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DIRECT OBSERVATIONS ON THE CONTACTS MADE BETWEEN Ia AFFERENT FIBRES AND α-MOTONEURONES IN THE CAT'S LUMBOSACRAL SPINAL CORD

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SUMMARY

1. The enzyme horseradish peroxidase was injected into identified lumbosacral α-motoneurones and Group Ia afferent fibres in cats anaesthetized with chloralose and paralysed with gallamine triethiodide. Subsequent histological examination allowed the determination of (*a*) the extent of the motoneuronal dendritic trees, (*b*) the number and location of Ia synapses upon the motoneurones.

2. α-motoneurones had seven to eighteen primary dendrites and each produced daughter branches up to the fourth to the sixth order. At dendritic bifurcations Rall's 3/2 Power Law was obeyed. There was little or no dendritic tapering up to about 800 μm from the soma. Beyond this distance, however, there was considerable tapering.

3. Horseradish peroxidase injections revealed that motoneuronal dendrites are much longer than previously thought. Individual dendrites could be traced for up to 1600 μm from the soma and dendritic trees were usually 2–3 mm from tip to tip. Nearly all the motoneurones had dendrites that entered the white matter of the cord. Dendrites could also reach as far dorsally as laminae V and VI.

4. Ia synapses upon motoneuronal somata were examined in cords counterstained with cresyl violet or methylene green. About 10% of Ia boutons in lamina IX were on somata and each Ia collateral terminated on 3·66 motoneuronal somata or the most proximal (30 μm) dendrites, with on average about two contacts per motoneurone.

5. Ten Ia afferent fibre-motoneurone pairs were injected with horseradish peroxidase. The following conclusions were drawn: (i) only one collateral of any given Ia axon makes contact with a motoneurone even though other collaterals from the same axon might pass through the dendritic tree, (ii) usually all contacts made between a Ia fibre and a motoneurone are at about the same geometrical distance from the soma, even when on different dendrites, (iii) between two and five contacts are made upon the dendritic tree (average 3·4) at distances of between 20 and 820 μm from the soma.

6. The results are discussed in relation to previous anatomical and electrophysiological work.

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INTRODUCTION

Knowledge of the detailed organization of the monosynaptic reflex arc is based largely on indirect evidence. Experiments have been of two main kinds: anatomical, in which the identity of the elements examined is in some doubt, and electrophysiological, where inferences are drawn without the benefit of precise anatomical data.

The anatomical data are, surprisingly, more incomplete than the electrophysiological. Even the extent of the dendritic trees of alpha motoneurones is not clear. Thus examination of Golgi-stained material from adult cats and dogs (Aitken & Bridger, 1961; Gelfan, Kao & Ruchkin, 1970) showed two to fourteen primary dendrites per cell (mean about seven) and geometrical dendritic lengths of up to 1 mm. Injection of Procion Yellow into motoneurones (Barrett & Crill, 1974) revealed eight to twenty-two primary dendrites per cell but total dendritic lengths were only 300–800 μm. The dye injection therefore showed more dendrites but these were presumably not stained for all their length. Injection of tritiated glycine into motoneurones (Lux, Schubert & Kreutzberg, 1970) showed similar numbers of dendrites to the Procion Yellow results and similar dendritic lengths to the Golgi studies. It will be shown in the present paper that all previous studies produced underestimates of geometrical dendritic length.

Anatomical studies of primary afferent fibre terminations upon motoneurones have been complicated by the recent demonstrations that axons from spindle secondary endings excite motoneurones monosynaptically (Kirkwood & Sears, 1975; Stauffer, Watt, Taylor, Reinking & Stuart, 1976) and that these axons project into the motor nuclei (Fu, Santini & Schomburg, 1974; Fu & Schomburg, 1974; Fyffe, 1979). Primary afferent fibre terminals upon motoneurones can no longer be assumed to belong to axons from spindle primary endings. The injection of horseradish peroxidase into identified Ia afferent fibres (Brown & Fyffe, 1978*a*; Ishizuka, Mannen, Hongo & Sasaki, 1979) has allowed some conclusions to be drawn concerning the possible numbers of contacts made by these fibres upon motoneurones.

