

THE SIZE-PRINCIPLE: A DETERMINISTIC OUTPUT EMERGES FROM A SET OF PROBABILISTIC CONNECTIONS

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SUMMARY

Orderly, size-related recruitment of motoneurons (MNs) illustrates how hundreds of cells operate as a functional entity to produce a highly deterministic output. The coherent action of the pool depends largely on the distribution of input to its members through the connections of afferent fibres. Three types of spike-triggered averaging have been utilized to study these connections. (1) Impulses in individual Ia afferents elicit excitatory postsynaptic potentials ('single-fibre' EPSPs) in about 80 % of homonymous MNs. After spinal transection 100 % may respond, suggesting that Ia fibres project anatomically to all homonymous MNs. Functionally absent Ia connections are due to transmission failure. (2) The sum of all the EPSPs elicited in a large population of MNs was recorded electrotonically from ventral roots. The mean amplitudes of these 'postsynaptic population potentials' (PSPPs) were correlated with the conduction velocities (CVs) of the Ia or spindle group II fibres. The greater the distance between the spinal entry point of a Ia fibre and the ventral root, the smaller was the PSPP. (3) Tape recording of multiple afferents and the responses of up to 24 MNs permitted study of as many as 264 possible connections in a single, acute experiment. Construction of wiring diagrams and connectivity matrices from the data showed that functional connectivity is influenced by afferent fibre size, the effect of branching on fibre size, MN size and probably transmission failure, but that on a cell-to-cell level, connectivity does not follow strict, deterministic rules. The results raise the question of how probabilistic connections between afferent fibres and MNs give rise to deterministic outputs from the whole pool.

To understand how even a single muscle is controlled, we must understand how a large ensemble of neurons can generate a complex function through the collective action of its members. The orderly recruitment of motoneurons (MNs) and motor units according to their sizes or tensions (Henneman, 1957; Henneman, Somjen & Carpenter, 1965*a,b*) is the product of collective action, illustrating how hundreds of MNs operate as a functional entity to control a muscle. Fig. 1 reproduces the recruitment of motor units recorded from a rabbit's diaphragm during a series

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of progressively stronger inspirations (G. Yasargil, unpublished). With each contraction larger spikes were recorded in orderly sequence, indicating the discharge of larger motor units. Nearly invariant recruitment orders have been recorded from the MNs or muscles of more than 20 species (Henneman & Mendell, 1981).

If the monosynaptic output of an entire MN pool is recorded from a ventral root and simultaneously on a second channel the responses of a single MN in the same pool, it is apparent that each MN fires only when the total output of the pool equals a certain percentage of the maximum. The fixed rank order in the pool, which this indicates, is not altered by inhibitory inputs. The firing of a particular MN, of course, implies that all smaller cells in the pool are also discharging (Henneman, Clamann, Gillies & Skinner, 1974).

The coherent action of the MN pool depends largely on the distribution of inputs to its members through the connections of afferent fibres. The highly deterministic output of the pool, which we seek to explain, is due to the collective action of both afferent and efferent neurones. Three types of spike-triggered averaging (STA) have been utilized to investigate the connections between them.

To learn how individual stretch-afferent fibres of a muscle are connected with its MNs, impulses were recorded from dorsal root filaments (DRFs) containing single Ia fibres from that muscle and used to trigger the sweep of an averager. The tiny,

