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STRUCTURAL AND TOPOGRAPHICAL INFLUENCES ON FUNCTIONAL CONNECTIVITY IN SPINAL MONOSYNAPTIC REFLEX ARCS IN THE CAT

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SUMMARY

1. A greatly expanded version of spike-triggered averaging (Mendell & Henneman, 1971), performed off-line on tape-recorded signals, was utilized to determine the presence or absence of functional connexions between stretch-afferent fibres and homonymous motoneurones.

2. As many as 264 possible connexions between eleven Ia or spindle group II fibres and twenty-four motoneurones were studied in each single, acute experiment. Morphological and topographical factors influencing functional connectivity were analysed with the aid of wiring diagrams and connectivity matrices.

3. In all experiments the greater the conduction velocity (i.e. diameter) of a Ia or group II fibre, the higher was the probability of its having functional connexions with homonymous motoneurones. The greater the longitudinal distance between the spinal entry points of Ia fibres and the location of a motoneurone, the less was the same probability.

4. The influence of axonal conduction velocity of motoneurones on functional connectivity was apparent in some experiments, but not in others. In pooled data large motoneurones received functional connexions from a higher percentage of group II fibres than did small cells. The projection percentage reached 100 only when both Ia fibres and motoneurones were large, suggesting that motoneurone size influences the probability of functional connexions from group Ia as well as group II fibres.

5. On a cell-to-cell level, connectivity apparently does not follow strict, deterministic rules. The results raise the question of how probabilistic connexions between afferent fibres and motoneurones give rise to deterministic outputs from the whole pool.

INTRODUCTION

A major problem in neurobiology is that of understanding how complex functions are generated by the collective action of large ensembles of neurones. The orderly, size-related recruitment of motoneurones in response to various inputs (Henneman,

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1957; Henneman, Somjen & Carpenter, 1965*a*, *b*) is the product of collective action, illustrating how hundreds of neurones operate as a functional entity to regulate tension in a single muscle. The coherent action of the motoneurone pool depends largely on the connexions of afferent fibres with the individual members of the pool and the functional distribution of their input (Henneman & Mendell, 1981). To understand how any circuit functions, a wiring diagram is needed. Unfortunately, none that are available for this reflex system are sufficiently detailed and comprehensive. Moreover, it must be emphasized that how inputs to the motoneurone pool are organized is not a purely anatomical question. Although Ia fibres send terminals to a very high percentage of homonymous motoneurones (Mendell & Henneman, 1971; Scott & Mendell, 1976), the extent to which this connectivity is expressed depends on the state of the nervous system. Spinal cord transection can lead to an immediate increase in the so-called 'projection frequency', i.e. to a higher percentage of functional projections from a single Ia fibre to its homonymous motoneurones (Nelson, Collatos, Niechaj & Mendell, 1979). These observations suggest that axonal conduction or synaptic transmission may fail completely in the projection of Ia fibres to some homonymous motoneurones and that this failure may be relieved by spinal transection. Regardless of the cause of what is hereafter referred to as 'transmission failure', its occurrence and its relief indicate that functional connectivity is by no means fixed or invariant, but reflects dynamic, state-dependent processes.

This report describes a new method of investigating functional, as contrasted with anatomical, connectivity; this greatly enlarges the scope of the spike-triggered averaging technique of Mendell & Henneman (1971). Large quantities of data on the direct, functioning connexions between single Ia fibres or group II spindle-afferent fibres and homonymous motoneurones, or on the absence of such connexions, were obtained in single acute experiments. From these data, wiring diagrams and connectivity matrices have been constructed, illustrating the functional connexions between as many as eleven stretch-afferent fibres of the medial gastrocnemius (m.g.) muscle and twenty-four of its α-motoneurones (i.e. a total of 264 possible connexions). The results illustrate the extent to which the morphological and topographical factors that influence functional connectivity are apparent in cell-to-cell relations. Among these factors are the diameters of the afferent fibres, the sizes of the motoneurones, the topographical relations between these neurones and, by implication, the effects of transmission failure. Some of the findings reported here have been published in abstract or letter form (Mathis, Henneman & Lüscher, 1982; Lüscher, Mathis & Henneman, 1984).

Terminology

Depending on the technique employed to record excitatory post-synaptic potentials (e.p.s.p.s) different types of responses can be distinguished, for which the following terms will be used in this paper.

(1) Composite e.p.s.p.: the synaptic potential produced in a motoneurone by synchronous activation of many of the Ia fibres of a muscle nerve. This term is synonymous with 'aggregate e.p.s.p.', which has been used by some authors.

(2) Single-fibre e.p.s.p.: the synaptic potential elicited by a single afferent impulse or the spike-triggered average of many such potentials produced by a series of impulses in the same fibre. 'Individual e.p.s.p.' has been used as a synonym.

(3) Post-synaptic population potential (p.s.p.p.): the post-synaptic potential elicited in a large population of motoneurones by impulses in a single afferent fibre and recorded electrotonically via their ventral roots, which are surrounded by sucrose (Lüscher, Ruenzel, Fetz & Henneman, 1979*a*).

(4) Unit e.p.s.p.: an all-or-none component of a single-fibre e.p.s.p.

METHODS

Adult cats of either sex were anaesthetized initially with sodium pentobarbitone (40 mg/kg). Later supplements were administered to maintain deep anaesthesia as judged by immobility, absence of corneal reflexes, pupillary diameter, and lack of response to pinch. After development of methods in pilot experiments, data from six of the most successful experiments carried out were chosen for presentation because they provided larger yields of homonymous motoneurones and afferent fibres. The remaining experiments produced results in support of those presented, but are not discussed in detail because they supplied smaller and, therefore, less valuable samples of data.

Surgical procedures. The lower lumbar and sacral segments of the spinal cord were exposed by dorsal laminectomy. The left hind limb was denervated except for the m.g. muscle, whose nerve was mounted on bipolar electrodes for electrical stimulation or recording (Fig. 1: S2 and R7). Input to the lumbar and sacral spinal cord was restricted almost exclusively to afferents from the m.g. muscle by extensive denervation of the tail, buttock and hip. The gluteal muscles were dissected to visualize the entire course of the sciatic nerve in the hip, so that all of its collateral branches could be severed.

Skin flaps were pulled up to form pools for mineral oil to cover the exposed spinal cord and popliteal fossa. The core temperature and that of the spinal oil pool were maintained at $37 \pm 0{\cdot}5$ °C. The popliteal fossa was held at $35 \pm 0{\cdot}5$ °C. The Achilles tendon was freed and separated from its insertion, leaving a chip of the calcaneous bone attached. Stretch was applied to the m.g. muscle (Fig. 1: S1) with a rack and pinion attached by a strong thread to the bone chip.

Isolation and recording of afferent units. Five pairs of small silver hook electrodes (Fig. 1: R2–R6) were used to record stretch-evoked activity in Ia and spindle group II fibres in five uncut dorsal root filaments which had been dissected until each contained two to six spindle afferents from the m.g. muscle. Stretch of the m.g. was adjusted to elicit continuous discharge in all of these fibres, as monitored on an oscilloscope. The remaining filaments in L7 and S1 dorsal roots were cut. In addition, the L6 dorsal root was severed and retracted to afford free access to the spinal cord. A small silver ball electrode (Fig. 1: R8) was used to record from the dorsal surface of the spinal cord cranial to the entry points of the dorsal root filaments to check by spike-triggered averaging (Mendell & Henneman, 1971) for possible blocking of impulse conduction in afferent fibres after their entry into the cord (see below). The spinal entry levels of the five dorsal root filaments from which recordings were taken were identified and measured precisely.

Intracellular recording. Intracellular recording from m.g. motoneurones, identified by antidromic volleys from the m.g. muscle nerve, was carried out with micro-electrodes pulled from borosilicate 'omega dot' glass tubing and filled with 3 M-KCl. The tips of the electrodes were sometimes modified by breaking them, bevelling them or etching them with hydrofluoric acid (Lüscher, Ruenzel & Henneman, 1983*b*). Final tip resistances ranged from 4 to 20 MΩ. Micro-electrodes were mounted on a Burleigh Inchworm (U.S.A.) piezoelectric microdrive. Potentials were measured with an electrometer (WPI, U.S.A.). Current could be injected into the impaled cell by means of a balanced bridge circuit. Cells with resting potentials less than -55 mV were not accepted for study. In order to rank the sizes of the motoneurones, the conduction velocities of their axons were measured. In some experiments the input resistance was also determined (1) by measuring the effect of hyper- and depolarizing current pulses on the amplitude of the antidromically evoked action potentials (Frank & Fuortes, 1956) and (2) by recording the voltage response to long current pulses (Kernell, 1966).