Electrophysiological experiments have privided much of the information about the spatial distribution and possible numbers of contacts made by Ia afferent fibres upon motoneurones. There is now a wealth of data suggesting a wide distribution of Ia synapses upon the motoneuronal somatic and dendritic surface, including the distal parts of the dendritic tree (see for example, Burke, 1967; Rall, Burke, Smith, Nelson & Frank, 1967; Jack, Miller, Porter & Redman, 1971; Mendell & Henneman, 1971; Iansek & Redman, 1973; Burke & Rudomin, 1977). More direct anatomical information is required to supplement these studies and the experiments to be reported in this paper were designed to provide it. The enzyme horseradish peroxidase has been injected into indentified Ia afferent fibres and alpha motoneurones in the same preparation and the contacts between the two have been analysed.

Some preliminary results were presented to the Physiological Society at Cambridge in July 1978 (Brown & Fyffe, 1978*b*). Burke and his co-workers (Burke, Walmsley & Hodgson, 1979) have recently published some preliminary results of similar experiments and their findings are in agreement with ours.

METHODS

The experiments were performed on twelve adult cats (2·0–2·9 kg) anaesthetized with chloralose (70 mg . kg^{-1}), after induction with 4 % halothane in a nitrous oxide : oxygen mixture, and paralysed with gallamine triethiodide. The state of anaesthesia was monitored by continuous recording of arterial blood pressure, by frequent examination of the pupils of the eyes and by allowing the effects of the paralysing agent to wear off from time to time. Additional doses of chloralose (25–50 mg . kg^{-1}) were given if required.

Full details of the methods used for intra-axonal and intracellular injection of horseradish peroxidase and its subsequent localization have been published previously (Snow, Rose & Brown, 1976; Brown, Rose & Snow, 1977; Brown & Fyffe, 1978, 1979). Briefly, micro-electrodes filled with a 6–8 % solution of horseradish peroxidase were used to impale identified Ia axons excited by stimulation of the nerves to the medial gastrocnemius Mg, lateral gastrocnemius-soleus Lg–S muscles and muscles innervated by the posterior tibial PT nerve. The axons were impaled in segments L_7 and caudal L_6 and injected with a total of between 130 and 270 nA . min of current. α-Motoneurones were identified on the basis of their peripheral conduction velocity (60–80 m. sec^{-1}) and antidromic activation from the appripriate peripheral nerves. The antidromic nature of the action potential was confirmed upon intracellular penetration of the motoneurone by its origin from a flat membrane potential, that is without a preceding synaptic potential.

Usually one or two alpha motoneurones were injected with horseradish peroxidase between 3 and 8 hr after injection of the Ia afferent fibre. After a further period of 2–4 hr the spinal cord was perfused (from the abdominal aorta) with heparinized saline and then 3·0 % paraformaldehyde and 1·0 % glutaraldehyde in phosphate buffer (pH 7·6). Serial, frozen, 100 μm transverse or parasagittal sections of cord were cut and subsequently treated for the demonstration of horseradish peroxidase by the method of Hanker, Yates, Metz & Rustioni (1977). Some sections were counterstained with either cresyl violet or methylene green. The sections were examined with the light microscope and reconstructions made with the aid of a camera lucida attachment at magnifications of × 100–1000. Sections were also examined using differential interference optics on the Vickers M17 microscope. Measurements were made using a Joyce-Loebl MAGISCAN system.

RESULTS

The structure of α-motoneurones

The present experiments were not designed originally to study the anatomy of motoneurones. It became apparent very early, however, that intracellular injection of horseradish peroxidase was providing a much more complete picture of these cells than had been previously observed (see also Cullheim & Kellerth, 1976; Burke *et al.* 1979). For this reason we include some data on the motoneurones.

Thirty-four α-motoneurones were stained. In Fig. 1 six motoneurones innervating the triceps surae muscle group are shown and information on these cells is summarized in Table 1. The injection current was kept at 60–120 nA . min in order to minimise damage to the cell and also to limit staining of motor axon collaterals (such staining might have complicated reconstruction of the terminal arborizations of the Ia axon collaterals). Even so the motoneuronal dendritic trees were very extensive.

Number of dendrities and dendritic branching

Between seven and eighteen (mean, 11·6) primary dendrites originated from the soma and each usually gave rise to daughter branches up to the fourth to the sixth order. Although dendrites generally bifurcated at branch points there was a relatively high number of trifurcations. Of 200 branch points in eight motoneurones ten were trifurcations representing 5 % of the total.

