The summated EPSPs recorded from a root in this way are called postsynaptic population potentials (PSPPs). The amplitude and time course of a PSPP permit inferences regarding the total synaptic effect of a single Ia impulse on a large number of motoneurons belonging to several different pools. This study reveals a clear relationship between the total synaptic effect of the Ia impulse and the diameter of the fiber transmitting it. Evidence will also be presented that the distribution of the active terminals of group Ia and group II spindle fibers on the surface of motoneurons is governed by the size principle. Some of the findings reported here have been published in abstract form (27).

Terminology

The following terminology will be used in this paper to distinguish three kinds of EPSPs and the new potential (PSPP) described here for the first time.

1) Unit EPSP will refer to an all-or-none potential resulting from release of one quantum of transmitter. The size of a unit EPSP may vary with the site of the synapse and the properties of the motoneuron, as Kuno and Miyahara (21, 22) demonstrated.

2) Individual EPSP will designate the response to a single afferent impulse or the averaged response to many afferent impulses in the same fiber. An individual EPSP consists of a variable number of unit EPSPs. The term unitary EPSP, used in some recent publications, does not refer to unit EPSPs and should therefore be avoided or precisely defined.

3) Aggregate EPSP will designate an EPSP evoked by a synchronous volley in all of the Ia fibers of a muscle nerve (7). It represents the sum of many individual EPSPs recorded intracellularly.

4) PSPP will designate the composite of many individual EPSPs recorded from a large population of motoneurons via their ventral root. In this paper it will refer only to the averaged response elicited by many impulses in the same afferent fiber.

METHODS

Experiments were carried out on adult cats anesthetized with α-chloralose (70 mg/kg iv). The initial surgery was done under ether anesthesia. A laminectomy was performed to expose the sacral and lower lumbar segments of the spinal cord. The left hindlimb was denervated

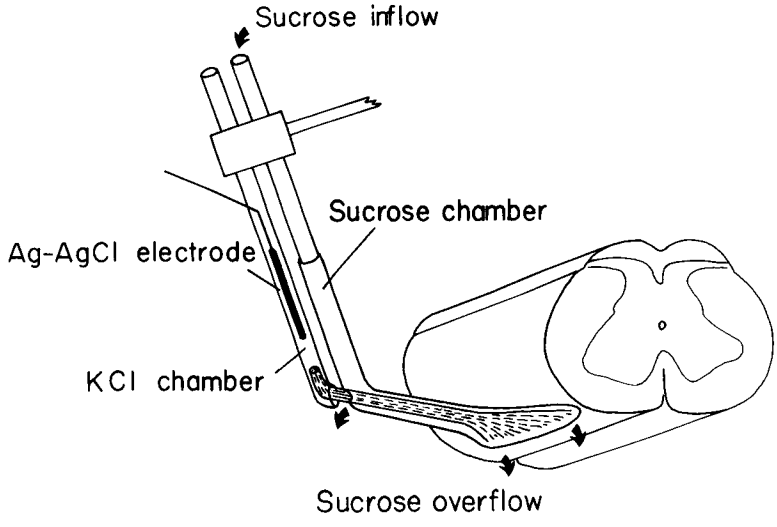

FIG. 1. Schematic view of the sucrose perfusion apparatus in situ. The device was immersed in the oil pool. The sucrose overflow collected in the deepest part of the oil pool, where it was sucked away when necessary.

except for the medial gastrocnemius (MG) and lateral gastrocnemius-soleus (LG-S). A very extensive denervation of the tail, buttock, and hip was also performed. The ventral roots (VRs) S_1, L_7, and L_6 were cut distally. S_1VR and L_7VR were dissected out of the cauda equina and the exit of the root was fully exposed to reveal the shape and extent of its exit zone. One of several sucrose baths was selected for each VR, according to the appearance of the exit zone, in order to get the best possible match of sucrose bath and VR.

The MG and LG-S muscle nerves were mounted on pairs of silver wires, which could be used either as stimulating or recording electrodes. Mineral oil pools were formed by pulling up skin flaps around the exposed tissues in the leg and spinal cord. Body temperature was held constant at 37°C by means of a heating pad and a thermostatically controlled heating lamp. Stretch was applied to the muscles with a rack and pinion, which was connected by means of strong thread to the bony attachment of the Achilles tendon.

The sucrose baths were constructed from polyethylene tubing of different sizes (Fig. 1). The tubes were formed to the appropriate shape by heating the polyethylene. After cooling, the shape of the polyethylene remained stable and slightly flexible. The end of the tube was flared into a flat funnel that was brought into contact with the VR exit zone, where rootlets usually fanned out over 2–3 mm. A hole was punched in the elbow of the tube. A set of such perfusing baths, each with a hole of different diameter, was prepared to cover the range of expected ventral root sizes. A KCl bath was constructed from glass tubing and formed into a simple suction electrode. The sucrose bath and suction electrode were mounted on a common stage (Fig. 1). The ventral root, usually S_1 but in a few cases the lower portion of L_7, was pulled very gently into the apparatus by means of a fine silk thread previously passed through the elbow hole of the polyethylene tube. In two experiments, duplicate baths and suction electrodes were applied to two roots. The apparatus was positioned so that the polyethylene funnel was actually touching the cord surface without exerting pressure on it. Care was taken to avoid twisting or pulling on the root, which is very susceptible to injury at the exit zone. Sucrose flow was maintained by means of a drip bottle at an approximate rate of 0.1 ml/min. The sucrose solution was prepared with distilled water, which had been further purified with an ion exchanger. A similar sucrose bath suitable for recording from ventral and dorsal roots in vivo has been described by Roberts and Wallis (39). More detailed information about the technique is discussed by Wallis et al. (46).

After setting up the sucrose apparatus, the cord was covered with warm mineral oil, held in a pool by skin flaps. The sucrose overflow collected in the deepest part of the oil pool and was sucked away when necessary.

Isolation of afferent units

By separating L_7 or S_1 dorsal root filaments with small glass hooks and pointed needles under a dissecting microscope, filaments small enough to contain only one to three stretch-sensitive muscle afferents were isolated. It was easier to

dissect the roots very close to their entry points where the filaments are spread out in a thin layer and natural dividing lines were easy to recognize. As many as 38 single muscle afferents were "isolated" in continuity in a single experiment. The filaments were lifted onto small platinum hooks for recording. After preamplification, the signals were led to a window discriminator (Haer) so that spikes of different amplitudes could be selected as trigger pulses for the signal averager. Better discrimination between afferent units in a single filament could often be achieved by first differentiating the signal (5). The shapes of the action potentials, rather than their amplitudes, were then used as criteria for discrimination. This procedure often resulted in a more stable trigger with less jitter. After their use, each filament was cut and put aside to get better access to new filaments.

Recording and averaging

For recording from the ventral root, a Ag-AgCl electrode was used in conjunction with a high-gain (10 K) and high-impedance (100 MΩ) AC preamplifier (Princeton Applied Research), which had a bandpass filter set at 3 Hz to 3kHz. A large Ag-AgCl electrode, wrapped in a piece of gauze soaked in physiological NaCl solution and placed under the skin on the side of the animal, served as an indifferent electrode. The preamplified signal was led to a signal averager (HP 5480A signal analyzer), which was triggered by the output of the window discriminator. With this recording arrangement, the synaptic effect of a single afferent impulse on the whole population of motoneurons in one spinal cord segment could be extracted from the physiological and electrical noise in the recording system. Potentials with the general waveform of an EPSP, but of somewhat longer duration, could be recorded. Potentials as small as 0.1 μV were recorded with a reasonable signal-to-noise ratio; 4,096 sweeps were usually averaged for Ia effects and 8,192 sweeps for the much smaller spindle group II effects. It was very important to make the triggering derived from the afferent spike as secure and consistent as possible to avoid "time-locking" between any 60-Hz hum in the afferent record and the ventral root recording.

Determination of conduction velocity of afferent impulse

To obtain the conduction time of the individual muscle afferents, spike-triggered averaging was used to extract an action potential from the neurogram recorded from the intact MG and LG-S muscle nerves. The trigger signal used for this purpose was the same as in averaging from the ventral root. In order to do this "back averaging," the signals from the dorsal root filament and from the muscle nerves were delayed by means of a tape recorder or a two-channel analog delay line (Midgard) (18, 19). This procedure not only yielded a reliable measurement of the conduction time of the afferent impulse, but also provided information about the muscle origin of the afferent impulse. In addition, phase locking between the triggering spike and any other potentials could be revealed by this back averaging (47). No evidence of additional phase-locked impulses was ever noted. At the end of each experiment, the sciatic nerve was exposed for its entire course from the triceps surae up to the spinal cord in order to measure the conduction distance. The maximal error in calculating the conduction velocity by this technique was estimated to be less than 5%.

All the statistical tests were carried out by the Dept. of Biostatistics (Harvard School of Public Health) on the Sidney Farber Cancer Institute computing facilities.