Fig. 1 illustrates the experimental plan. Trains of afferent impulses were recorded from the five dorsal root filaments (R2–R6). Activity was also recorded from the muscle nerve (R7). Signals from the impaled motoneurones were recorded intracellularly (R1) with an a.c. coupled high gain amplifier. All of these signals were stored concurrently for later off-line analysis on seven channels of the frequency-modulated tape recorder (Honeywell 101, U.S.A.) at 30 inches per second (upper frequency limit: 10 kHz). In the most successful experiments all of the Ia and group II fibres on

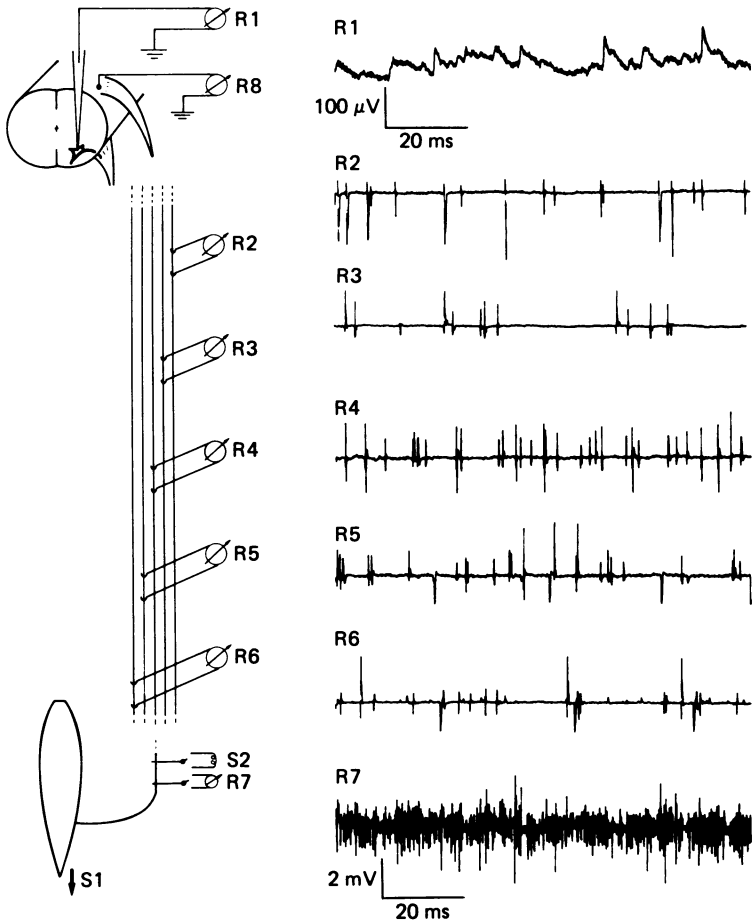

Fig. 1. Experimental plan (left) and corresponding recordings (right). R1: intracellular recording from motoneurone. R2–R6: stretch-evoked impulse activity recorded from five dorsal root filaments. R7: recording from nerve to medial gastrocnemius (m.g.). R8: surface electrode just rostral to entry zone of dorsal root filaments. S1: static stretch of m.g. muscle. S2: electrical stimulation of m.g. nerve. All recordings stored simultaneously on magnetic tape (see Methods).

the R2–R6 electrodes remained functional throughout the recording session, while intracellular recordings were made successively from as many m.g. motoneurones as possible.

Data analysis. The multi-unit spike sequences recorded from each of the five dorsal root filaments were decomposed, using a window discriminator (Lüscher, Mathis & Schaffner, 1983*a*) with two time–voltage 'windows' whose dimensions and positions could be adjusted independently. Whenever an afferent signal crossed one of the two windows, a trigger pulse was generated. It could be led separately from either window, or pulses from the two windows could be combined by 'AND' logic. This discriminator made it possible to isolate one afferent spike train from as many as five others recorded simultaneously from the same filament. A detailed description of the discriminator and its use are given in the reference cited.

During repeated play-back of the taped recordings, the signals from one dorsal root filament were led to the discriminator to derive the trigger pulses for the sweep of an averaging computer (Tracor Northern TN-1710 Modular Analyzer, U.S.A.). Whenever an impulse with the selected characteristics

passed through the discriminator, it triggered one sweep of the averager, which sampled the taped membrane potential of the motoneurone immediately following each trigger pulse. The e.p.s.p.s evoked by the selected afferent impulses were thus time-locked with the signals that triggered the sweep and therefore occurred with the same delay on each successive sweep. These e.p.s.p.s were summed in phase in the memory of the averager, whereas the e.p.s.p.s elicited by impulses in other

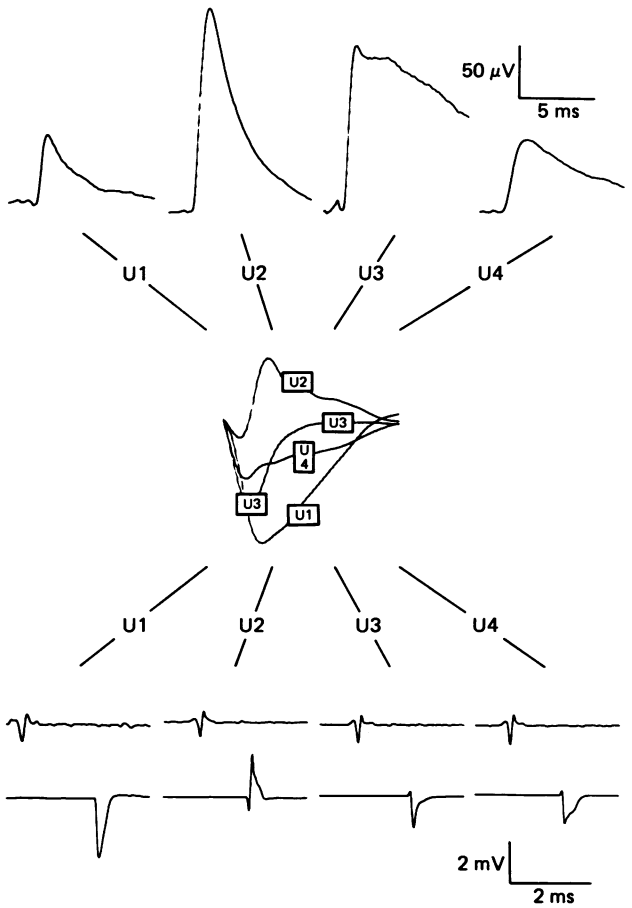

Fig. 2. Four single-fibre e.p.s.p.s (above) evoked in the same motoneurone by impulses in four different afferent fibres (U1–U4). Oscillographic display of four different afferent impulses recorded from one dorsal root filament (middle), together with the placement of the 'windows' needed to select any of the units for triggering. Upper of two lowest traces displays averaged action potentials recorded from m.g. nerve; lower trace displays corresponding action potentials recorded from dorsal root, permitting measurement of conduction times between the two recording sites (see Methods).

fibres of the same filament occurred randomly with respect to the trigger, generating a relatively flat base line. Each train of sensory impulses was used in turn for spike-triggered averaging, as originally described by Mendell & Henneman (1971). In general, 1024–4096 sweeps were averaged. The amplitude resolution of the averager was about 5 μV under these circumstances. Smaller potentials were interpreted as 'no response'. Fig. 2 (middle) is an example of how four different impulses recorded from one filament were displayed superimposed at high sweep speed, together with the positions of the windows used to isolate each spike for triggering purposes. In order to

separate the small spike (U3) nearly superimposed on two other signals (U1 and U4), the two windows were combined with AND logic as noted above. The four single-fibre e.p.s.p.s elicited concurrently in a motoneurone by the four afferent units (U1–U4) are illustrated in the upper row of Fig. 2. In the present experiments this technique enabled us to study single-fibre e.p.s.p.s evoked concurrently in m.g. motoneurones by impulses in up to twelve different Ia and spindle group II fibres. Summation averaging was utilized throughout.

During the averaging procedure the trigger pulses were also used to generate an interpulse-interval histogram. Triggering on two discharging afferents, which is unacceptable, results in a histogram with entries at short intervals. In contrast, a single discharging unit always generated a histogram whose amplitude fell to zero for very short intervals. Only units whose histograms met this requirement were accepted for study.

Determination of conduction velocity of afferent fibres. To obtain the conduction velocities of the afferent fibres, the same spike-triggered averaging technique was used to extract action potentials from the complex activity recorded from the muscle nerve (Fig. 1: R7). The trigger signals used for this purpose were the same as for averaging single-fibre e.p.s.p.s. In order to do this 'back-averaging' of signals that were temporally reversed, corresponding action potentials from the dorsal root fibres and the muscle nerve were delayed in a two-channel analog delay line. As illustrated in the two lowest traces in Fig. 2, this procedure displayed the action potentials recorded from the m.g. muscle nerve (above) and the dorsal root fibre (below), permitting reliable measurement of the conduction time between the two. At the end of each experiment the conduction path was exposed for its entire course from the recording site on the muscle nerve to that on the dorsal root filaments, allowing accurate measurement of the conduction distances.

'Back-averaging' should also reveal possible phase-locking between the triggering spike and any other impulse having a different conduction velocity. No examples of phase locking between afferent impulses were ever noted, however.