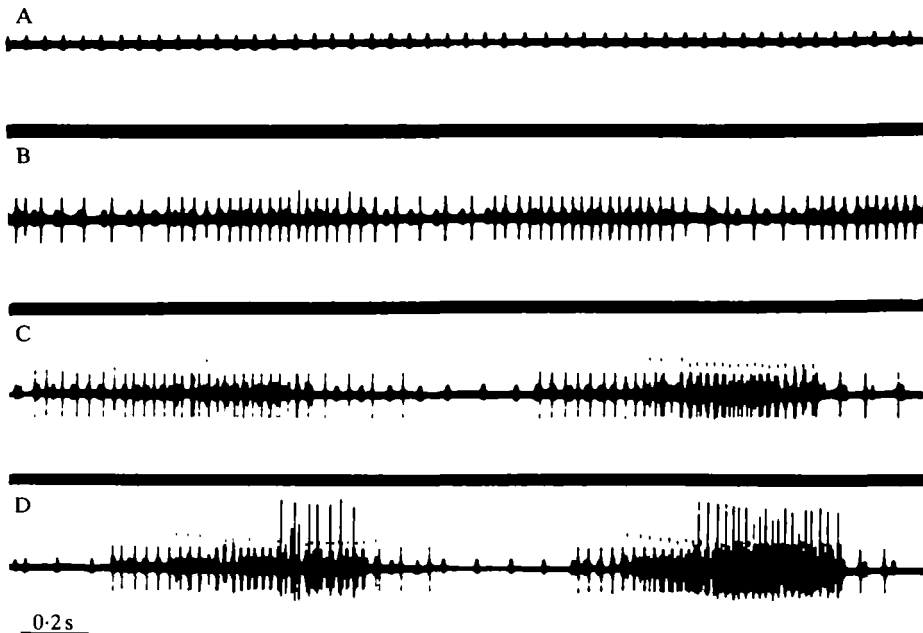


Fig. 1. Electromyographic recording from rabbit's diaphragm, showing discharges of progressively larger spikes in A–D during a series of increasingly strong inspirations after a period of apnoea caused by hyperventilation. The smallest unit discharges spontaneously (A). Recruitment of larger motor units, firing more rapidly, occurs in B, C and D. During each inspiration in D, the same motor units are recruited in the same orderly sequence (from unpublished records of G. M. Yasargil).

generally indistinguishable, excitatory postsynaptic potentials (EPSPs) elicited in an MN by each impulse were fed to the input of the averager. By summing a few hundred of these small responses, each time-locked with the signal that triggered the sweep, larger, so-called 'single-fibre' EPSPs were generated, indicating that a direct, functional connection existed between the afferent fibre and the MN. The larger the diameter of the afferent fibre, as judged by its conduction velocity (CV), the greater was the mean amplitude of the EPSP (Mendell & Henneman, 1971). In the original study single Ia fibres projected to 93 % of the homonymous MNs examined. It was speculated that the true projection was 100 %, but in six other laboratories the mean was about 80 %. This widespread projection, suggesting that the majority of the MNs in a pool are excited by each afferent impulse at about the same time, obviously helps to explain the coherent action of the MN pool. In 1979 it was discovered that the projection probability of single Ia fibres rose from 80 % to about 100 % immediately after low spinal transection (Nelson, Collatos, Niechaj & Mendell, 1979). Mendell was properly cautious in interpreting this surprising finding, which suggests that axonal conduction or synaptic transmission may fail somewhere in the projection of Ia fibres to MNs. Recent morphological studies indicate that every Ia fibre probably projects anatomically to every homonymous MN (R. E. Burke, personal communication), which supports the electrophysiological evidence of transmission failure. Regardless of the site of failure or its cause, the occurrence of transmission failure and its relief indicate that functional connectivity is by no means fixed or invariant, but reflects dynamic, state-dependent processes. This conclusion is also emerging from laboratories in Canberra (Jack, Redman & Wong, 1981), Zurich (Henneman, Lüscher & Mathis, 1984) and Boston (Lüscher, Ruenzel & Henneman, 1979, 1983).