RESULTS

Conduction velocities of afferent impulses

Two-hundred thirteen afferent fibers that were sensitive to muscle stretch were isolated in continuity in L_7 and S_1 dorsal root filaments. Of these, 159 came from the MG, 54 from the LG-S. Of the MG units recorded, 81% came from S_1DR filaments and the remainder from L_7DR, whereas only 30% of the LG-S units were isolated from S_1DR. In Fig. 2, the conduction velocities of these afferent fibers, grouped by pentads, are plotted against the percentage of the total number (n) of fibers. The distribution is skewed and unimodal, with a peak at 85–90 m/s. Following Hunt (14), the dividing line between group Ia and group II fibers was set at a conduction velocity of 72 m/s. In general, units with conduction velocities below 72 m/s showed a very regular firing pattern characteristic of secondary endings (44). However, the variability in discharge pattern was not studied systematically. Afferent fibers were classified solely on the basis of conduction velocity.

General appearance of PSPPs

The waveform of a PSPP closely resembles that of an individual EPSP, but has a somewhat longer duration and a considerably slower rising phase. The size of the PSPPs recorded in these experiments, however, was much smaller than that of the EPSPs

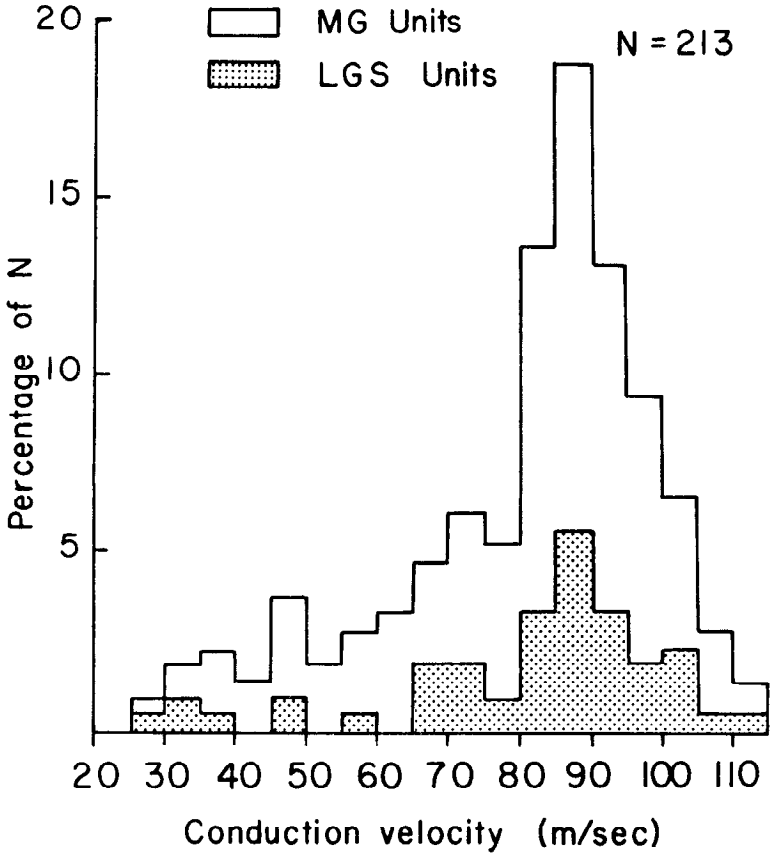

FIG. 2. Histogram of the conduction velocities of all the afferent fibers investigated, grouped in pentads.

elicited by impulses from Ia (31) or spindle group II afferents (19, 43). The mean amplitude of 174 PSPPs evoked by Ia impulses was 1.28 ± 0.71 μV, whereas that of 57 PSPPs elicited by group II impulses was only 0.37 ± 0.19 μV. A representative sample of PSPPs elicited by Ia impulses from MG is reproduced in Fig. 3. All of them were recorded from S_1VR. Since the afferent impulses were recorded very close to the point where the dorsal root filament entered the spinal cord, the delay from the onset of the sweep to the beginning of the rising phase of the PSPP must be due entirely to the sum of the central conduction time of the afferent fiber and the synaptic delay. The rise times of PSPPs are much less variable than those of individual EPSPs (31, 34, 41, 47), but they are not identical for different afferent units.

PSPPs were often recorded with slower sweep speeds than in Fig. 3 in order to examine their full time course and any subsequent deflections. In the great majority of cases, the falling phase reached the base line and showed no sign of an undershoot. In several instances, however, the PSPP was followed by a clear late hyperpolarization with a slow time course, a sequence that has not been observed with individual EPSPs. Figure 4 shows three examples of a late hyperpolarization.

In six cases, impulses in Ib fibers were used to trigger the averager. In general, no obvious response was observed or a very small hyperpolarization was recorded. Figure 5 illustrates the best example of a probable inhibitory PSPP that was obtained. Its latency is 1.0–1.5 ms. The time course of these inhibitory PSPPs was considerably prolonged compared with that of IPSPs recorded intracellularly.

Figure 6 shows a collection of PSPPs produced by impulses from slowly conducting afferent fibers, which probably had their ori-

gin in secondary endings. The waveform of these responses did not differ much from that of PSPPs elicited by Ia impulses, except for their much lower amplitude. These group II responses were usually averaged over 8,192 sweeps in order to improve the signal-to-noise ratio. Late hyperpolarization was never detected in group II PSPPs.

The firing rates of the afferent units from which the trigger pulses were derived varied widely from one unit to another. No attempt was made to adjust the muscle stretch in order to get a similar firing rate for each unit. However, several PSPPs evoked by the same unit were recorded at different firing rates. No obvious change in the shape or the amplitude of the PSPPs due to a change in the firing rate of the afferent unit was observed.

Latency: central conduction time and synaptic delay

Latency measurements have frequently been used, not only in intracellular recordings but also in ventral root responses (1, 24), to determine how many synapses there are in a neuronal circuit. The problems of doing so have been discussed in a great number of studies (1, 7, 8). Figure 7 shows latency histograms constructed from measurements of 176 Ia responses and 52 group II responses. Histograms were constructed separately for Ia and group II responses and are shown superimposed. The latency plotted in Fig. 7 is the time elapsing from the start of the sweep to the beginning of the rising phase of the PSPP. The sweep of the averager was triggered by afferent spikes recorded as much as 5 mm distal to the cord entry point of the filament. This additional conduction distance may account for the 0.1 ms longer mean latency in Ia responses observed in our study compared with the mean latency found by Watt et al. (47). The shortest latency for both Ia and group II responses was 0.4 ms, whereas the mean values were 0.85 $\pm$ 0.27 and 1.19 $\pm$ 0.41 ms, respectively. Statistical testing revealed that the difference in latency of Ia and group II PSPPs was significant ($P < 0.001$).

The latency of a PSPP in these experiments is presumably a composite of all the

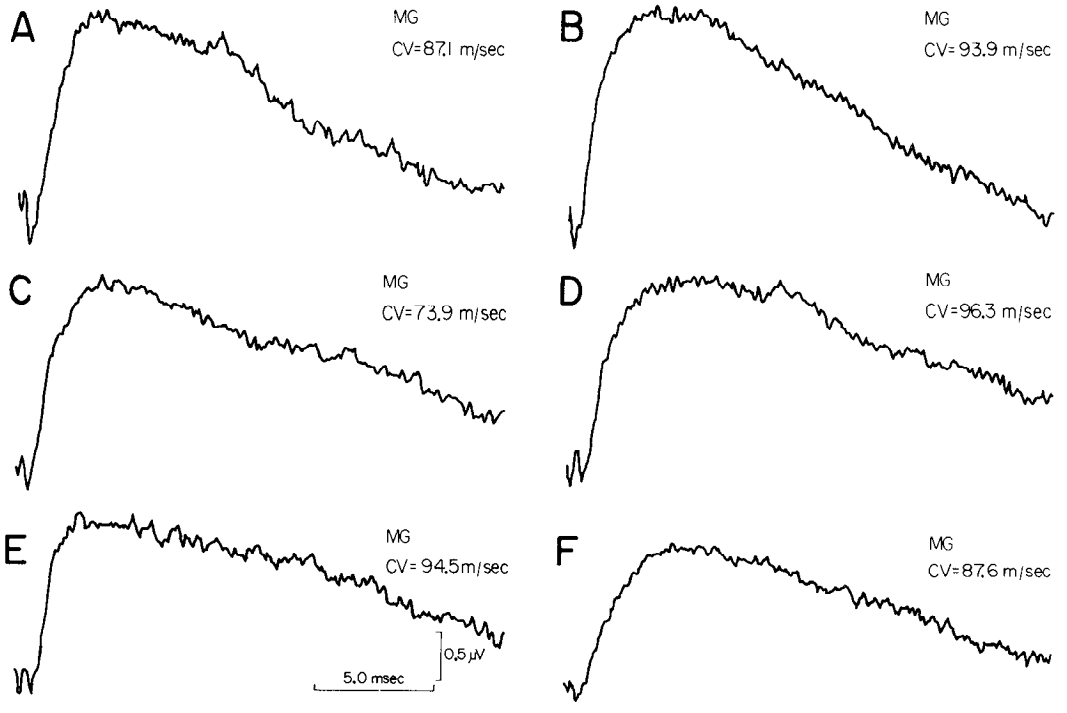

FIG. 3. Examples of typical PSPPs elicited by impulses in MG Ia fibers and recorded in S_1VR. Each PSPP was averaged over 4,096 sweeps.