RESULTS

General orientation. Fig. 3 is a schematic wiring diagram intended only to provide a brief orientation for the design of the experiments. It illustrates the functional connexions of five Ia and six group II spindle fibres from the m.g. muscle with fifteen m.g. motoneurones in the seventh lumbar and first sacral segments of the spinal cord. Five dorsal root filaments (F1–F5), containing one to three afferent units each (U1–U3), were isolated and mounted on recording electrodes. Heavy lines denote Ia fibres and their branches; lighter lines indicate group II fibres. A micro-electrode was placed successively inside motoneurones 1–15, whose locations are shown. The multi-unit trains of signals recorded from filaments F1–F5 were played back from a tape recorder and used to generate single-fibre e.p.s.p.s in each motoneurone, as described in the Methods. The wiring diagram indicates which afferent fibres established functional connexions with each motoneurone. Detailed analyses of this and other similar experiments will be given after data on the afferent fibres and motoneurones studied in this series of experiments have been presented.

Sample of afferent fibres and motoneurones investigated. The primary goals of this study were to investigate the functional projections of as many Ia and spindle group II fibres as possible to single motoneurones and to compare how the same fibres projected to motoneurones of different size and location. It was, therefore, essential to distinguish the impulses of each afferent fibre in a dorsal root filament unmistakeably throughout the entire experiment. Experiments in which the identity of afferent fibres was lost during many hours of recording contributed to the general fund of knowledge, but could not be included in the final analysis. Identification of the afferent fibres within a dorsal root filament was based on three criteria: (1) their

conduction velocities, (2) the amplitudes and shapes of their action potentials as recorded from the dorsal root filaments and (3) the amplitudes and shapes of their action potentials as recorded by 'back-averaging' from the m.g. muscle nerve. In general, it was not feasible to follow the identities of more than six afferents within one dorsal root filament throughout an experiment.

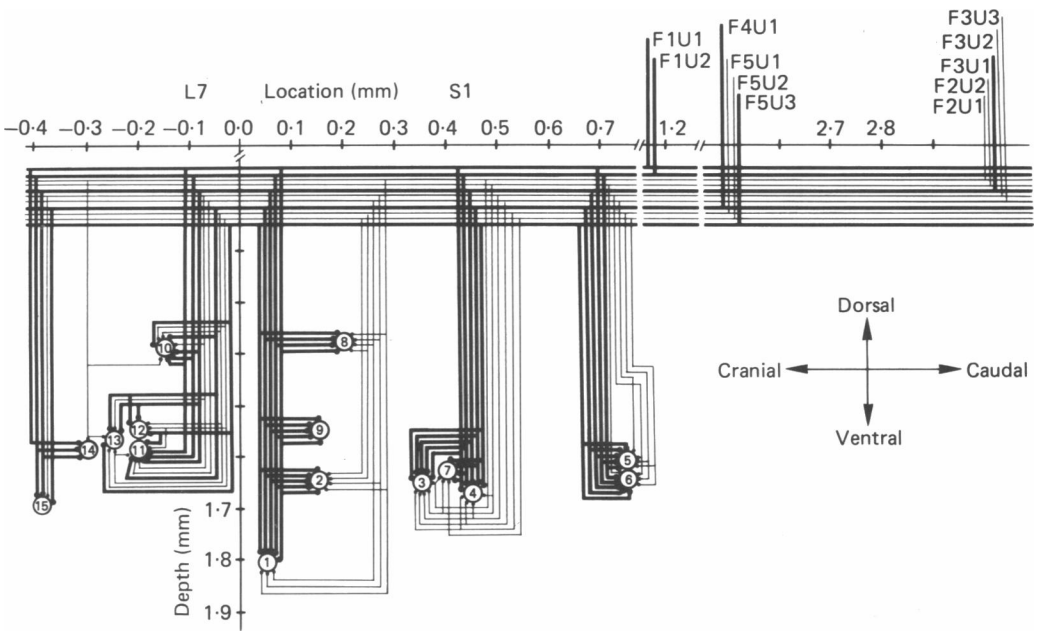

Fig. 3. Wiring diagram of direct, functional connexions between afferent fibres from stretch receptors in the m.g. muscle of a cat and fifteen motoneurones that innervate it, as established by spike-triggered averaging. Heavy lines represent group I a fibres and their branches, lighter lines group II spindle fibres. Numbered circles represent fifteen m.g. motoneurones from which intracellular recordings were made in sequence. The locations of afferent collaterals are merely diagrammatic (see text).

The two histograms in Fig. 4 illustrate the distributions of conduction velocities in the afferent fibres and axons of motoneurones that were studied in six experiments that comprise our definitive data. Following Hunt (1954), afferents were classified into group I a and spindle group II fibres on the basis of their conduction velocities, as well as on their responses to stretch and twitch contraction of the m.g. muscle. The dividing line between the two groups was set at 72 m/s (Hunt, 1954; Lüscher *et al.* 1979*a*).

The input resistances of some of the motoneurones were measured with two different techniques, as noted in the Methods. Although the input resistances were often found to be related inversely to the conduction velocities of the corresponding motor axons as reported by several authors (Kernell, 1966; Barrett & Crill, 1971; Fleshman, Munson, Sypert & Friedman, 1981), the correlation was troublingly unsatisfactory for our purposes. Despite the great care with which they were done, estimates of the input resistance of the same motoneurone sometimes differed by as

much as 50 % when determined with the direct and indirect techniques (see Methods). The conduction velocity of the motor axon was, therefore, taken as the most reliable indication of motoneurone size and was used throughout the study (see Henneman & Mendell, 1981, for discussion of this matter and references).

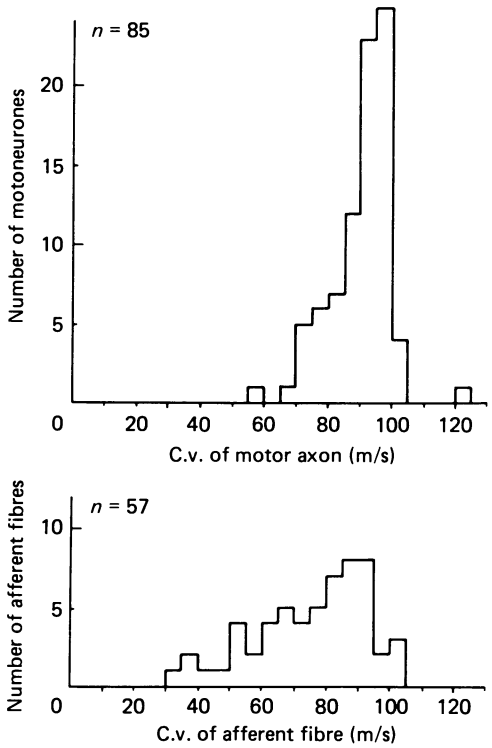

Fig. 4. Histograms illustrating the distribution of conduction velocities (c.v.s), grouped in pentads, of the afferent fibres and axons of motoneurones that were studied in six definitive experiments.

A summary of data on the afferent fibres and motoneurones studied in the six most complete experiments, on which this paper is based, is presented in Table 1.

Examples of single-fibre e.p.s.p.s used to establish functional connexions. Two sets of single-fibre e.p.s.p.s recorded from two different motoneurones in the same experiment (STA 13) are reproduced in Fig. 5. Those from motoneurone 12 (C12), which had an axonal conduction velocity of 91 m/s are shown in column *A*, while those recorded from motoneurone 7 (C7), with a conduction velocity of 75 m/s, are displayed in column *B*. The e.p.s.p.s in the two columns were elicited by impulses in the same eleven afferent fibres (ten group Ia and one group II). The identifying labels and the conduction velocities (m/s, in parentheses) of these eleven fibres are given between the two columns of responses. The conduction velocities are arranged in descending order from 94 to 32 m/s. A vertical line is drawn through small deflexions that we believe signal the arrival of the afferent impulse in the volume of the spinal cord. Identification of these potentials was not certain in some traces; hence the latencies

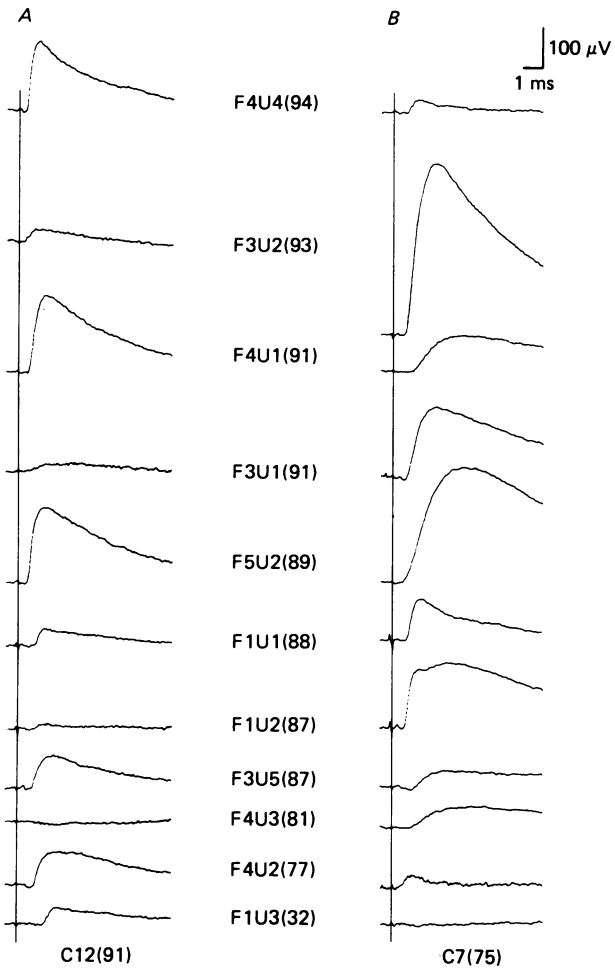

Fig. 5. Single-fibre e.p.s.p.s evoked by eleven afferent fibres in two motoneurones in the same experiment (STA 13). The motoneurone in *A* (C12) had an axonal conduction velocity of 91 m/s; that in *B* (C7) had 75 m/s. The identifying labels and conduction velocities (m/s, in parentheses) of the eleven afferent fibres are given between the two columns of responses.