Dendritic bifurcations were examined to see if Rall's (1959) Power Rule was obeyed. This rule is a constraint necessary for the dendritic tree to be reduced to an equivalent cylinder and requires that the sum of the diameters of two daughter branches each raised to the power 3/2 should equal the diameter of the parent branch, also raised to the power of 3/2. Measurements were made of dendritic diameters before and after fifteen bifurcations in each of the stained motoneurones. The branches were chosen

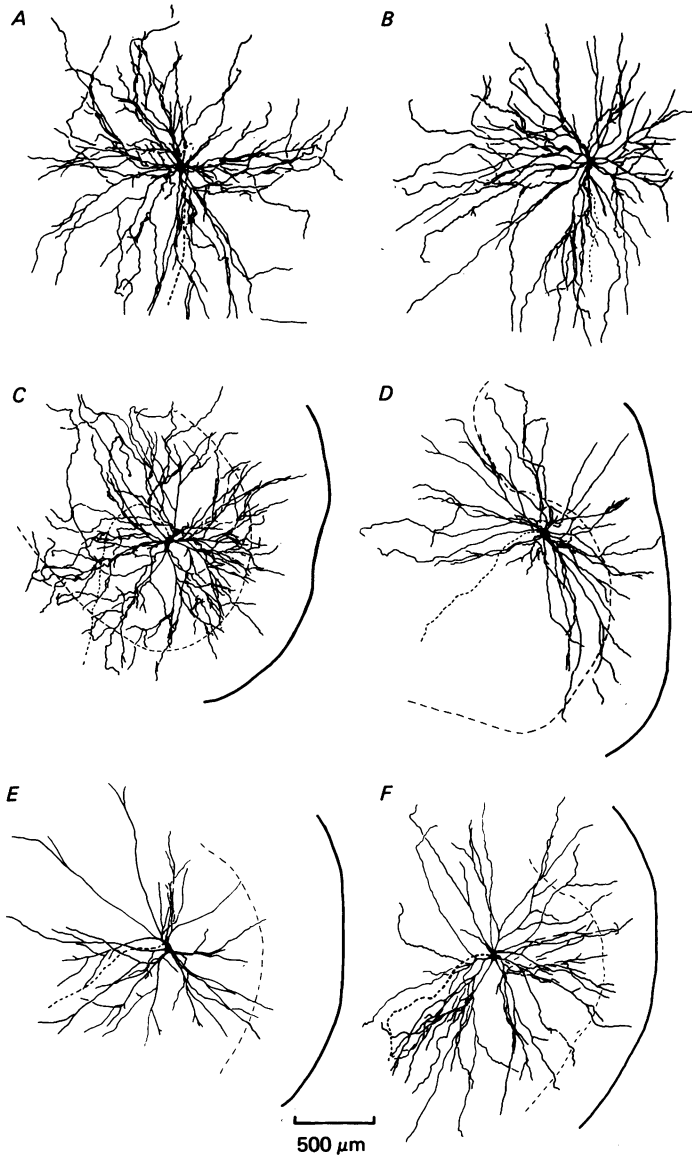

Fig. 1. Reconstructions of six horseradish peroxidase labelled triceps surae α motoneurones. *A* and *B* were drawn from 100 μm thick serial sections (rostral to the left), *C–F* reconstructed from transverse sections of cord. Motoneurones *A*, *C* and *D* innervated Lg–S; *B*, *E* and *F* innervated Mg. Motor axons are shown as dashed lines from the somas. In transverse reconstructions the outline of the ventral horn and surface of the cord are indicated by dashed and continuous lines respectively. For further descriptions see text.

to include proximal and distal branch points, where there was no evidence of possible dendritic flattening and where the dendrites were situated well within the thickness of the section. In our sample of ninety bifurcations the ratios of the sum of the daughter branch diameters (to the power of 3/2) to the parent branch diameter (to the power 3/2) ranged from 0·65 to 1·7 with a mean of $1{\cdot}06 \pm 0{\cdot}15$ (S.D.). These values range rather more widely than those of Lux *et al.* (1970) but provide support for Rall's simplifying assumption, at least for individual branch points.