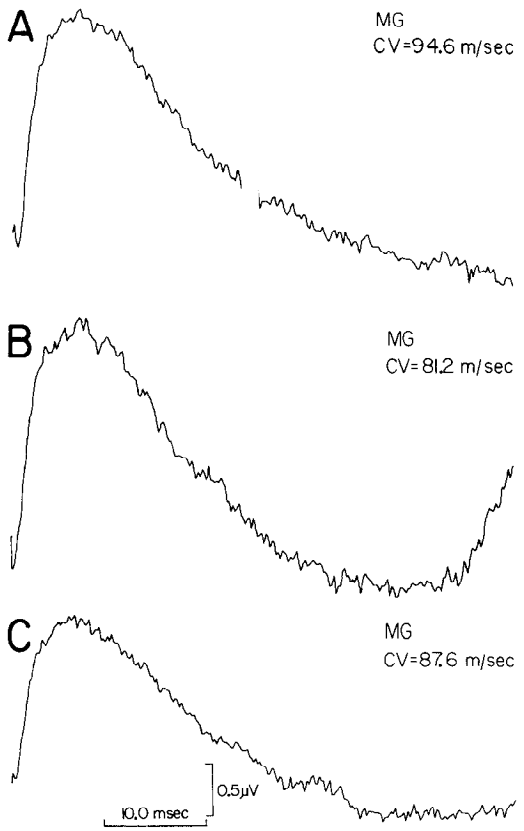

FIG. 4. Examples of PSPPs elicited by impulses in Ia fibers of MG and recorded in S_1VR, which exhibit a clear late hyperpolarization. Each PSPP was averaged over 4,096 sweeps.

latencies in the whole population of neurons, but it is determined chiefly by the EPSPs with the shortest delays. Ia impulses are transmitted directly to motoneurons (7) and the most direct pathway for group II impulses is also monosynaptic (18, 19, 26, 44). It was surprising, therefore, to find latencies as long as 2.2 ms for some Ia responses and 3.0 ms for group II responses. It is very unlikely that any Ia or group II afferent would make no excitatory monosynaptic connections with a large population of motoneurons; hence, these longer latencies were attributed to unusually slow conduction in the spinal cord rather than to disynaptic connections.

In 65 Ia responses and 20 group II responses, a small, usually triphasic wave preceded the PSPP. This wave was considerably broader in group II responses than in Ia responses. A similar wave seen in intracellular recordings (43, 45) has been attributed to the arrival of the impulse in the presynaptic axon or its terminals (16). Stauffer et al. (43) refer to that wave as the presynaptic spike (Pre-SS). The Pre-SS permits one to divide the latency, measured from the arrival of the afferent impulse to the start of the PSPP, into "central conduction time" and "synaptic delay." Figure 8*A* and *D* illustrates how the central conduction time (CT) and the synaptic delay (SD) were determined in Ia and group II responses, respectively. Note that the Pre-SS is broader in the group II response (Fig. 8*D*) than in the Ia responses (Fig. 8*A*).

Histograms of the synaptic delay (Fig. 8*C*, *F*) show the most common value to be 0.5 ms for both Ia and group II afferents; the mean values are 0.57 ± 0.16 and 0.67 ± 0.22 ms, respectively. This small difference in synaptic delay is significant by t test ($P < 0.04$). Histograms of the central conduction times are shown in Fig. 8*B* and *E*. The most common values are 0.3–0.4 and 0.4–0.6 ms for Ia and group II afferents, respectively; the mean values are 0.29 ± 0.12 and 0.55 ± 0.16 ms for Ia and group II responses. The central conduction time is, thus, about 0.26 ms longer for group II than for Ia afferents. The mean latency for all ($n = 176$) Ia PSPPs is 0.85 ms and for all ($n = 52$) spindle group II afferents is 1.19 ms, as previously described. The difference in latency of Ia and group II PSPPs, which is 0.34 ms, therefore consists of the difference in synaptic delay (0.09 ms) plus the difference in central con-

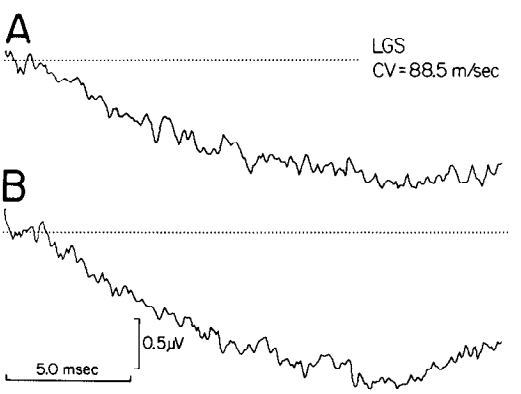

FIG. 5. Examples of PSPPs elicited by impulses in a Ib fiber of LG-S. Simultaneous recordings were made from S_1VR (*B*) and the lower part of L_7VR (*A*) and averaged over 4,096 sweeps.

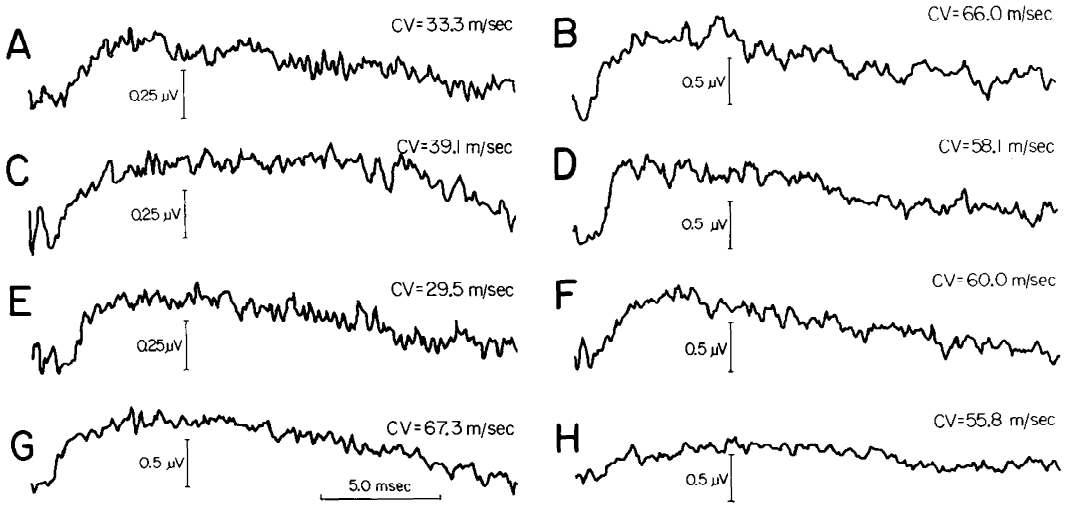

FIG. 6. Examples of PSPPs elicited by impulses in group II fibers of MG, recorded in S_1VR. Note different voltage calibrations but equal time scales. PSPPs in *A*, *C*, *E*, and *H* were averaged over 8,192 sweeps, the remainder over 4,096 sweeps.

duction time (0.26 ms). Slower central conduction accounts chiefly for the longer central latency of group II responses. This agrees with the data presented by Stauffer et al. (43) and Fu et al. (9). Strangely, neither group found any correlation between peripheral conduction velocity and central conduction time. The present data, based on 84 measurements of central conduction times, suggest a weak but significant correlation ($r = -0.7206$; $P < 0.001$) as would be expected, the central conduction time being longer for slowly conducting afferents than for faster ones (Fig. 9). Even taking the two groups separately, a significant correlation between peripheral conduction velocity and central conduction time was apparent, the correlation coefficients for Ia and group II being -0.3556 ($P = 0.0025$) and -0.6891 ($P < 0.001$), respectively.

Rise time of PSPPs

The great variety in the shapes of individual EPSPs (31) was attributed to differences in the location of the active synapses on the motoneuron surface. Rall (38) has calculated that synapses located on the cell body or proximal dendrites give rise to fast-rising EPSPs, whereas remotely located synapses elicit EPSPs with slow-rising phases. There is no anatomical or physiological evidence that the terminals given off by a particular afferent fiber to a large number of cells are distributed to the same compartment of each motoneuron. On the contrary, Fig. 6 in Mendell and Henneman's paper (31) shows that the EPSPs evoked in

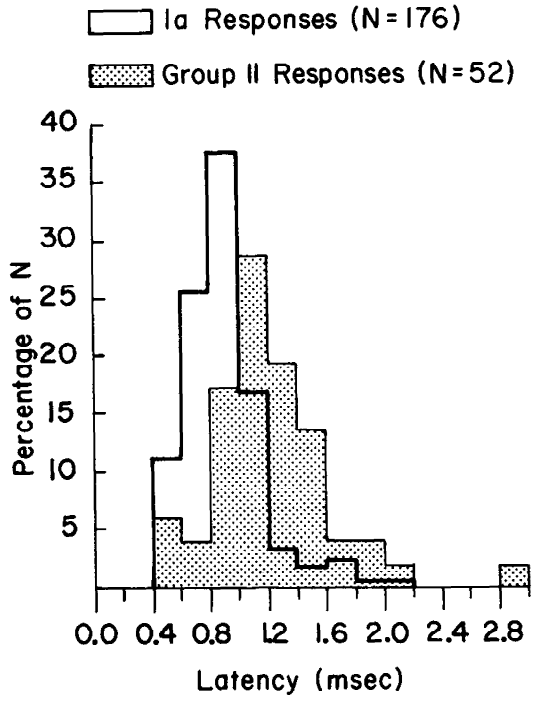

FIG. 7. Histograms of latencies of all PSPPs evoked by impulses in groups Ia and II afferents studied in this paper.