TABLE 1. Summary of afferent–motoneurone connexions studied

Experiment	No. of motoneurones	No. of afferents			No. of connexions studied			
		Total	Ia	II	Total	Active	Inactive	Not analysed
FOA 14	15	11	5	6	165	112	53	0
FOA 20	11	6	3	3	66	50	15	1
FOA 21	10	6	3	3	60	44	14	2
STA 8	13	11	6	5	143	85	48	10
STA 13	24	11	10	1	264	206	56	2
STA 14	12	12	9	3	144	105	33	6
Totals	85	57	36	21	842	602	219	21

of the e.p.s.p.s measured from this line may not all be reliable. In general, the smaller e.p.s.p.s with slower rising phases have longer latencies, as Mendell & Henneman (1971) reported, which is consistent with Rall's (1967) theoretical analysis. The relatively long base line preceding the e.p.s.p.s is a result of passing the intracellularly recorded signals through an analog delay line before averaging. This procedure helped to distinguish very small e.p.s.p.s from no apparent responses. Fig. 5 illustrates well the great variety of amplitudes and shapes of e.p.s.p.s elicited in the same motoneurone by impulses in different afferent fibres. Several morphological and topographical factors apparently influence the amplitude of single-fibre e.p.s.p.s. We will attempt to identify and evaluate these factors as we continue. Responses with time courses that were obviously composite, such as C7 F1U2, were seldom observed, as Mendell & Henneman (1971) reported in their original study of single-fibre e.p.s.p.s. In a paper to follow, the amplitudes and shapes of the large sample of e.p.s.p.s obtained in this series of experiments will be analysed more rigorously and more definitive conclusions regarding e.p.s.p.s with compound time courses will be presented.

Each of the two columns *A* and *B* in Fig. 5 has one trace with no e.p.s.p. One of these exceptions is associated with F4U3 (a Ia fibre with a conduction velocity of 81 m/s), the other with F1U3 (a small group II fibre conducting at 32 m/s). Much of the present paper will be concerned with identifying factors associated with such absences of functional connectivity.

Despite the obvious importance of afferent fibre size in determining e.p.s.p. amplitude, which will be demonstrated clearly in this study, a comparison of the individual responses in columns *A* and *B* of Fig. 5 indicates that factors other than the conduction velocity of the afferent fibre are quite sufficient to overshadow its influence on single-fibre e.p.s.p.s.

Functional connectivity between afferent fibres and motoneurones. Fig. 6 is designed to illustrate exactly which of the eleven afferent fibres in FOA 14 made functional connexions with each of the fifteen motoneurones studied in that experiment and which ones did not. The dorsal root fibres entering the S1 segment of the spinal cord are labelled with the filament (F) and unit (U) numbers. Ia fibres are represented by continuous lines and spindle group II fibres by dashed lines. It is known from anatomical studies (Scheibel & Scheibel, 1969) that as each Ia fibre passes up the dorsal columns (the shaded longitudinal tract), it gives off a series of ventrally directed primary collaterals. The courses of these collaterals, which make direct connexions with homonymous motoneurones, are not known in these experiments. The large, ventrally directed arrow leaving the dorsal column is not intended to suggest that these collaterals all run together. The rostro-caudal and dorso-ventral locations of the motoneurones in this experiment are shown with numbers that indicate the order in which they were impaled (see scales for distances). The rostro-caudal levels of the cells are accurate to within about $\pm 0{\cdot}10$ mm, but the dorso-ventral locations are less certain, because the advancing micro-electrode may distort spinal tissues causing misleading read-out of depth from the micromanipulator. Zero on the longitudinal scale marks the boundary between the L7 and S1 segments. Nine motoneurones are situated in the rostral part of S1 and six in the caudal part of L7. The motoneurones were arbitrarily divided into two sizes on the basis of their axonal conduction velocities. Those drawn with larger cell bodies and greater total

length of cell body and dendrites had axonal conduction velocities from 99 to 86 m/s. Those with smaller cell bodies and shorter dendrites had axonal conduction velocities from 85 to 73 m/s. Functional connexions or their absence are indicated by the presence or absence of triangles at the ends of the afferent terminals where they contact the dendrites. The terminals of the afferent fibres are represented in the same

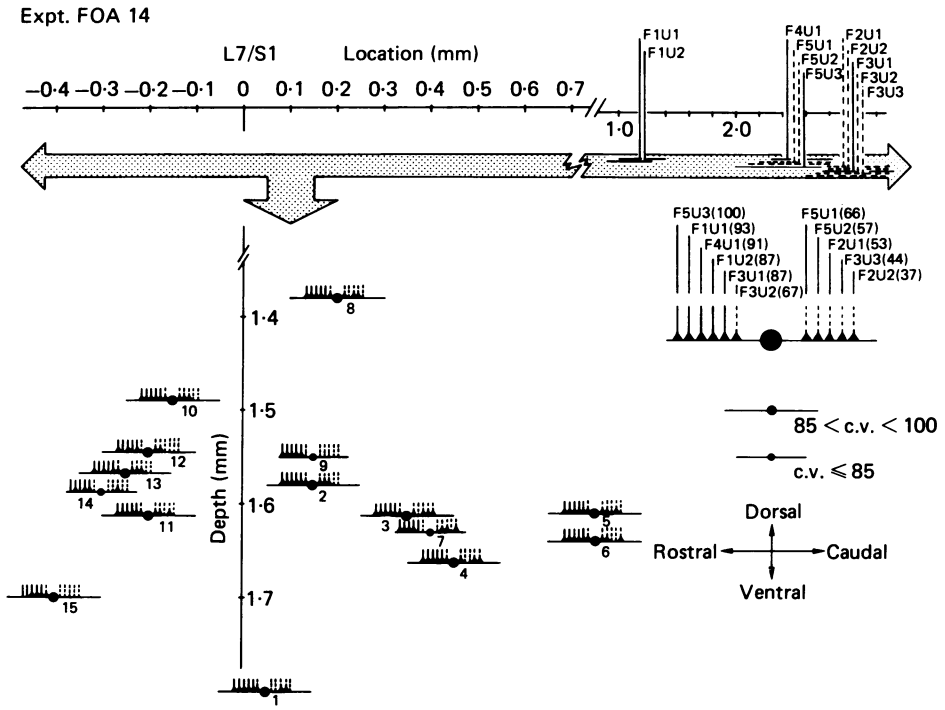

Fig. 6. Diagram illustrating which of the eleven afferent fibres in expt. FOA 14 made functional connexions with each of the fifteen motoneurones and which ones did not. Three motoneurones with conduction velocity (c.v.) of 85 m/s or less are represented by smaller cell bodies and shorter dendrites. Those with conduction velocities of 86–99 m/s are represented by larger cell bodies and longer dendrites. The terminals of the eleven afferent fibres are shown in the same fixed order on all motoneurones and can be identified on the labelled inset. Functional connexions or their absence are indicated by the presence or absence of triangles at the ends of the afferent terminals where they contact the dendrites of each cell.

fixed order on all the motoneurones in Fig. 6. This standard order is indicated on a larger, schematic drawing of a motoneurone, on which the afferent terminals are labelled. The conduction velocities of the afferent fibres are arranged in decreasing order from left to right. Thus, four of the five Ia fibres have functional connexions with motoneurone 8 (C8) and one does not. Four of the six group II fibres have functional connexions with this cell and two do not. Although each triangular 'connexion' in Fig. 6 may suggest a single synapse, it is intended, rather, to symbolize a variable number of synapses formed by the terminal arborization of a single afferent fibre. The number of synapses in such a contact 'system' may range from three to

forty according to recent anatomical studies with horseradish peroxidase or cobalt staining (Iles, 1976; Brown & Fyffe, 1978, 1981; Burke, Walmsley & Hodgson, 1979; R. E. Burke, personal communication). Recent electrophysiological evidence (Henneman, Lüscher & Mathis, 1984) indicates that impulses in a single afferent fibre do not necessarily activate all of the synapses which that fibre forms on a motoneurone, but may repeatedly fail to activate some endings during prolonged spike-triggered averaging, while consistently activating others from the same fibre. The number of synapses activated by an impulse in a single afferent fibre is presumably one of the important factors determining the amplitude of the e.p.s.p. that is evoked.