TABLE 1. Some geometrical and electrophysiological data for the six horseradish peroxidase labelled motoneurones shown in Fig. 1

Motoneurone		Soma diameter (μm)	Conduction velocity ($m.sec^{-1}$)	No. of 1° dendrites	Dendritic spread (μm)		
					rostro-caudal	medio-lateral	dorso-ventral
A	Lg–S	60 × 40	83	11	2131	2000	1938
B	Mg	45 × 50	90	14	1969	1800	2063
C	Lg–S	48 × 45	74	8	2600	1720	1915
D	Lg–S	45 × 50	86	11	2600	1975	2185
E	Mg	75 × 50	92	9	1100	1688	1625
F	Mg	45 × 55	76	11	2100	1781	2371

Dendritic tapering

Dendrites were examined for tapering between branch points. Because there were often slight swellings of the dendrites at branch points, especially at trifurcations, measurements were made at distances of 10–20 μm from the branch points or origin at the soma, and the termination at branch points or end points. In the present material there was little or no tapering within the dendritic tree up to about 800 μm from the soma, although a very few individual dendritic trunks tapered rapidly along their length. The lack of obvious tapering in most of these proximal dendrites may be seen in the figures of Pl. 2*A*, 3 and 4*A–C*. There was, however, rather more tapering along the course of the most distal dendrites towards their termination.

One complicating factor to the measurement of dendritic diameters, particularly troublesome in some distal dendrites, was the presence of beading (see Pl. 4*D*, *F*). These dendrites were excluded from the diameter measurements. Beaded dendrites have also been seen in other neurones and by other workers after horseradish peroxidase injection (unpublished work from the authors' laboratory; Cullheim & Kellerth, 1976, 1978; Burke *et al.* 1979). Beaded dendrites are also observed with silver staining techniques (see Cajal, 1952). The nature of these structures requires further study.

Dendritic length

Horseradish peroxidase injections into motoneurones revealed much more extensive dendritic trees than either silver stains (Aitken & Bridger, 1961; Gelfan *et al.* 1970) or intracellular injection of tritiated glycine (Lux *et al.* 1970) or Procion Yellow (Barrett & Crill, 1974). Dendrites were demonstrated up to 1600 μm in length from origin at the soma to their apparent termination (Fig. 1, Table 1). This produced dendritic trees with spreads of between 2 and 3 mm. Triceps surae motoneurones (Fig.

1) had dendrites that radiated out from the cell body more or less equally in all directions, unlike the motoneurones innervating flexor muscles; these tend to have dendritic trees oriented mainly in the longitudinal axis of the cord (Scheibel & Scheibel, 1969; A. G. Brown & R. E. W. Fyffe, unpublished results).

The dendritic trees were not restricted to the grey matter of the ventral horn. Nearly all motoneurones had dendrites that penetrated into white matter of either the lateral or ventral funiculi (Fig. 1C–F, Pl. 4*D*) sometimes to within less than 100 μm of the cord surface. Many dendrites, upon reaching the border between grey and white matter turned and ran, in the longitudinal direction, close to this interface. Dorsally drected dendrites often penetrated through the intermediate region of the grey matter to reach the base of the dorsal horn (laminae V and VI of Rexed, 1952).

Dendritic spines

Only very occasionally were dendritic spines observed on motoneurone. Some are shown in Pl 4*A–C*. All were on the proximal half of the dendritic tree. They occurred at widely separated sites on the dendrites and were carried on short (2–5 μm) stalks.

Ia terminations upon motoneurones

Terminations upon motoneuronal somata and the most proximal parts of the dendritic tree

When both the Ia afferent fibre and the motoneurones had been injected with horseradish peroxidase difficulty was experienced in determining whether there were contacts on the motoneuronal soma. This was because of the intense staining of the cell body and the absence of contrast between it and the Ia terminal arborizations. In order to examine for somatic contacts and determine their frequency, selected sections of spinal cord containing well-stained Ia terminal branches were counterstained with either cresyl violet or methylene green. The counterstaining demonstrated the motoneuronal somata and up to 30 μm of the main dendritic trunks. Within the motor nuclei the large cells were presumed to be α-motoneurones and it was only with these profiles that the Ia boutons were associated, the vast majority of Ia boutons within the confines of the motor nuclei were not in any apparent association with counterstained neurones.

Pl. 1 shows the relationships between some Ia afferent fibre terminals and counterstained motoneurones. Up to six boutons were observed on the soma and proximal 20–30 μm of dendrite (see for example Pl 1*D*) although there were usually only two or three. The boutons were of both the en passant variety (Pl. 1*C*) and the terminal type and all boutons contacting a single motoneuronal soma from a single Ia collateral were not always necessarily from the same collateral branch (Pl. 1*D*).