To learn more about the connections of afferent fibres with MNs, another, quite different method of spike-triggered averaging was developed (Lüscher, Ruenzel, Fetz & Henneman, 1979) to study the effects of impulses in single Ia or group II fibres on large populations of MNs. Of course, impulses in single fibres are not sufficient to discharge any MNs. They do, however, elicit EPSPs in all MNs receiving direct, functional connections from a particular fibre. The sums of all such EPSPs, which were called postsynaptic population potentials (PSPPs), were recorded electrotonically from ventral roots surrounded by isotonic sucrose with the aid of STA. The mean amplitudes of PSPPs were highly correlated with the CVs of the afferent fibres, i.e. with their diameters, as Fig. 2 illustrates. The slopes of the relationships were different for Ia and group II fibres. The results also revealed that the greater the longitudinal distance between the spinal entry point of a Ia fibre and the ventral root, the smaller was the PSPP (Lüscher, Ruenzel & Henneman, 1980). From these results it was inferred that the number of synapses a Ia fibre can give off to a population of MNs depends on its diameter. The number it can supply to a single MN depends upon the diameter of the primary collateral sending terminals to that cell. The diameters of the primary collaterals decrease at greater distances from the entry point of the afferent fibre because successive branchings reduce the size of the parent fibre and the diameters of the collaterals it gives off as it ascends the dorsal

columns. These findings have added to the generality of the size-principle by showing how afferent fibre size and branching influence functional connectivity.

All techniques have their limitations. Spike-triggered averaging reveals the functional connections between a single stretch-afferent fibre and a number of homonymous MNs, or between a single afferent and a very large population of MNs. Neither approach permits construction of a wiring diagram of sufficient scope to be useful for research purposes. By expanding the usual single channel technique into multiple channels (Lüscher, Mathis & Henneman, 1984), as many as 264 possible connections have been investigated in a single, acute experiment (11 afferent fibres projecting to 24 MNs), permitting construction of representative wiring diagrams. To obtain multiple inputs, impulses elicited by stretching a muscle were recorded from five uncut dorsal root filaments (DRFs), each containing one to five Ia or spindle group II fibres. The afferent activity from each DRF was stored in a separate channel of a tape recorder. All other DRFs were cut to reduce input to this part of the spinal cord. The EPSPs evoked by long trains of impulses in all five DRFs were recorded intracellularly from a single MN and stored on a sixth channel of tape. Keeping the same set of inputs throughout the experiment, the microelectrode was placed successively inside as many motoneurons as possible.