In experiment FOA 14 there were 165 (11 × 15) possible contact systems. Functional connexions were demonstrated by spike-triggered averaging in 112 of them. Inspection of Fig. 6 reveals a clear correlation between somatotopic factors and functional connectivity. Motoneurones 5 and 6, closest to the spinal entry levels of the eleven afferent fibres, each received functioning connexions from nine of them. Motoneurones 3, 4 and 7, somewhat farther away, received connexions from slightly fewer fibres (average = 8·67). The average number of functioning connexions received by cells 1, 2, 8 and 9, even farther away, was still smaller (7·50) and for cells 10–13 it was the same (7·50). The motoneurones located most rostrally (14 and 15) received fewer functional connexions (four each) than any cell that was closer to the entry levels of the afferent fibres. These findings are consistent with observations of Lüscher, Ruenzel & Henneman (1980), showing that the amplitudes of post-synaptic population potentials elicited in a ventral root by impulses in single Ia fibres decrease as the distance between the entry level of the afferent fibre and the ventral root increases.

Before drawing any firm conclusions about the relation between distance and connectivity in experiment FOA 14 the influence of motoneurone size on connectivity should be considered as well. Motoneurones 5 and 6, both relatively large as judged by their axonal conduction velocities (89 and 97 m/s) each received nine functional connexions. In the next group of cells (3, 7 and 4), the two motoneurones with faster axonal conduction velocities, numbers 3 (91 m/s) and 4 (97 m/s) received ten and nine functional connexions respectively, while the smaller cell (number 7) with an axonal conduction velocity of 82 m/s, situated between them, received only seven functional projections. In the next group of cells the three larger ones (numbers 8, 2 and 1), with axonal conduction velocities of 99, 88, and 91 m/s, received eight, nine and eight connexions respectively, while the smaller motoneurone, C9 (73 m/s) received only five. Motoneurones 10 (96 m/s), 11 (97 m/s), 12 (90 m/s) and 13 (93 m/s), all relatively large, received nine, nine, five and seven connexions respectively, whereas the smaller motoneurone C14 (84 m/s) nearby received only four. Cell 15 (88 m/s), a larger cell but the most distant, received only four functional connexions.

If only the larger motoneurones in the five groups are considered, the average number of functional connexions decreases with distance as follows: 9·0, 9·50, 8·33, 7·50 and 4. If only the smaller motoneurones are considered, the average number of functional connexions also decreases with distance (from seven to five to four).

Analysis of data with connectivity matrices. The factors that appeared to influence functional connectivity in Fig. 6 were evaluated further by entering the data from

that experiment in a connectivity matrix that could be arranged in various ways. In Fig. 7 the existence of functional connexions between individual afferent fibres and motoneurones is indicated by filled circles, and absence of connexions by blank spaces. In both matrices the eleven afferent fibres are arranged from left to right in

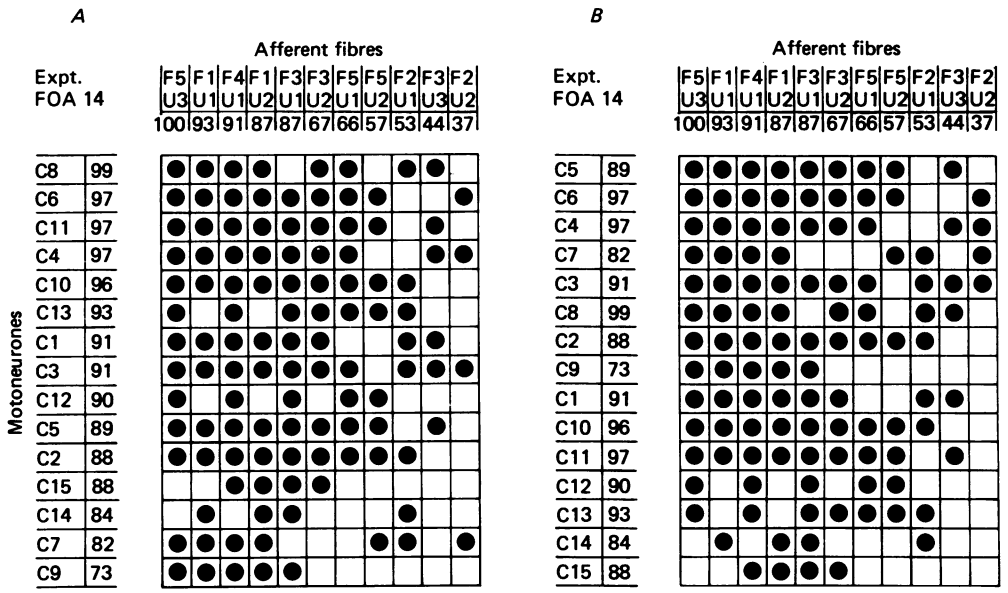

Fig. 7. Connectivity matrices illustrating how the axonal conduction velocities of the motoneurones and afferent fibres in expt. FOA 14 are related to the presence (●) or absence (blank squares) of functional connexions between them. In both *A* and *B* the eleven afferent fibres are arranged from left to right in order of decreasing conduction velocity. Motoneurones are listed from top to bottom in order of decreasing axonal conduction velocity in *A* and in order of increasing longitudinal distance from the entering dorsal root fibres in *B*.

order of decreasing conduction velocity. The fifteen motoneurones to which they project are listed from top to bottom in order of decreasing axonal conduction velocity in Fig. 7 *A* and in order of increasing longitudinal distance from the entering dorsal root fibres in Fig. 7 *B*. It is readily seen that the five Ia fibres, with conduction velocities ranging from 100 to 87 m/s, had functional connexions with most of the fifteen motoneurones. As the conduction velocities of the six group II fibres decreased from 67 to 37 m/s, however, the number of motoneurones receiving connexions from them decreased from eleven to ten to eight to eight to six to four. Hursh (1939) showed that conduction velocity was directly correlated with fibre diameter. It may be inferred that the larger the diameter of a stretch-afferent fibre, the greater is its capacity for establishing functional connexions with homonymous motoneurones. The considerable influence of afferent fibre diameter on the patterns of connectivity in Fig. 7 *A* and *B*, however, is not sufficient by itself to ensure functional connectivity between all large afferent fibres and all motoneurones. There are ten blank squares in the five vertical columns on the left sides of the two matrices, where the Ia fibres

are placed. Even the largest fibre, with a conduction velocity of 100 m/s, did not make functional connexions with motoneurones 14 and 15. A reasonable explanation for some of these ten blanks, as already noted, is that factors other than afferent-fibre diameter may have considerable influence on functional connectivity.

Analysis of Fig. 6 suggested that the distance between the spinal entry point of an afferent fibre and the location of a particular motoneurone, and the size of the motoneurone, both influenced the probability of functional connexions between them. The two matrices in Fig. 7 are arranged so that the influence of each of these factors can be seen more readily. If the five vertical columns, in which the connexions of the Ia afferents are tabulated, are compared, it is seen that the blank spaces are located in different places in the two matrices. In Fig. 7*B*, where the motoneurones are arranged according to their distances from the entering dorsal root fibres, eight of the ten blank spaces fall in the four lowest horizontal rows, whose motoneurones are the most 'distant'. This distribution of blanks occurred despite the fact that three of the four cells in the lowest rows were relatively large according to their axonal conduction velocities. Distance, in this case, was evidently a more potent factor in causing blanks than large motoneurone size was in preventing them. In the same five vertical columns of Fig. 7*A*, where the motoneurones are arranged according to their axonal conduction velocities, only five of the ten blank spaces fall in the four bottom rows where the smallest motoneurones are located. Four of these five blanks are associated with C15 and C14, both 'distant' cells. C7 and C9 are both smaller than C15 and C14, but together they have only one blank in their ten left-hand spaces. Both were closer to the entering fibres than C15 and C14, as can be seen in Fig. 7*B*. The other blank squares in the five Ia columns of Fig. 7*A* are found in the row of cell 8 (despite its large size), in the row of C13 (a large, but 'distant' cell) and in the row of C12 (also a large, but 'distant' cell). All of these observations suggest that distance has a greater effect on functional connectivity than motoneurone size. It would be premature, however, to minimize the possible influence of motoneurone size at this stage. Only one blank space occurs in the 5×5 block of squares in the upper left corner of Fig. 7*A*, which is associated with the five largest motoneurones in this experiment. Although cells 10 and 11 are both 'distant' and might, therefore, have failed to receive functional connexions, their large size was perhaps sufficient to ensure connexions. Their size, in fact, may have been a favourable factor in enabling them to receive functional connexions from all afferents except two group II fibres.