In six collaterals from different lateral gastrocneumius-soleus Ia afferent fibres 500 synaptic boutons were counted within the motor nuclei (lamina IX). Only forty-five of these boutons were in close association with the somata and proximal dendrites of motoneurones. A total of twenty-two motoneurones received these forty-five contacts. Thus each collateral terminated upon, on average, 3·66 motoneuronal somata or proximal dendrites and only 9 % of the Ia boutons within lamina IX made somatic or proximal dendritic contacts. For those motoneurones receiving such proximal contacts the number of boutons per cell was 2·05.

Terminations upon dendritic trees

(*a*) *Descriptive anatomy.* As shown above, 90% of Ia synaptic boutons within the motor nuclei did not end in relation to motoneuronal somata or proximal dendrites. Most, if not all of these, probably contacted motoneuronal dendrites. In order to examine the terminations of Ia fibres upon the dendritic trees of alpha motoneurones and to provide direct estimates of the locations and frequencies of such contacts, horseradish peroxidase was injected into both identified Ia axons and identified motoneurones in the same preparation.

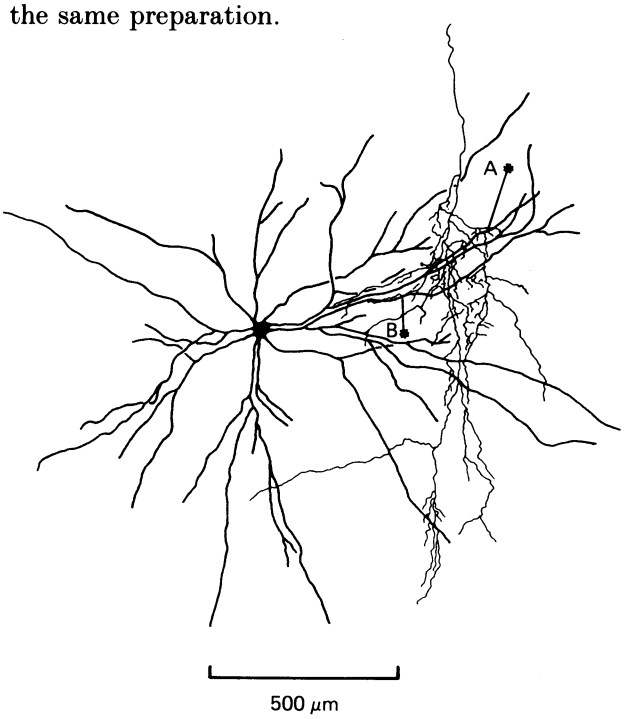

Fig. 2. Reconstruction, in the sagittal plane, showing the locations of four synaptic contacts made by a Lg–S Ia afferent collateral on two different distal, caudally directed dendrites. One contact was made at site A*, three at site B*. See text for details.

In the absence of electronmicroscopical examination of the present material some assumptions have had to be made about presumed contacts. All presumed contacts satisfied the following criteria. (1) All stained contacts were traced back to the identified Ia axons on the one hand and to the labelled motoneurone on the other. (2) Contacts were only counted if the axonal profile exhibited a clear bouton-like swelling. (3) There was no sign, under oil immersion examination at $\times 1000$ magnification, of any gap between the two elements. Although functional synapses may not necessarily involve axonal swellings (boutons) we have limited ourselves to instances where a swelling was apparent. Therefore we may have underestimated the numbers of functional contacts between Ia afferent fibres and motoneurones.

Ten Ia afferent fibre-motoneurone pairs were suitable for detailed examination in that both the axon terminal arborizations and the motoneurones were intensely

stained, and there were contacts between the two. Fig. 2–7 and Pl. 2 and 3 show details of some of the material from eight of these pairs and the diagram of Fig. 8 summarizes the arrangements of the contacts in diagrammatic fashion. In the camera lucida reconstructions only those motoneuronal dendrites that received the contacts have been drawn in full and dendrites not receiving any contacts have been omitted or shortened for the sake of clarity in the Figures. Most of the Ia collateral branches ventral to lamina VI are shown in the figures except in those at the higher magnifications where only the final preterminal parts are shown.