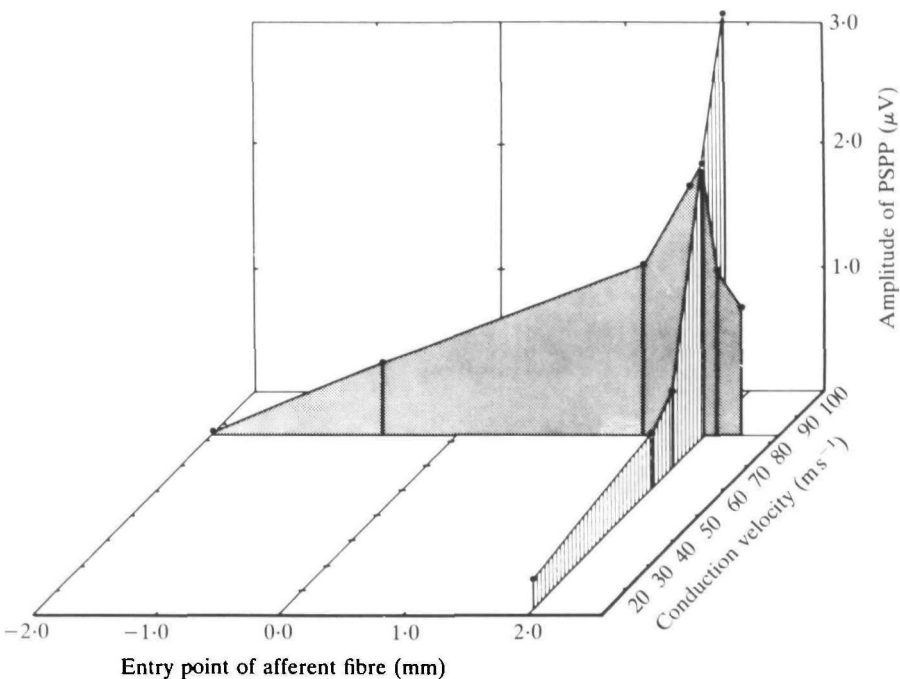


Fig. 2. Graphical representation of the relationships between the amplitudes of postsynaptic population potentials (PSPPs), and the conduction velocities and spinal entry points of the afferent impulses evoking the responses. Peak amplitudes of PSPPs were averaged 4096 times. Six units with the same entry levels (2.0 ± 0.2 mm) were used to illustrate the effect of afferent conduction velocity on PSPP size. Seven units with the same conduction velocities (84 ± 1.0 m s^{-1}) show the effects of different spinal entry levels on PSPP size (from Lüscher, Ruenzel & Henneman, 1980).

During playback of tape-recorded data, the stored trains of impulses from the five DRFs were displayed one at a time on an oscilloscope. A special window discriminator was used to select the impulses from any particular afferent fibre as triggers for averaging their corresponding single EPSPs. Each train of sensory impulses was used in turn for spike-triggered averaging. To obtain the conduction velocities of the afferent fibres, stored signals from the dorsal root and muscle nerve were averaged.

With this technique, the single-fibre EPSPs elicited in many homonymous MNs by impulses in the same set of afferent fibres could readily be compared. It was immediately obvious that impulses in a particular afferent fibre evoked completely different responses in different MNs. Both the amplitudes and the time courses of the EPSPs elicited by impulses in a given fibre varied widely, suggesting that the locations of the responsible synapses and their functional efficacy differed on each MN, but not in a recognisably systematic manner. Similarly, when the EPSPs evoked in a single MN by 10–20 afferents were compared, there was no uniformity in the shapes or sizes of the responses. The variations in size and shape of single-fibre EPSPs in these large samples can only be described as random. This implies that on a cell-to-cell level the monosynaptic connections mediating these responses are distributed with a considerable degree of randomness. Despite the randomness, it was apparent that the size of the afferent fibres had a definite influence on the amplitude of the EPSPs. Impulses in large afferent fibres elicited small, medium or large EPSPs, whereas impulses in small fibres evoked only small responses. Clearly, impulses in a large fibre are necessary to evoke large EPSPs, but they are by no means sufficient to do so in many instances. It may be inferred that only a large fibre can supply an MN with enough synapses to evoke a large EPSP.

Using single-fibre EPSPs to identify functional connections, a wiring diagram was constructed (Fig. 3) to illustrate the connections of five Ia and six spindle group II fibres from the medial gastrocnemius (MG) muscle with 15 MG MNs in the 7th lumbar and 1st sacral segments of the spinal cord. In this experiment, each of the five DRFs (F1–F5) contained one to three afferent fibres from muscle spindles in MG. Heavy lines denote Ia fibres and their branches; lighter lines indicate group II fibres. A recent study (Henneman *et al.* 1984) suggests that the connections shown as single contacts in Fig. 3 may, in some instances, represent multiple contact systems or clusters of synapses, some of which are active, while others from the same fibre are inactive.

Inspection of the wiring diagram reveals that MNs 5 and 6, which were closest to the spinal entry levels of the 11 afferent fibres, each received functioning connections from nine of them. MNs 3, 4 and 7, a little further away, received slightly fewer connections (average, 8.67). For MNs 1, 2, 8 and 9, still more rostral, the average was again smaller (7.50) and for cells 10–13 it was the same (7.50). The most distant MNs (14 and 15) received fewer functional connections (4 each) than any cell closer to the entry levels of the afferent fibres. These findings suggest that, as the diameters of more distant collaterals decreased, their capacity to give off functional terminals decreased correspondingly. Morphological studies confirm the progressive decrease in the diameters of more distant collaterals (Ishizuka, Mannen, Hongo & Sasaki, 1979).