In short, Fig. 7 indicates that the diameter of an afferent fibre definitely influences the probability that it will establish functional connexions with motoneurones. Increasing distance between its entry point and the location of particular motoneurones decreases this probability. It is possible that larger motoneurones have a greater chance of receiving functional connexions, but this will require further investigation.

In order to throw further light on some of the factors that may influence functional connectivity, a three-dimensional graph (Fig. 8) was constructed. The amplitude of each single-fibre e.p.s.p. recorded in experiment FOA 14 is plotted against the axonal conduction velocity of the motoneurone and that of the afferent fibre. This Figure illustrates that, in general, the amplitudes of individual e.p.s.p.s are directly related to the conduction velocities, and, therefore, to the diameters of the afferent fibres. No obvious correlation between e.p.s.p. amplitudes and axonal conduction velocities

of motoneurones can be seen. As noted previously, it is apparent that impulses in large afferent fibres evoke both large and small e.p.s.p.s, whereas those in small fibres elicit e.p.s.p.s that are generally small, but occasionally of medium amplitude. Only the larger afferent fibres project functionally to the smallest motoneurones in this

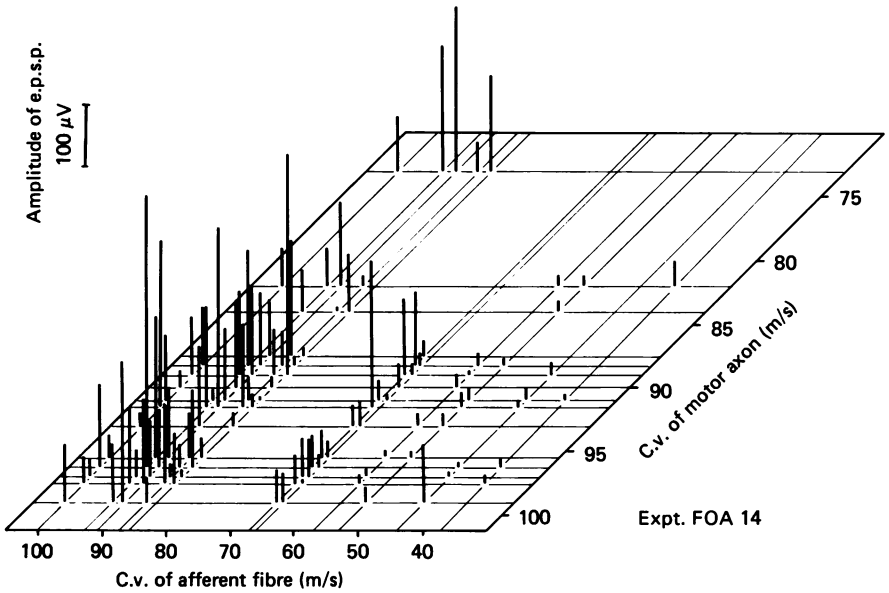

Fig. 8. Linear three-dimensional graph of the relations between amplitude of e.p.s.p.s, conduction velocity (c.v.) of afferent fibres and conduction velocity of motor axons. Data from a single experiment (FOA 14).

experiment. This Figure also illustrates the enormous variations in e.p.s.p. amplitude which make identification of systematic relationships difficult, even though the experiments were designed to minimize most of the extraneous factors which might contribute to variability.

If each of the three factors we have identified could be weighted according to the degree of influence it exerts, the pattern of connectivity could probably be explained more precisely. Certain exceptions to the trends we have noted, however, could not be explained by any combination of the three factors. For example, cell 3, which is both smaller and more distant than C6 and C4, has only one blank, while the latter each have 2. A group II fibre with a conduction velocity of 57 m/s failed to elicit an e.p.s.p. in C3, causing this blank, while three other group II fibres which were more slowly conducting and had somewhat more caudal entry points all evoked e.p.s.p.s in C3. Experimental errors do not account for the frequent exceptions to the orderly trends we have identified in the data. It may be concluded (1) that factors not yet identified exert significant effects on functional connexions and/or (2) that there is a considerable degree of uncertainty in the process by which connexions are established.

Functional connexions of a large sample of Ia fibres. Only five Ia fibres were isolated in experiment FOA 14. Each of them had functional connexions with most of the

fifteen motoneurones. This small sample of fibres and their rather limited range of conduction velocities (87–100 m/s) did not provide a satisfactory opportunity to analyse the influence of I a fibre size on connectivity. An unusually good opportunity to do so, however, arose in a later experiment (STA 13) in which ten I a fibres were isolated and intracellular recordings were obtained from twenty-four homonymous

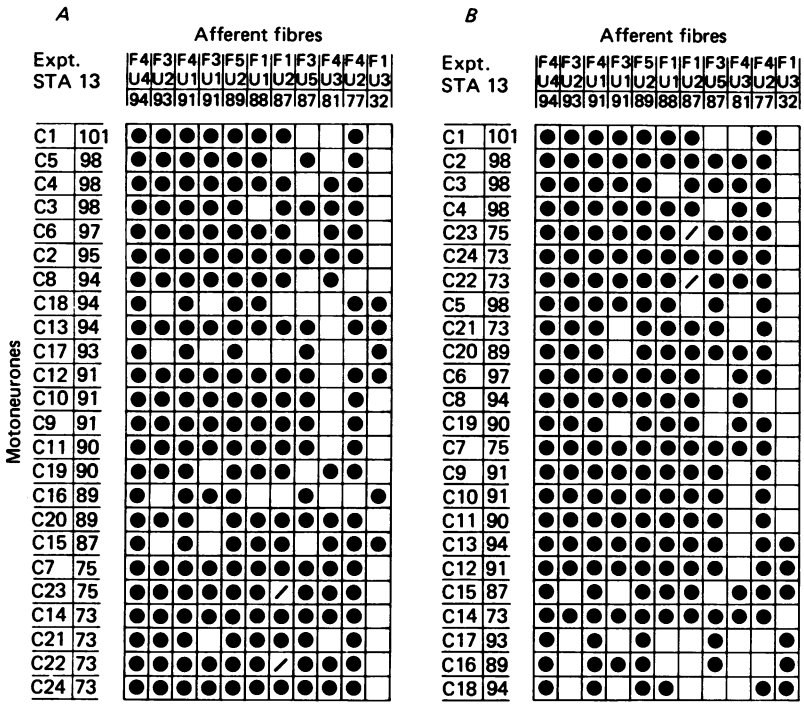

Fig. 9. Connectivity matrices for expt. STA 13, illustrating the functional connexions of ten group I a fibres and one group II fibre from the medial gastrocnemius muscle with twenty-four of its motoneurones. Two versions of a connectivity matrix, organized as in Fig. 7 *A* and *B* are shown. Connexions marked with diagonal lines could not be analysed because the afferent fibre failed to discharge temporarily.

motoneurones. Only one group II fibre (conduction velocity, 32 m/s) was isolated in this experiment. Little attention will be paid to it, except to note that it made fewer functional connexions with the twenty-four motoneurones than any of the I a fibres. Without including the group II fibre, there were 240 (10×24) possible I a contact systems. In 38 of these no e.p.s.p. was elicited. Two versions of a connectivity matrix for this experiment, organized as in *A* and *B* of Fig. 7, are shown in Fig. 9.

In general, but with notable exceptions, the number of blank spaces in the vertical columns increased from left to right as the conduction velocities of the ten afferent fibres decreased. In the three vertical columns on the left side of the matrices, where the conduction velocities of the afferent fibres averaged 92·7 m/s, 5·6% of the seventy-two squares were blank. In the next three vertical columns, where conduction velocities averaged 89·3 m/s, 12·5% of the seventy-two squares were blank, and in

the next four columns, where conduction velocity averaged 83·0 m/s, 26·6 % of the ninety-four squares investigated were blank. The combinations of vertical columns selected for averaging were necessarily arbitrary in this case and the results no doubt reflect this arbitrariness. In order to verify the results more rigorously, the percentage of blank spaces was also calculated by 'boxcar averaging'. The results were as follows: vertical columns 1–5 on the left, 8·3 % blanks; 2–6, 10·8 %; 3–7, 10·8 %; 4–8, 16·9 %; 5–9, 21·2 %; 6–10, 23·7 %. The range of conduction velocities available in the ten afferent fibres was relatively limited (17 m/s) in this experiment. Nevertheless, with the larger sample of data, it was obvious that, with some exceptions, the larger the Ia fibre was, the more functional connexions it had with homonymous motoneurones.

In Fig. 9*B*, with the twenty-four motoneurones arranged in order of increasing distance from entering dorsal root fibres, the number of blank squares increased as the distance increased. In the upper eight horizontal rows, representing connexions of motoneurones that were closest to the entry points of the afferent fibres, only 7·7 % of the seventy-eight squares were blank (two contact systems could not be investigated and group II blanks were not counted). In the next eight rows 12·5 % of the squares were blank and in the lowest eight rows 27·5 % of the squares were blank. Clearly, 'distant' cells have a lower probability of receiving functional connexions from Ia fibres than nearer cells.