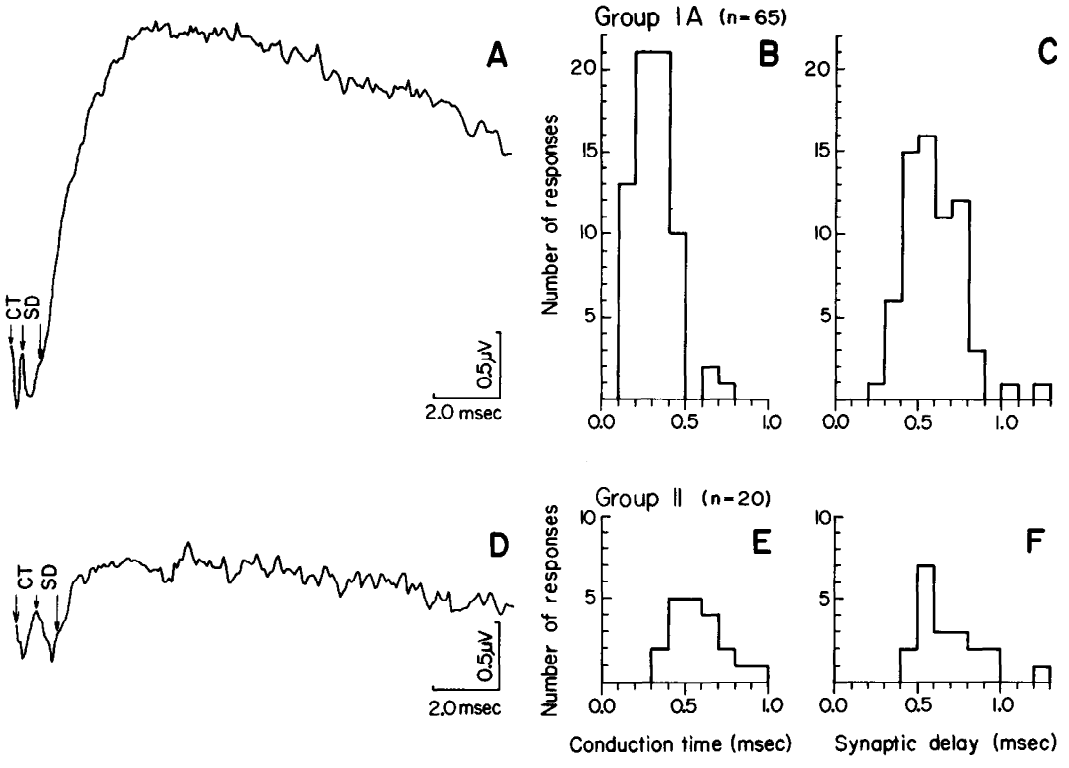

FIG. 8. Measurement of central conduction time (CT) and synaptic delay (SD) in a Ia- (*A*) and a group II- (*D*) elicited PSPP. Note the broader presynaptic spike in the group II PSPP. Histograms of central conduction time for Ia PSPPs (*B*) and group II PSPPs (*E*). Histograms of the synaptic delay for group Ia PSPPs (*C*) and group II PSPPs (*F*).

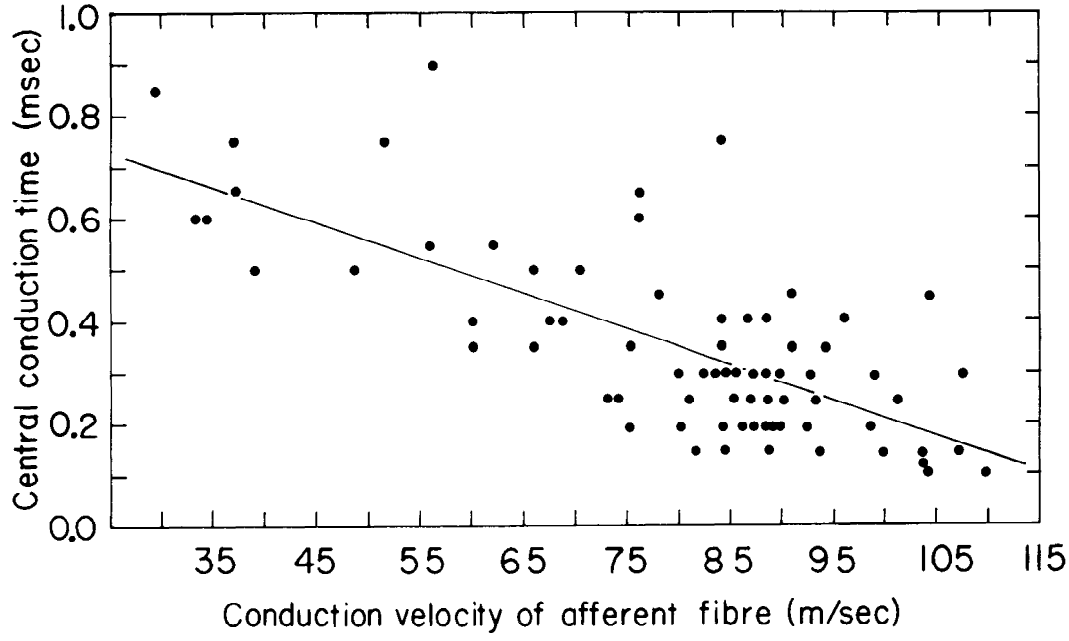

FIG. 9. Plot of central conduction time versus peripheral conduction velocity of afferent fiber. The straight line was fitted by the method of least squares.

six different motoneurons by impulses in the same Ia fiber have shapes that differ widely. This suggests that the synapses on the six motoneurons were located on different compartments. If the synapses given off by Ia fibers from a particular muscle are distributed randomly over the cell bodies and dendrites of a large population of motoneurons, the rise times of the PSPPs elicited by impulses in any one of them should be approximately the same since they represent a composite of all the rise times occurring in the population. The rise time of a PSPP should be considerably slower than the rise time of an individual EPSP, however, because of the additional electrotonic conduction distance introduced by recording from the ventral root. The distance from the cell body to the cord exit of the axon is different for each cell in the population, but the average distance is always the same in a given experiment and should not contribute to systematic variations in rise time. Differences in rise times, therefore, probably reflect differences in the average location of the synapses on the motoneurons.

As expected, the mean rise times of Ia PSPPs and spindle group II PSPPs were considerably longer than the rise times observed in individual EPSPs. The mean rise time for Ia PSPPs was 2.54 ± 1.05 ms, whereas Stauffer et al. (43) give a value for individual EPSPs of 1.0 ± 0.5 ms. The value given by the same authors (43) for spindle group II EPSPs was 1.59 ± 0.36 ms, whereas the mean value for group II PSPPs was 3.17 ± 1.25 ms. Figure 10 shows the superimposed histograms of the rise times of all Ia- (n = 171) and all group II- (n = 47) evoked PSPPs. The histogram for the group II re-

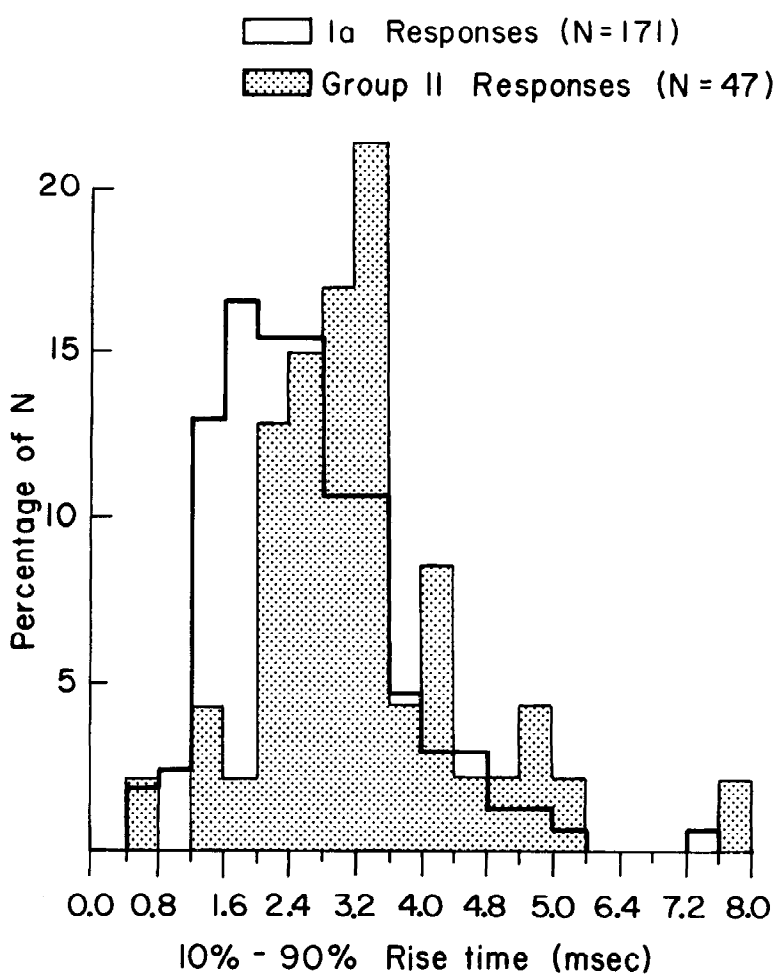

FIG. 10. Histograms of the rise times of all Ia and group II PSPPs studied in this paper.