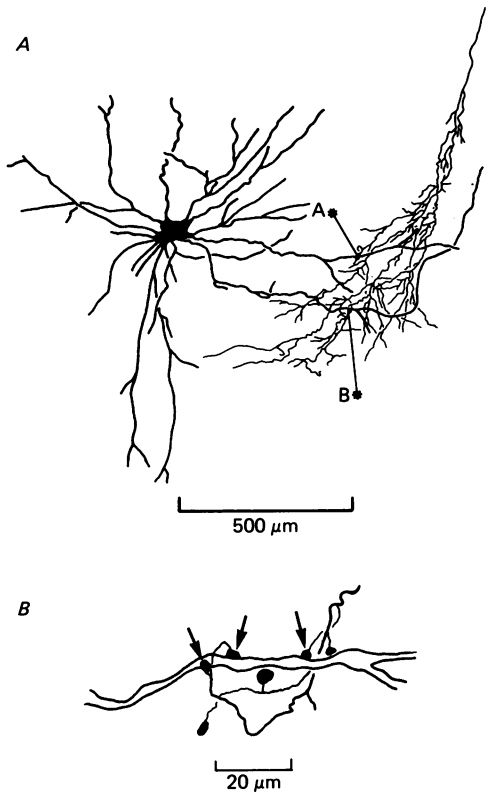

Fig. 3. *A*, reconstructions, from sagittal sections, of a Ia collateral and an α motoneurone whose axons lay in the posterior tibial nerve. Again (see Fig. 2) four contacts were observed at some distance from the soma, on caudally oriented dendrites. *B*, this higher magnification reconstruction shows the arrangement of the three contacts made at site B*. Further details in the text.

Fig. 2 shows the overall anatomy, from sagittal sections, of a lateral gastrocnemius-soleus Ia afferent fibre collateral and its target lateral gastrocnemius-soleus motoneurone. This collateral intersected the distal parts of the caudally directed components of the dendritic tree. (Branches of this collateral extended for over 800 μm in the rostro-caudal plane and overlapped with parts of the adjacent collaterals). The next most rostral collateral from the same Ia axon intersected the cranially directed dendrites of this motoneurone but no contacts were made between then. On the caudally directed dendrites four synaptic contacts were observed, at two

widely separated locations on the branches of two different primary dendrites. At *A* in Fig. 2 (see also Fig. 8*E*) there was a single contact made by a bouton de passage at about 765 μm from the soma on a third order dendrite. At *B* in Fig. 2 there was a group of en passant boutons 330–385 μm from the soma on a second order dendrite. Photomicrographs of these four contacts are shown in Pl. 4*E*, *G*.

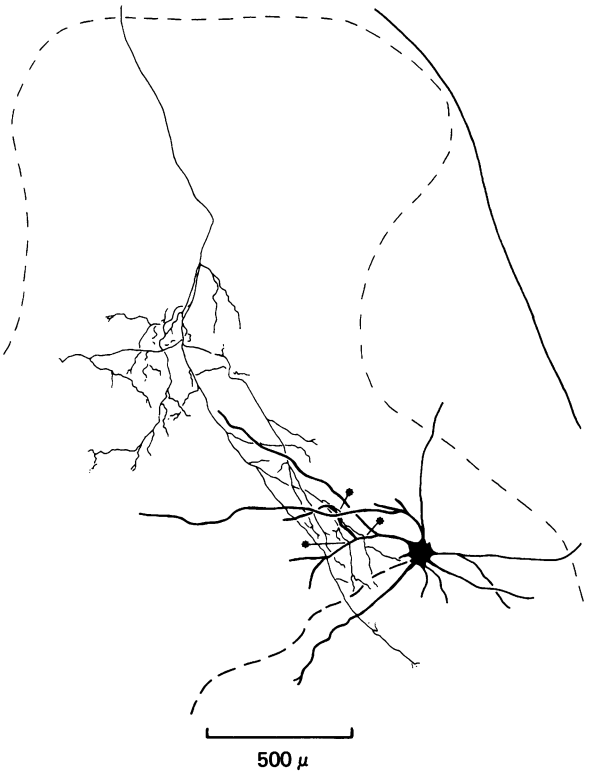

Fig. 4. Reconstructions from transverse sections of cord showing a Lg–S Ia collateral and a Lg–S motoneurone. At three sites (*), medial and just rostral to the soma, a total of five contacts were made. See text and Fig. 5 for further details.