In experiment FOA 14 it appeared that the largest motoneurones received the most functional connexions from Ia fibres. In Fig. 9*A*, however, with a considerably larger sample of potential connexions on which to base conclusions, there is little evidence that motoneurone size exerts an influence on the connectivity of Ia fibres.

Again, as in Fig. 7, the connectivity pattern in Fig. 9*B* reveals the unmistakable influence of afferent fibre size and topography, as well as significant individual departures from the clear statistical trends.

Connectivity matrix for a group of cells lying close together. In experiments FOA 14 and STA 13 the motoneurones from which e.p.s.p.s were recorded were spread out over a longitudinal distance of 1·2–1·3 mm. Despite their close proximities to each other, the relatively small differences in their distances from dorsal root entry levels were evidently sufficient to produce definite effects on the number of functional connexions they received.

In experiment STA 14 recordings were made from twelve motoneurones that were unusually close together and therefore were of special interest. Eleven of the cells had longitudinal separations no greater than 0·35 mm and one was 0·2 mm farther away. Two versions of a connectivity matrix for this experiment, organized as in Figs. 7 and 9, are shown in Fig. 10. Once again, the influence of afferent fibre size is apparent in the connexions of the Ia fibres alone as well as in the matrix as a whole. Boxcar averaging of the vertical columns in groups of six, as in experiment STA 13, gave the following results: columns 1–6 on the left, 11 % blanks; 2–7, 22·9 %; 3–8, 22·9 %; 4–9, 25·7 %; 5–10, 27·1 %; 6–11, 28·6 %; 7–12, 36·8 %.

The distances of the motoneurones from the dorsal root entry levels were clearly correlated with their functional connectivities. Boxcar averaging of the horizontal rows in groups of six gave the following results: rows 1–6 at the top, 16·7 % blanks; 2–7, 20·8 %; 3–8, 22·2 %; 4–9, 27·8 %; 5–10, 28·2 %; 6–11, 32·9 %; 7–12, 31·8 %.

In Fig. 10*A* there is no apparent correlation between motoneurone size and functional connectivity for either Ia or group II fibres.

In short, although the total longitudinal extent of the group of motoneurones in experiment STA 14 was much less than that in the experiments previously described, the influences of afferent fibre size and topography on functional connectivity were still apparent.

Data on motoneurone size pooled from six experiments. Findings in individual experiments have not established conclusively whether the size of a motoneurone

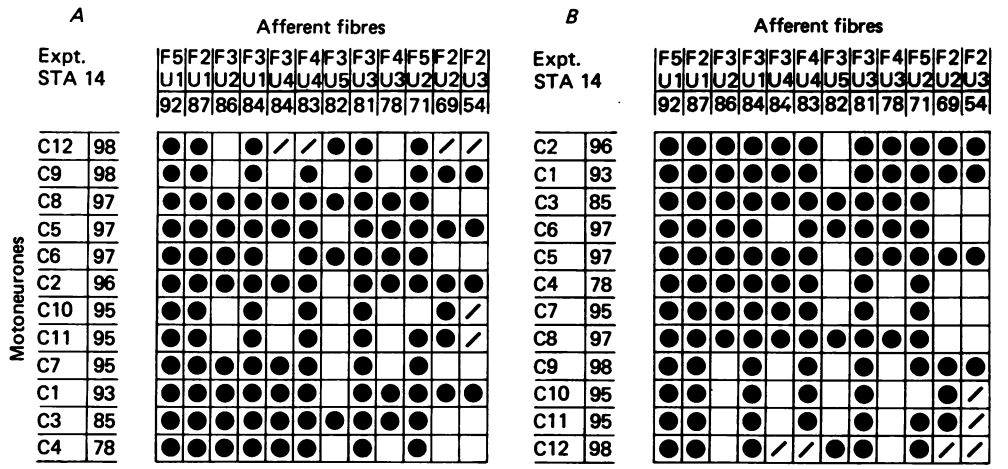

Fig. 10. Connectivity matrices for expt. STA 14. Eleven of the motoneurones in this experiment were within 0·35 mm of each other (longitudinally) and one (C12) was 0·2 mm farther away. *A* and *B* are organized as in Figs. 7 and 9.

influences the number of functional connexions it receives from stretch-afferent fibres. In order to bring more data to bear on this question, the findings from all six of our definitive experiments were pooled. A three-dimensional histogram (Fig. 11) was constructed, illustrating how the percentages of functional connexions varied with the axonal conduction velocities of the motoneurones and afferent fibres. The combined influence of these two variables is easy to visualize in the twelve groups of pooled data. The numbers of connexions represented by each group are indicated at the top of each column.

Motoneurones with axonal conduction velocities in the ranges of 59–85 m/s and 86–100 m/s had projection percentages that increased progressively with the conduction velocities of the afferent fibres. A similar trend, but with a less even progression, is apparent for motoneurones with more rapidly conducting axons.

The influence of motoneurone size on projection percentage is clear for group II projections. As the axonal conduction velocities of the motoneurones increased in three steps, there was a corresponding increase in the percentage of group II fibres projecting to them. This stepwise increase was seen for both the slower half of the group II fibres (32–51 m/s) and the faster half (52–71 m/s). With Ia afferents, the influence of motoneurone size was not clearly demonstrated by the pooling procedure. However, the trends in the pooled data are obvious. The lowest projection percentage

was obtained in a group of thirty-four connexions between the smallest afferent fibres and the smallest motoneurones. In general, this percentage increased as the size of the afferent fibres or the motoneurones increased from group to group, finally reaching 100% for the largest motoneurones receiving projections from the largest

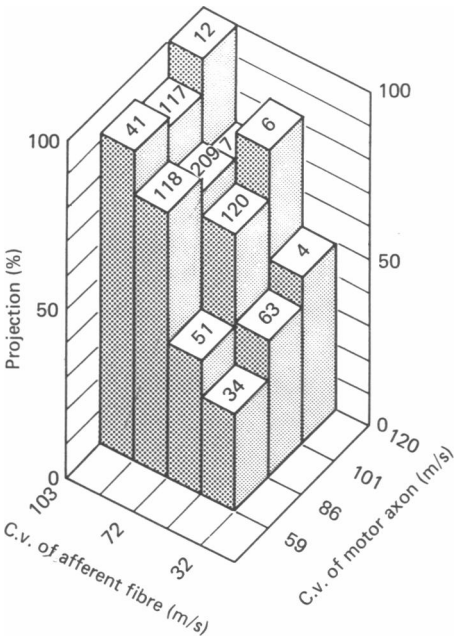

Fig. 11. Three-dimensional histogram illustrating how the percentage of functional connexions in the pooled data from six experiments varied with the axonal conduction velocity (c.v.) of the motoneurones and afferent fibres. The numbers of connexions represented by each group are shown at the top of each column.

afferent fibres. We conclude that motoneurone size definitely influences the percentage of the functional projections from group II fibres and that this influence probably extends into the range of group Ia fibres.

DISCUSSION

To simplify initial analysis of the experimental findings it was expedient to define connectivity qualitatively, i.e. simply by the occurrence of single-fibre e.p.s.p.s, postponing quantitative analysis of the shapes and sizes of the responses used to identify connexions for a subsequent study.

Failure to evoke a single-fibre e.p.s.p. in a motoneurone by spike-triggered averaging does not necessarily indicate that there is no anatomical projection from that fibre. When horseradish peroxidase is injected into single Ia fibres and homonymous motoneurones, evidence of a presumed anatomical connexion between the two can always be found (R. E. Burke, personal communication). Some of these connexions are apparently subject to what is usually called 'transmission failure'.

According to Mendell (1984), in preparations anaesthetized with barbiturates the proportion of homonymous motoneurones in which single-fibre e.p.s.p.s were evoked averaged about 80% in six different laboratories. Evidence has been accumulating that not only may transmission fail in Ia projections, but also that failure can be relieved by experimental procedures (Edwards, Redman & Walmsley, 1976*a*, *b*; Lüscher, Ruenzel & Henneman, 1979*b*, 1983*b*, *c*; Jack, Redman & Wong, 1981*a*, *b*; Hirst, Redman & Wong, 1981). In most cases the failures have presumably involved part of the terminal arborization of a Ia fibre on a single motoneurone or some of its synapses, the clearest example of partial failure being that recently reported by Henneman, Lüscher & Mathis (1984). Following low spinal transections, there is an increase in the percentage of motoneurones to which single Ia fibres project functionally (Nelson *et al.* 1979). This evidently indicates that pre-existing anatomical connexions, which were completely non-functional in untransected cords, became active after transection. Since there was no way of identifying anatomical connexions that were inactive in the present study, the term 'functional connexions' has been used to avoid ambiguity throughout this paper.

Influence of afferent fibre diameter on connectivity. In all experiments it was obvious that the diameter of a stretch-afferent fibre has considerable influence on the probability of its establishing functional connexions with homonymous motoneurones. Even with the largest afferent fibres, however, this influence was not strong enough to ensure functional connexions with all motoneurones. An element, which can only be identified as chance or the operation of some probabilistic mechanism was evident in all the results. There were, for example, two Ia fibres (F4U1 and F3U1) with conduction velocities of 91 m/s in Fig. 9. The more 'distant' afferent fibre (F4U1) had functional connexions with all of the twenty-four motoneurones investigated. The nearer one lacked connexions with six of these same cells.