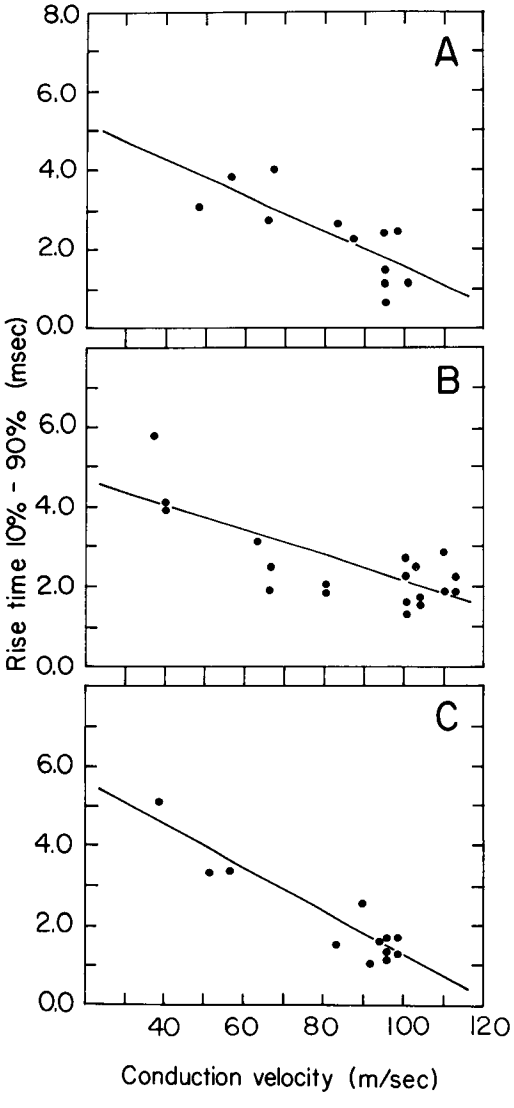

FIG. 11. Plots of the relation between the rise time of the PSPP and the conduction velocity of the afferent fiber in three different experiments. Straight lines were fitted by the method of least squares.

sponses is slightly shifted to higher values. Statistical testing revealed that this difference in rise times was significant ($P < 0.001$).

If the rise times of PSPPs are plotted against the conduction velocities of the afferent fibers in each case, a relationship between the two variables becomes apparent. In Fig. 11*A–C*, the data of three experiments are represented graphically. The straight lines drawn through each set of data points were plotted by the method of least squares. In other experiments, the correlation was less apparent. Figure 12 illustrates the correlation between these variables in data pooled from 215 PSPPs. The straight line was drawn by the method of least squares. Statistical analysis revealed a weak but significant correlation ($r = -0.3331$; $P < 0.001$) between conduction velocity and rise time. Very small PSPPs evoked by group II afferents were not accepted in the data pool because it was almost impossible to measure their rise time with accuracy and reliability. Consequently, the data points on the left half of the plot were subjected to a selection process that favored PSPPs with faster rise times. This tended to weaken the apparent relationship between these variables in the pooled data.

Amplitude of PSPPs

In a recent paper by Mendell and Henneman (31) it was shown that the amplitude of individual EPSPs is related to the conduction velocity of the Ia impulses evoking them. The hypothesis advanced to explain this relationship was that a faster and, therefore, larger Ia fiber gives off more terminals to a single motoneuron than a slower and smaller fiber does. It is uncertain whether this inference is correct, however, because the amplitude of an individual EPSP may be strongly influenced by the input resistance of the motoneuron (3, 17) and the location of the synaptic terminals (38). Since a PSPP is a composite of individual EPSPs recorded from a large number of motoneurons, the effects of individual variations in input resistance and synaptic location are averaged and the total impact of a given impulse on the whole population of motoneurons can be determined and related to the number of terminals.

In Fig. 13, the amplitude of each PSPP is plotted against the conduction velocity of the afferent fiber. All the 117 data points shown were obtained with MG muscle afferents, which entered the spinal cord through S_1DR, and all PSPPs were recorded from S_1VR. Although the degree of correlation was not exactly the same from one experiment to another, the conduction velocity of the afferent fiber and the amplitude of the PSPP were obviously related in all 16 experiments in which enough units were examined to establish a trend.

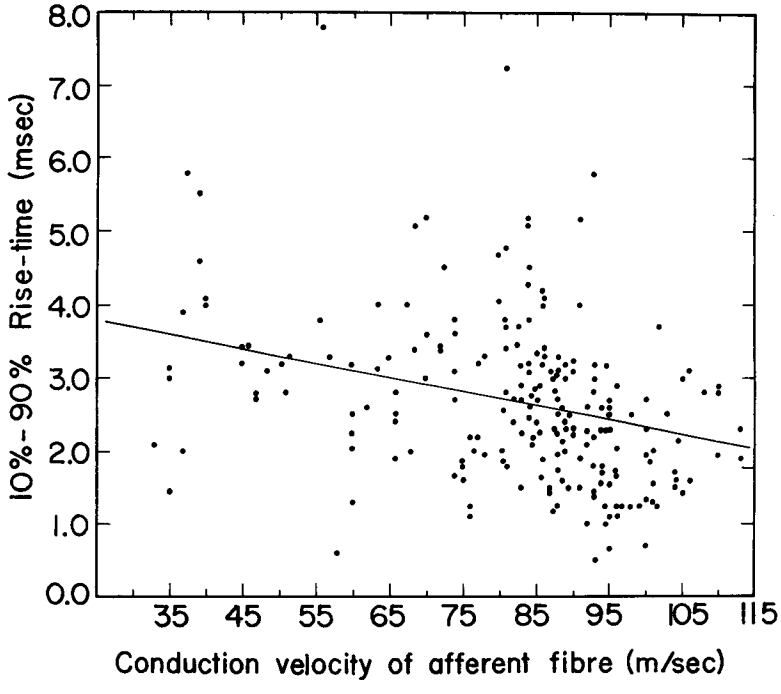

FIG. 12. Plot of the rise times of 215 PSPPs versus the conduction velocities of the afferent fibers. The straight line was fitted by the method of least squares.

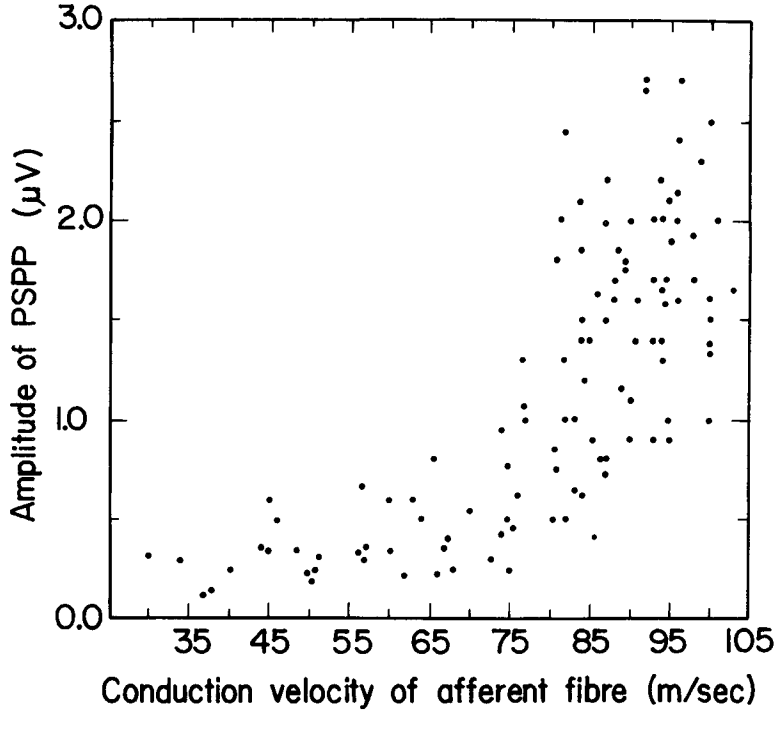

FIG. 13. Plot of the peak amplitudes of the PSPPs versus the conduction velocities of the afferent fibers. All PSPPs were recorded from S_1VR and were elicited by impulses in MG muscle afferents.