Fig. 4 is a matrix relating the axonal conduction velocities of the individual MNs and afferent fibres to the presence or absence of connections between them. Filled circles represent combinations in which an EPSP was evoked. Empty squares indicate that no detectable response was obtained. The matrix is arranged so that the CVs of the afferent fibres decrease from left to right, while those of the MNs decrease from above downward. In the upper left corner of the matrix, where both MNs and afferent fibres were large, there are few blanks, but in the lower right corner where both neurones are small, there are many blanks. The large, fast-conducting Ia fibres had actively functioning connections with most of the 15 MNs. However, as the CVs of the afferent fibres decreased from 67 to 37 ms^{-1} , the numbers of MNs receiving connections from them decreased from 11 to 10 to 8 to 8 to 6 to 4.

MN size also influences connectivity, but not so strongly. The seven smaller MNs with CVs of 73–90 m s^{-1} received functional connections from fewer afferent fibres (average, 6.3) than the eight larger MNs with CVs of 91–99 m s^{-1} (average, 8.5). The larger an MN, the greater is its chance of receiving functional connections, but this relationship is highly probabilistic, just as the influence of afferent fibre size on connectivity is.

If the MNs are arranged in order of increasing distance from the entering afferent

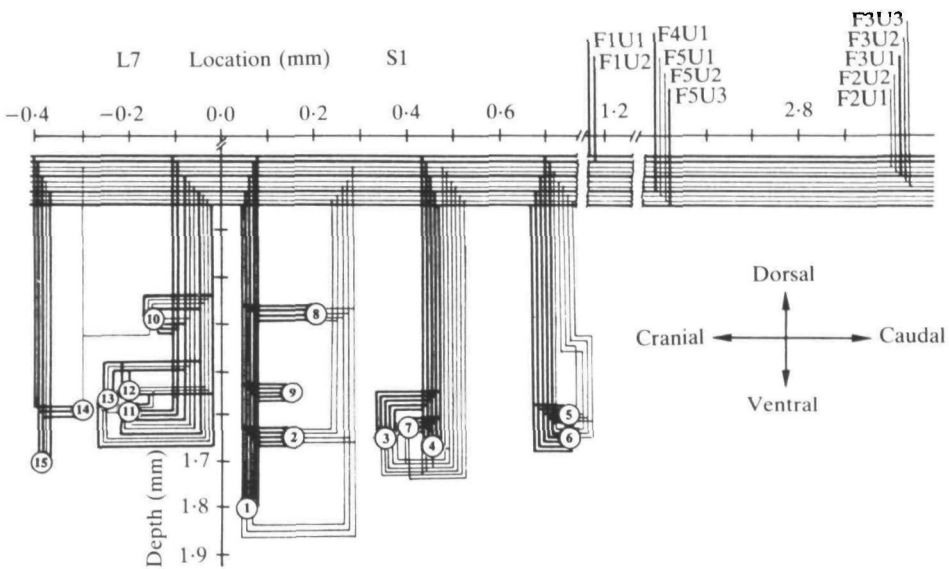


Fig. 3. Wiring diagram of direct, functional connections between 11 Ia and spindle group II fibres of the medial gastrocnemius (MG) muscle and 15 of its motoneurons, as established by spike-triggered averaging. Heavy lines indicate Ia fibres and their collaterals, lighter lines group II spindle fibres. Numbered circles represent 15 MG motoneurons from which intracellular recordings were made in sequence. Rostro-caudal locations of these cells were obtained from surface measurements, dorso-ventral positions from microelectrode coordinates. F1–F5 indicate dorsal root filaments from which stretch-evoked impulses were recorded. U1–U3 denote afferent units in each filament. The entry point of each afferent fibre is shown at its actual rostro-caudal level; the courses of the afferent collaterals are merely diagrammatic. In most cases individual contacts between afferent fibres and motoneurons probably represent groups or clusters of synapses (from Lüscher, Mathis & Henneman, 1984).

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