It is easy to suggest why larger fibres might have a greater capacity for forming functional connexions. As Cajal (1909) was the first to note, large fibres in all parts of the mammalian nervous system give off more branches and terminals than small fibres. There seem to be no striking exceptions to this scaling principle. To meet the demands of axoplasmic flow in all of its many branches and terminals, a large parent fibre requires a thick axon. The analogy with a system of branching water pipes is obvious. The amplitude of single-fibre e.p.s.p.s associated with large and small fibres suggests that the diameter of the parent fibre is finally expressed in the size of the terminal arborizations it forms on individual motoneurones. Fig. 8 reveals that impulses in large fibres evoke large, medium or small e.p.s.p.s, whereas those in small fibres elicit relatively small responses. It is reasonable to infer that many active synapses are required (though not sufficient) to evoke a large individual e.p.s.p. and that these can only come from a large terminal arborization formed by a large afferent fibre. The more extensive terminal arborizations stemming from large fibres should have a greater likelihood of coming into close proximity with motoneurones in their vicinity and, thus, a better chance of establishing connexions with them. The large element of chance in such a process would offer an explanation for the random way in which the influence of fibre size is expressed in the experimental results.

Interpretation of the distance effect. Figs. 5, 6, 7, 9 and 10 all illustrate what we have called the 'distance effect', namely that the distance between the spinal entry point

of a Ia fibre and the location of a particular motoneurone has considerable influence on the probability of functional connexions between them. Before accepting the apparent significance of the observations, however, a previously neglected aspect of the experiments should be considered. In most, but not all experiments, the micro-electrode was moved, after each period of recording, to progressively more rostral levels farther from the entry points of the dorsal root fibres. As a result, the most distant motoneurones were often the last to be studied. Conceivably, the results might therefore be attributable to progressive deterioration of the spinal cord which chiefly affected observations made near the ends of the experiments.

There are many examples in the connectivity matrices where the distribution of responses and blanks could hardly be due to deterioration. In Fig. 9*B*, for example, there were numerous blanks in the three lowest rows of the matrix. It is unlikely that these blanks were due to deterioration, however, because in three of the five vertical columns on the left side of the matrix there was not a single blank. It would be inconsistent to explain the blanks in the three horizontal rows on the basis of deterioration when e.p.s.p.s were being recorded simultaneously from the same motoneurones by impulses in other fibres. Failure of conduction in the intraspinal part of the parent fibres or in the preterminal parts of their collaterals is also an unlikely explanation, because of the order in which the failures occurred. F3U1 had blanks in C15 and C17, but not in C16, which was recorded after C15 and before C17. Similarly, F1U1 had blanks in C16 and C17, but not in C18.

Perhaps the strongest evidence against deterioration as a cause of the distance effect comes from observations on motoneurones that were investigated at the end of an experiment, but were located closer to the entering dorsal root fibres than cells which had many blanks. Cells 22, 23 and 24, for example, were the last three motoneurones studied in experiment STA 13, but there were no blanks in any of the Ia projections to them. It would be unreasonable to conclude that the numerous blanks in the Ia projections to C16, C17 and C18 were due to some type of deterioration, when cells 22, 23 and 24, which were recorded even later in the experiment, responded so well. For similar reasons it could hardly be argued that the blanks in F3U2, F3U1, F1U5, F3U5 and F4U3 were due to conduction failures anywhere in the courses of the afferent fibres, except possibly in the terminal arborizations, when impulses in these same afferent fibres elicited e.p.s.p.s in C22, C23 and C24 at the end of the experiment.

A consideration of the intraspinal course of Ia fibres and their collaterals leads to a reasonable explanation of the intriguing distance effect. As each Ia fibre passes up the dorsal columns, it gives off a series of primary collaterals that run ventrally to make connexions with motoneurones. Recently Lüscher *et al.* (1980) recorded the post-synpatic population potentials (p.s.p.p.s) evoked in ventral roots by impulses in single Ia fibres. The amplitude of the post-synaptic population potentials was greatest when the afferent fibre entered the spinal cord at the level of the ventral root, and decreased progressively as the distance between the ventral root and the entry point increased. This finding suggested that the number of endings given off to motoneurones by primary collaterals is greatest near the entry point and that the number given off by successively more distant collaterals decreases progressively. From this it may be inferred that as the total number of collaterals given off by a

Ia fibre increases with distance from the entry point, the diameter of the Ia fibre and its collaterals decreases progressively. In their study of the intraspinal course of Ia fibres, Ishizuka, Mannen, Hongo & Sasaki (1979) provide morphological support for this inference. In Figs. 2*A* and 3*A* of their paper, primary afferent collaterals of Ia fibres from m.g. and soleus are represented along with measurements of their initial diameters. Collaterals from both the ascending and descending branches of the parent fibre decrease in initial diameter as the distance from the spinal entry point increases. As noted in the preceding section, the diameter of a primary collateral is presumably reflected in the extent of the terminal arborizations it gives off. A series of progressively thinner collaterals should, therefore, have successively less extensive terminal arborizations, whose capacities to form functional connexions should decrease accordingly. The rather abrupt increase in the number of blanks in the bottom three rows of Fig. 9*B* suggests that progressive thinning of the collaterals may reach a critical diameter at some distance from the spinal entry level, beyond which the capacity to make functional connexions declines rapidly.

The distance effect, it would seem, is simply a corollary of the fibre size effect. As might be expected, the distribution of functional connexions influenced by distance apparently has the same randomness as the distribution of connexions associated with afferent fibres of different diameter.

The effects of successive branching on fibre diameter are apparently superimposed on the limitations set by the diameters of the parent fibres and their primary collaterals. As might be expected, the thinner the parent fibre, the more susceptible it is to the distance effect in its capacity for forming functional connexions. These conclusions do not necessarily apply to group II fibres. In them fibre diameter has the same influence on connectivity as in Ia fibres. Data on the distance effect in group II fibres, however, are not conclusive.

Influence of motoneurone size on connectivity. The projection percentage in Fig. 11 reached 100 only when both Ia fibres and motoneurones were large. The greater surface area of a large motoneurone would presumably increase the probability of a chance contact with any terminal arborization within its domain. If a large surface area were distributed throughout a corresponding large volume of neural tissue, however, its density might not be sufficient to ensure a 100% projection from the largest afferent fibres. A recent morphological study (Westbury, 1982) provides evidence that the density of dendrites around a motoneurone is, in fact, related to the conduction velocity of its axon. The measured extents of the dendrites of individual motoneurones were found to differ very little, even between α- and γ-motoneurones, i.e. the volumes of tissue 'occupied' by large and small cells were not significantly different. However, the complexity of the dendritic tree was clearly related to the axonal conduction velocity of the cell. Both the number of dendritic terminals and the number of branchings they had undergone increased with the axonal conduction velocity. As would follow from these observations, there was also a clear relation between the total surface area of the cell and its axonal conduction velocity. If the extents of the dendritic trees are about equal, but their complexity increases with conduction velocity, the density of dendrites within the cell's domain must increase with its total surface area or size. Accordingly, any terminal arborization developing within the domain of a large motoneurone should have a higher

probability of making contact with some part of its dendritic tree. This morphological evidence helps to account for the finding that the probabilistic nature of the process governing establishment of connexions varies with the sizes of the neurones involved.

Significance of results. These experiments have led to the identification of three morphological variables that influence functional connectivity on a cell-to-cell level. They will serve as guidelines in the next phase of this study, which deals with the quantitative distribution of input to motoneurones. The results were not unexpected in view of recent studies on the distribution of input from single afferent fibres to large populations of motoneurones (Lüscher, Ruenzel, Fetz & Henneman, 1979*a*, 1980). What was not anticipated was the finding that, on this level of analysis, connectivity apparently does not follow strict, deterministic rules, as the output of the motoneurone pool does. Only size-related 'influences' affecting the probability of connexions have been identified. It cannot be concluded with complete assurance, but it is probable that the connectivity matrices reflect a considerable degree of uncertainty in the process by which connexions are established. This interpretation concurs with the views of Weiss (1969) that the components (in this case, connexions) of a living system necessarily exhibit much more variability (i.e. less orderliness) than the behaviour characteristic of the system as a whole (i.e. orderly recruitment). It is possible, however, that the randomness that we preceive in connectivity matrices may be more apparent than real, just as the scattered pieces of a mosaic may look haphazard until enough are in place to form a pattern.

It would be naive to conclude that the morphological factors, which have been identified in this study, 'explain' connectivity. The influence which size exerts on projection probability suggests that it may play a critical role in bringing neurones into close proximity where short range physical or chemical forces can act effectively. Once proximity is achieved the extents of the presynaptic and post-synaptic surfaces available for contact may determine the number of synapses that develop between two neurones.

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