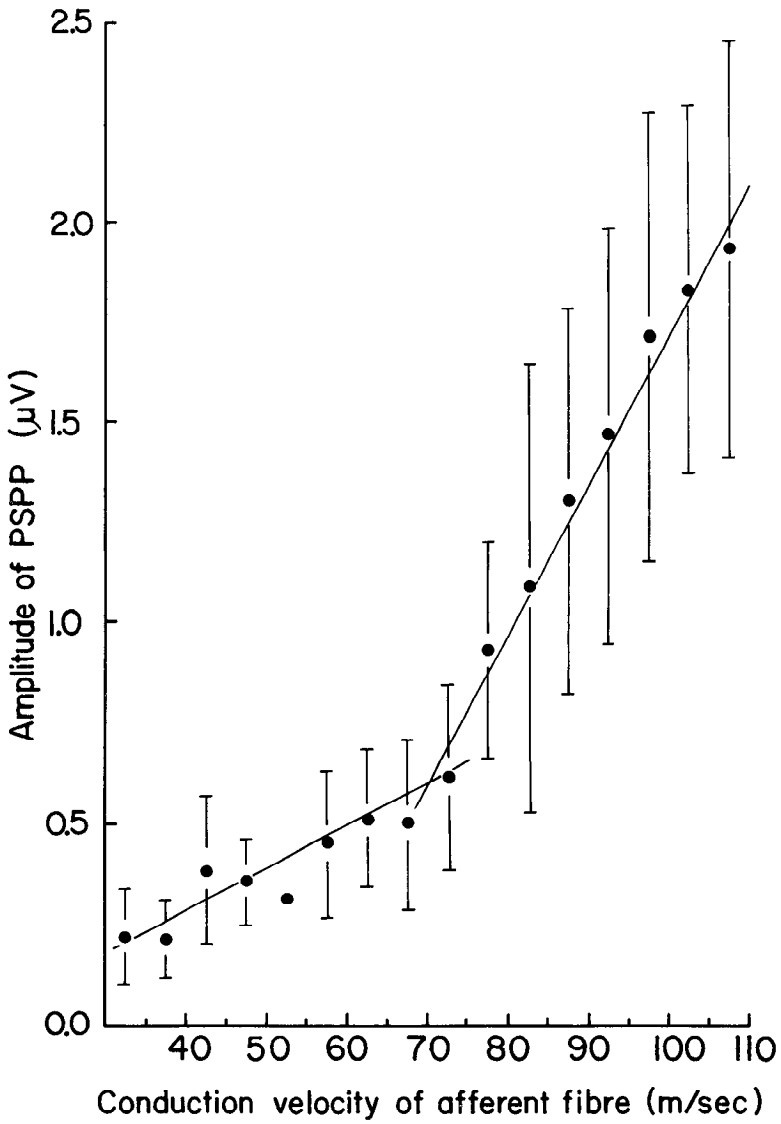

FIG. 14. Plot of the peak amplitudes of PSPPs versus the conduction velocities of the afferent fibers. PSPPs were recorded from S_1VR and L_7VR and elicited by impulses in MG muscle afferents. Data were grouped in pentads of conduction velocity and means ± SD were calculated for each group. The straight lines were fitted by the method of least squares.

Figure 13 shows a clear nonlinear relationship between the two variables. In an attempt to determine whether the data could be separated into two groups with a distinct dividing line, a method of "maximum likelihood estimates" described by Quandt (36) was used. By this method, using all 235 data points collected in this study, the "best" division point was found to be a conduction velocity of 73 m/s. An independent two-sample t test was performed to determine whether the slope for the group of data with conduction velocities below 73 m/s was equal to the slope for the data above the cutoff. The result was significant at the 5% level ($t = 22.39$, $df = 229.0$). The implication was that an appropriate cutoff point had been selected and that the two groups were different.

In Fig. 14, the mean amplitude of the PSPPs in each pentad of conduction velocity is plotted with its standard deviation. To construct this graph all PSPPs elicited by impulses in MG muscle afferents and re-

corded in S_1VR or the lower part of L_7VR ($n = 153$) were used. The data points again fell into two groups indicated by the lines that intersect at 70 m/s. The lines were drawn by the method of least squares and the data point at 72.5 m/s was used for the calculation of both lines.

These cutoff points at 73.0 and 70.0 m/s, found by two independent methods, are almost identical with the proposed dividing line of 72 m/s between group Ia and spindle group II afferents, which was based on the different anatomical and functional properties of the two types of endings (10, 14, 28, 44).

DISCUSSION

Sample of units investigated

The 213 afferent units investigated in this study were collected at random during the process of dissecting the dorsal roots. Group II afferents from spindles accounted for only 58 of these units; the remainder were Ia fibers by the criterion of conduction velocity. Anatomical studies have shown that each spindle contains only one primary ending, but the number of secondary endings may vary from 0 to 5 (29). The preponderance of group II endings should, therefore, lead to a larger percentage of group II fibers than were actually obtained. It was, however, much more difficult to isolate usable group II afferents because they were more susceptible to conduction block and their action potentials were smaller and more difficult to discriminate for triggering purposes. These technical problems, no doubt, account for the discrepancy between the actual and expected numbers of group II fibers in this study.

Since Romanes' (40) work, it has been known that the LG motoneuron pool is situated slightly rostral to the MG pool, with an extensive region of overlap. The soleus pool is also slightly rostral to the MG pool (4). The rostrocaudal positions of these pools are reflected in the cord entry points of MG and LG-S muscle afferents, the MG fibers being more abundant in S_1DR and the LG-S fibers in L_7DR.

General appearance and latency of PSPPs

Despite the fact that the PSPP is the composite of a large number of individual responses from subpopulations of motoneurons, it almost always has the appearance of a pure depolarizing potential. The rarely observed late hyperpolarization (Fig. 4*A–C*) may be taken as a manifestation of inhibitory action. The origin of such late hyperpolarization is not clear. Inhibitory actions on spinal motoneurons, which are produced by impulses in primary afferent fibers, generally require the presence of an interneuron (7) in the afferent pathway. The fact that the PSPP displays almost exclusively the excitatory effects of primary afferent impulses may be taken as an indication that the spike-triggered averaging technique, in combination with the present recording methods, does not, in general, reveal synaptic effects transmitted over more than one synapse. This is consistent with the conclusion (see RESULTS) that latencies that were longer than usual were due to slowing of intraspinal conduction velocity rather than to disynaptic connections.

If the present technique seldom discloses disynaptic connections, each spindle group II afferent must make monosynaptic excitatory connections with motoneurons in addition to the disynaptic excitatory and trisynaptic inhibitory connections reported by Lundberg et al. (25). The monosynaptic connection of group II afferents appears, therefore, to be more a rule than an exception. The technique, however, does not permit identification of the particular motoneurons receiving monosynaptic connections.

Rise time of PSPPs

The rise times of PSPPs are always somewhat longer than those of intracellularly recorded EPSPs due to the additional electrotonic conduction distance introduced by the recording technique, as has already been mentioned in the results. Rall et al. (38) have shown theoretically that inputs distributed to different portions of a motoneuron give rise to EPSPs with different time courses. With increasing distance from synapse to cell body, the rise time of the EPSP is prolonged. How the different rise times of the EPSPs occurring within a large population of motoneurons contribute to the composite rise time of the PSPP is not known. It may be assumed, however, that the rise time of a PSPP reflects the average location of all the terminals activated by the impulses that elicit the response. The difference in the mean rise times (Table 1) of Ia and group II

TABLE 1. *Summary of data on PSPPs*

Group	Amplitude, μV	Rise Time, ms	Latency, ms	Central Conduction Time, ms	Synaptic Delay, ms
Ia	1.28 ± 0.71 (174)	2.54 ± 1.05 (171)	0.85 ± 0.27 (176)	0.29 ± 0.12 (65)	0.57 ± 0.16 (61)
II	0.37 ± 0.19 (57)	3.17 ± 1.25 (47)	1.19 ± 0.41 (52)	0.55 ± 0.16 (20)	0.67 ± 0.22 (19)

Values are means ± SD. Figures in parentheses are numbers of records measured.

PSPPs suggests that the terminals given off by group II afferents are located on the average more distally on the dendritic tree than those from Ia fibers. The relationship between the conduction velocity of the afferent fiber and the rise time of the PSPP (Figs. 11*A–C* and 12) suggests that the average location of terminals may be determined by the size of the fiber giving off these terminals. The larger the afferent fiber, the closer to the cell body are its terminals. Evidence supporting the above-outlined hypothesis can be found in the paper by Mendell and Weiner (32), which shows that the peak amplitude of an individual EPSP, but not the average peak amplitude of the unit EPSP, is larger for more proximally generated EPSPs. They conclude that the mean number of quanta of neurotransmitter released by a single presynaptic impulse, which may be related to the size of the afferent fiber carrying that impulse (this paper), may be larger for somatic than for dendric synapses. Conradi (6) showed, by histological methods, that most Ia terminals are located on the proximal dendrites and that only a few synapses are located on distal dendrites. A study finding that group II fibers contact predominantly distal dendrites has not been reported previously.

Another possible explanation for the slower rise times of PSPPs elicited by impulses in slowly conducting afferent fibers may be slower intraspinal conduction in these fibers, leading to considerable temporal dispersion of the impulses in their widespread terminals. The broader Pre-SS in group II responses (Fig. 8*D*) is probably due to temporal dispersion also. Other examples are seen in Fig. 6.

On the other hand, theory (38) suggests that the latency from the event that generates the EPSP to the time at which it is recorded in the soma varies systematically with the distance from the synapses to the recording site. Mendell and Henneman (31) and Munson and Sypert (33) were able to confirm this theory experimentally. The significantly longer synaptic delay (Table 1), measured from the Pre-SS to the onset of the PSPP in the group II responses, supports the hypothesis that terminals from group II muscle afferents are located more distally on the dendritic tree than synapses from Ia afferents.

Amplitude of PSPPs

Many factors influence the experimentally determined amplitude of EPSPs and PSPPs, making it difficult to distinguish the significant physiological variables from effects introduced by experimental procedures. Individual EPSPs may be affected by variations in cell properties such as input impedance (2, 17), failure in synaptic transmission (20), or decrease in membrane potential following electrode penetration. Some possible sources of experimental error are minimized by the techniques used in this study. The resting membrane potentials of motoneuron cell bodies are stable and unaltered by electrode penetration in recordings from ventral roots. Even more important, the large population of motoneurons contributing to the composite PSPP has the beneficial effect of averaging out the variations in individual responses that tend to obscure relations between physiological variables.

Kuno and his colleagues (20, 21) have shown that the mean number of quanta of transmitter released by a presynaptic impulse is an important factor in determining the peak amplitude of the EPSP it produces.

Two recent studies support Kuno's observations. Mendell and Weiner (32) report a very weak negative correlation between mean peak amplitudes of individual EPSPs and their rise times. Their study, in which EPSPs elicited in the same motoneuron by impulses in two different Ia fibers were compared, indicates that, although the location of Ia synapses is important in determining the shape of EPSPs, it is not the major factor in determining their size. Iansek and Redman (15) found no correlation at all between peak EPSP amplitude and the estimated distance between active synapses and soma. These three studies agree that the amplitude of an individual EPSP is influenced more by the properties of the presynaptic fiber than by postsynaptic spatial factors. It is possible, however, that the inevitable scatter in the data, produced by pooling of results from intracellular recordings in these studies, has obscured the influence of other factors on EPSP amplitude. For example, if the number of terminals given off by an afferent fiber is a function of the size of that fiber, the relationship between conduction velocity of afferent fiber and amplitude of PSPP should be the same for both Ia and group II afferents. The different slopes, relating the conduction velocities of Ia and group II impulses to the PSPPs they elicit, suggest that factors other than the number of terminals alone influence the size of a PSPP.

In their study of individual EPSPs, Mendell and Henneman (31) showed that there was a correlation between the conduction velocity of Ia fibers and the amplitude of the EPSPs they elicited. The data presented in this paper indicate that this correlation applies not only to homonymous motoneurons within a given pool, but also to the large population of cells that send their axons out a single ventral root. The data obtained in this population study are far more extensive than those in the original study since one PSPP is a composite of more responses than there were in the entire series of individual EPSPs. As a consequence, the results are highly significant statistically and leave little doubt that a large, fast-conducting afferent fiber gives off more terminals to a population of motoneurons than a small, slowly conducting afferent fiber does.

The present technique does not answer the crucial question of whether a large fiber gives off more terminals to individual cells or simply distributes more terminals throughout a population of cells. Since the amplitude of individual EPSPs is directly related to the conduction velocity of the afferent fiber (31) and since the larger a Ia fiber is, the more motoneurons it projects to (L. M. Mendell, personal communication), it seems likely that the increased number of terminals is distributed in both ways.

Neurons of the dorsal spinocerebellar tract (DSCT) receive connections from primary afferent fibers from a variety of sensory receptors. It is useful, therefore, to compare their individual EPSPs with those in motoneurons. Aggregate EPSPs are much larger in DSCT neurons than in motoneurons and the mean amplitude of monosynaptic effects evoked by impulses in single fibers is also greater. Both of these differences could be due to the smaller size and greater input resistance of DSCT neurons, but these factors do not seem sufficient to account entirely for the larger EPSPs in DSCT neurons. The analysis of the DSCT by Kuno et al. (22) reinforces the general conclusions of this section by showing that the mean number of quanta of transmitter released by one impulse may vary over a 10-fold range. The average amplitude of individual EPSPs evoked in DSCT cells was significantly smaller for cutaneous fibers than for muscle or joint afferents, which are, in general, larger in diameter. There was a positive correlation between the amplitude and rise time of EPSPs in this study. It was suggested that the locations of synapses responsible for small and large EPSPs are intermingled and that large EPSPs are associated with a longer duration of transmitter action than small ones. It was concluded that the major factor responsible for the larger EPSPs in DSCT neurons is the greater number of quanta of transmitter released due to the number of multiple contacts formed by one afferent fiber.

Implications of results

Identification of general rules of organization are particularly significant in the nervous system because so few are known and they are so limited in scope. This paper expands the application of the size principle previously concerned with the properties of motoneurons and the muscle fibers they

innervate to primary sensory fibers that impinge directly on motoneurons. The data collected in this study indicate that neuronal size may have the same general implications for sensory cells as for motoneurons. The larger the Ia fiber, the greater and possibly the more widespread are the excitatory effects of its impulses. The data, furthermore, give some indication of how the size principle governs the amplitude of synaptic effects presynaptically as well as postsynaptically. The size of a sensory neuron not only determines the magnitude of the effect its impulses produce, but may also influence the locations where these effects are exerted postsynaptically.

The size of a primary stretch afferent fiber from muscle is correlated with its dynamic properties as well as the central effects it exerts. Differences in the dynamic properties of primary and secondary endings have been reported by Harvey and Matthews (10), Matthews (28), and Lennerstrand (23). Furthermore, Matthews (28) showed that the dynamic responses of primary and secondary endings were related to the conduction velocities of the afferent fibers. There was a clear tendency for their sensitivity to the velocity of stretch to increase with fiber size. No sharp division was apparent, however, between fibers with conduction velocities above or below 72 m/s. The velocity sensitivity of the spindle endings is believed to play an important role in the maintenance of stability in the servo control of movement. The dynamic sensitivity of primary endings counteracts the tendency of the feedback system to become unstable due to the delay between initiation of the error signal and the development of the appropriate corrective action (29). The fact that the larger and more rapidly conducting afferent fibers transmit bigger excitatory effects to the motoneuron pool may further improve the response time and stability of the system.

The evidence that the synapses from group II and Ia afferents are located on different parts of the somatodendritic membrane, in conjunction with a suggestion made by Rall (37), leads to some interesting physiological implications. Rall's theoretical studies suggest a functional distinction between dendritic and somatic activity. The more dendritically located synapses, generating slower EPSPs (measured at the soma), would be more appropriate for the maintenance and regulation of the general excitatory state of the motoneuron pool. They would be well suited to process input from secondary endings that exhibit a predominant static sensitivity. In contrast, the more proximally located input would be more apt to cause rapid triggering of reflex discharge. This would be an appropriate response to input from primary receptors, which are most sensitive to rapid changes in muscle length. The sustained synaptic activity in the dendrites, produced by input from secondary endings may, therefore, modify the effectiveness of the input from primary endings. The functional distinction in the central processing of signals from primary and secondary endings may shed some light on how inputs from different peripheral receptors can act together at the level of the motoneurons.

The brain and spinal cord are organized into systems, each consisting of a relatively large population of cells and fibers functioning in parallel. One of the difficulties with present-day electrophysiological techniques is that they deal with the nervous system one cell at a time. Although analysis of single neurons yields precise and specific information about their individual characteristics, it is usually impossible to define the capacities of an entire population from the properties of its members, which tend to vary quite widely. In a previous paper, a technique was introduced for recording the output of an entire pool of motoneurons (11) and relating the activity of its members to the output level of the population. This made it possible to analyze the organization of the motoneuron pool in new ways with quantitative rigor. The technique described in this paper deals with the intracellular events in even larger populations of motoneurons. As the initial results indicate, analyses of an entire population of cells has a number of important advantages and, of course, a few drawbacks. Only a few of the potentialities of this technique have been explored. In a succeeding study (unpublished observations), the technique has been used to study the geographical distribution of terminals from Ia and group II

fibers. With minor modifications the technique can, no doubt, be utilized for other types of experiments.

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REFERENCES

1. ARAKI, T., ECCLES, J. C., AND ITO, M. Correlation of the inhibitory post-synaptic potential of motoneurones with latency and time course of inhibition of monosynaptic reflexes. *J. Physiol. London* 154: 354–377, 1960.
2. BURKE, R. E. Group Ia synaptic input to fast and slow twitch motor units of cat triceps surae. *J. Physiol. London* 196: 605–630, 1968.
3. BURKE, R. E. AND NELSON, P. G. Synaptic activity in motoneurons during natural stimulation of muscle spindles. *Science* 151: 1088–1091, 1966.
4. BURKE, R. E., STRICK, P. L., KANDA, K., KIM, C. C., AND WALMSLEY, B. Anatomy of medial gastrocnemius and soleus motor nuclei in cat spinal cord. *J. Neurophysiol.* 40: 667–680, 1977.
5. CLAMANN, H. P. AND LAMB, R. L. A simple circuit for filtering single unit action potentials from electrograms. *Physiol. Behav.* 17: 149–151, 1976.
6. CONRADI, S. On motoneuron synaptology in adult cats. *Acta Physiol. Scand.* 78: Suppl. 332, 1970.
7. ECCLES, J. C. *The Physiology of Synapses*. Berlin: Springer, 1964.
8. FRANK, K. AND SPRAGUE, J. M. Direct contralateral inhibition in the lower sacral spinal cord. *Exp. Neurol.* 1: 28–43, 1959.
9. FU, T. C., SANTINI, M., AND SCHOMBURG, E. D. Characteristics and distribution of spinal focal synaptic potentials generated by group II muscle afferents. *Acta Physiol. Scand.* 91: 298–313, 1974.
10. HARVEY, R. J. AND MATTHEWS, P. B. C. The response of de-efferented muscle spindle endings in the cat's soleus to slow extension of the muscle. *J. Physiol. London* 157: 370–392, 1961.
11. HENNEMAN, E., CLAMANN, H. P., GILLIES, J. D., AND SKINNER, R. D. Rank order of motoneurons within a pool: law of combination. *J. Neurophysiol.* 37: 1338–1349, 1974.
12. HENNEMAN, E., SOMJEN, G., AND CARPENTER, D. O. Functional significance of cell size in spinal motoneurons. *J. Neurophysiol.* 28: 560–580, 1965.
13. HENNEMAN, E., SOMJEN, G., AND CARPENTER, D. O. Excitability and inhibitability of motoneurons of different sizes. *J. Neurophysiol.* 28: 599–620, 1965.
14. HUNT, C. C. Relation of function to diameter in afferent fibres of muscle nerves. *J. Gen. Physiol.* 38: 117–131, 1954.
15. IANSEK, R. AND REDMAN, S. J. The amplitude, time course and charge of unitary post-synaptic potentials evoked in spinal motoneurone dendrites. *J. Physiol. London* 234: 665–688, 1973.
16. JANKOWSKA, E. AND ROBERTS, W. J. Synaptic actions of single interneurones mediating reciprocal Ia inhibition of motoneurones. *J. Physiol. London* 222: 623–642, 1972.
17. KERNELL, D. Input resistance, electrical excitability and size of ventral horn cells in cat spinal cord. *Science* 152: 1637–1640, 1966.
18. KIRKWOOD, P. A. AND SEARS, T. A. Monosynaptic excitation of motoneurones from secondary endings of muscle spindles. *Nature London* 252: 243–244, 1974.
19. KIRKWOOD, P. A. AND SEARS, T. A. Monosynaptic excitation of motoneurones from muscle spindle secondary endings of intercostal and triceps surae muscles in the cat. *J. Physiol. London* 245: 64–66P, 1975.
20. KUNO, M. Quantal components of excitatory synaptic potentials in spinal motoneurones. *J. Physiol. London* 175: 81–99, 1964.
21. KUNO, M. AND MIYAHARA, J. T. Analysis of synaptic efficacy in spinal motoneurones from "quantum" aspects. *J. Physiol. London* 201: 479–493, 1969.
22. KUNO, M., MUÑOZ-MARTINEZ, E. J., and RANDIĆ, M. Synaptic action on Clarke's column neurones in relation to afferent terminal size. *J. Physiol. London* 228: 343–360, 1973.
23. LENNERSTRAND, G. Position and velocity sensitivity of muscle spindles in the cat. I. Primary and secondary endings deprived of fusimotor activation. *Acta Physiol. Scand.* 73: 281–299, 1968.
24. LLOYD, D. P. C. AND WILSON, V. J. Functional organization in the terminal segment of the spinal cord with a consideration of central excitatory and inhibitory latencies in monosynaptic reflex systems. *J. Gen. Physiol.* 42: 1219–1231, 1959.
25. LUNDBERG, A., MALMGREN, K., AND SCHOMBURG, E. D. Characteristics of the excitatory pathway from group II muscle afferents to alpha-motoneurones. *Brain Res.* 88: 538–542, 1975.
26. LUNDBERG, A., MALMGREN, K., AND SCHOMBURG, E. D. Comments on reflex actions evoked by electrical stimulation of group II muscle afferents. *Brain Res.* 122: 551–555, 1977.
27. LÜSCHER, H.-R., FETZ, E., RUENZEL, P., AND HENNEMAN, E. EPSPs elicited by single impulses in large populations of motoneurons. *Neurosci. Abstr.* 4: 568, 1978.
28. MATTHEWS, P. B. C. The response of de-efferented muscle spindle receptors to stretching at different velocities. *J. Physiol. London* 168: 660–768, 1963.
29. MATTHEWS, P. B. C. *Mammalian Muscle Recep-*

tors and Their Central Actions. London: Arnold, 1972.
30. McPHEDRAN, A. M., WUERKER, R. B., AND HENNEMAN, E. Properties of motor units in a homogeneous red muscle (soleus) of the cat. *J. Neurophysiol.* 28: 71–84, 1965.
31. MENDELL, L. M. AND HENNEMAN, E. Terminals of single Ia fibers: location, density and distribution within a pool of 300 homonymous motoneurons. *J. Neurophysiol.* 34: 171–187, 1971.
32. MENDELL, L. M. AND WEINER, R. Analysis of pairs of individual Ia-EPSPs in single motoneurones. *J. Physiol. London* 255: 81–104, 1976.
33. MUNSON, J. B. AND SYPERT, W. G. Latency-rise time relationship in unitary postsynaptic potentials. *Brain Res.* 151: 404–408, 1978.
34. NELSON, S. G. AND MENDELL, L. M. Projection of single knee flexor Ia fibers to homonymous and heteronymous motoneurons. *J. Neurophysiol.* 41: 778–787, 1978.
35. PETERSON, R. P. Cell size and rate of protein synthesis in ventral horn neurons. *Science* 153: 1413–1414, 1966.
36. QUANDT, R. E. The estimation of the parameters of a linear regression system obeying two separate regions. *J. Am. Stat. Assoc.* 53: 873–880, 1958.
37. RALL, W. Branching dendritic trees and motoneuron membrane resistivity. *Exp. Neurol.* 1: 491–527, 1959.
38. RALL, W. Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *J. Neurophysiol.* 30: 1138–1168, 1967.
39. ROBERTS, M. H. T. AND WALLIS, D. I. Dorsal and ventral root potentials recorded in vivo by the sucrose-gap method. *J. Physiol. London* 277: 42P–43P, 1978.
40. ROMANES, G. J. The motor pools of the spinal cord. In: *Progress in Brain Research. Organization of the Spinal Cord,* edited by J. C. Eccles and J. C. Schadé. Amsterdam: Elsevier, 1964, vol. 11, p. 93–119.
41. SCOTT, J. G. AND MENDELL, L. M. Individual EPSPs produced by single triceps surae Ia afferent fibers in homonymous and heteronymous motoneurons. *J. Neurophysiol.* 39: 679–691, 1976.
42. SOMJEN, G., CARPENTER, D. O., AND HENNEMAN, E. Responses of motoneurons of different sizes to graded stimulation of supraspinal centers of the brain. *J. Neurophysiol.* 28: 958–965, 1965.
43. STAUFFER, K. E., WATT, D. G. D., TAYLOR, A., REINKING, R. M., AND STUART, D. G. Analysis of muscle receptor connections by spike-triggered averaging. 2. Spindle group II afferents. *J. Neurophysiol.* 39: 1393–1402, 1976.
44. STEIN, R. B. AND MATTHEWS, P. B. C. Difference in variability of discharge frequency between primary and secondary muscle spindle afferent endings of the cat. *Nature London* 208: 1217–1218, 1965.
45. TAYLOR, A., WATT, D. G. D., STAUFFER, E. K., REINKING, R. M., AND STUART, D. G. Use of afferent triggered averaging to study the central connections of muscle spindle afferents. *Prog. Brain Res.* 44: 171–183, 1976.
46. WALLIS, D. T., LEES, G. M., AND KOSTERLITZ, H. W. Recording resting and action potentials by the sucrose-gap method. *Comp. Biochem. Physiol. C* 50: 199–216, 1975.
47. WATT, D. G. D., STAUFFER, E. K., TAYLOR, A., REINKING, R. M., AND STUART, D. G. Analysis of muscle receptor connections by spike-triggered averaging. 1. Spindle primary and tendon organ afferents. *J. Neurophysiol.* 39: 1375–1392, 1976.
48. WUERKER, R. B., McPHEDRAN, A. M., AND HENNEMAN, E. Properties of motor units in a heterogeneous pale muscle (m. gastrocnemius) of the cat. *J. Neurophysiol.* 28: 85–99, 1965.