Fig. 3 shows a collateral from a Ia fibre in the posterior tibial nerve and its contacts upon a motoneurone antidromically activated from the same nerve (see also Fig. 8*F*). Although three collaterals from this Ia afferent fibre passed through the dendritic tree, only the most caudal of the three had boutons contacting the motoneurone. Again, the contacts were made at two locations on the branches of two primary dendrites: a single en passant contact at 500 μm from the soma on a second order dendrite, and at about the same distance (500 μm from the soma) a more complex set of terminations involving three boutons along 25 μm of a third order dendrite. Photomicrographs (Pl. 4*F*) and camera lucida drawings Fig. 3*B*) show this latter contact site in more detail. It can be seen that two of the contacts were on beaded parts of the dendrite.

The reconstruction of Fig. 4, from serial transverse sections, shows perhaps a more familiar aspect of the antomical arrangements of Ia afferent fibre collaterals and

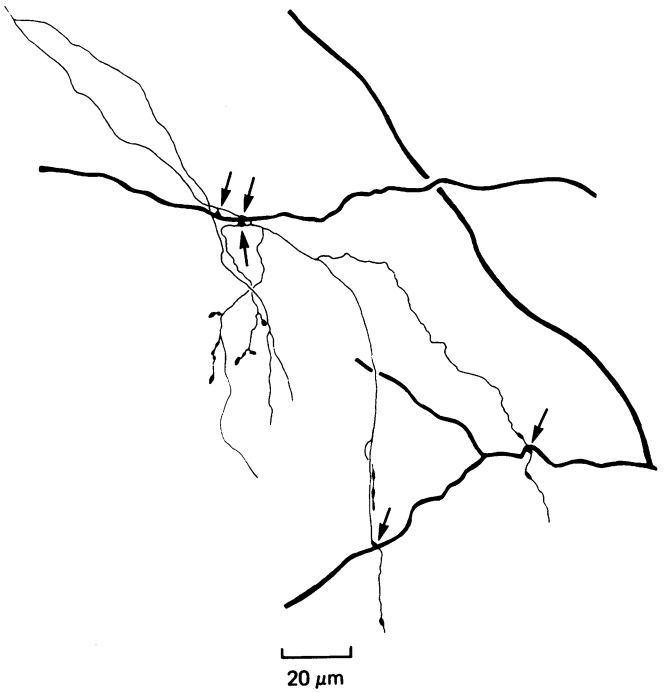

Fig. 5. Transverse reconstruction showing the arrangement of the contacts indicated in the previous figure. Contacts (arrows) were made on three branches from two different dendrites.

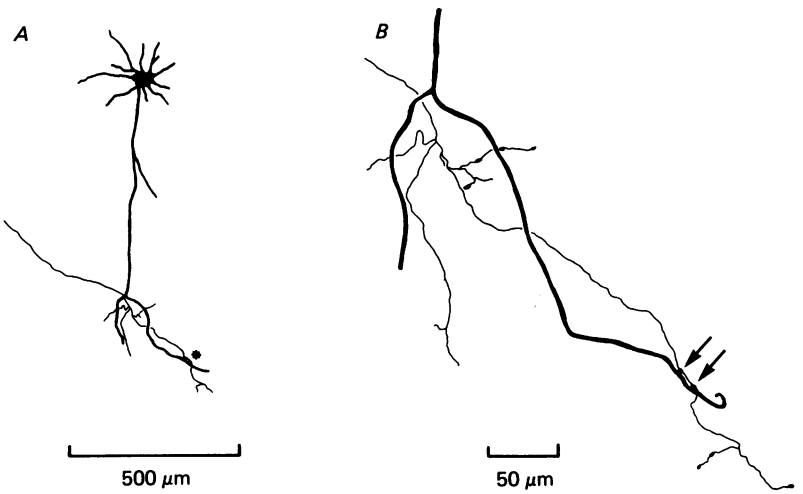

Fig. 6. *A*, transverse view reconstruction showing the location (*) of two contacts made on a distal, ventral dendrite of a Lg–S α-motoneurone by a Mg Ia afferent collateral. These two *en passant* contacts are shown in more detail in reconstruction *B*. See text for further details.

motoneurones. The Ia collateral (from lateral gastrocnemius-soleus) sends terminal branches to the intermediate region (lamina (VI) and to lamina VII before proceeding to the motor nuclei of triceps surae. Five contacts were made by this collateral upon the lateral gastrocnemius-soleus motoneurone shown in Fig. 4. The contacts (Figs 5 and 8*G*) were: three at 350–365 μm on a third order branch and two single contacts on different branches of another dendrite, one at 190 μm on a second order branch and one at 250 μm on a third order branch. Four of the contacts involved en passant boutons.

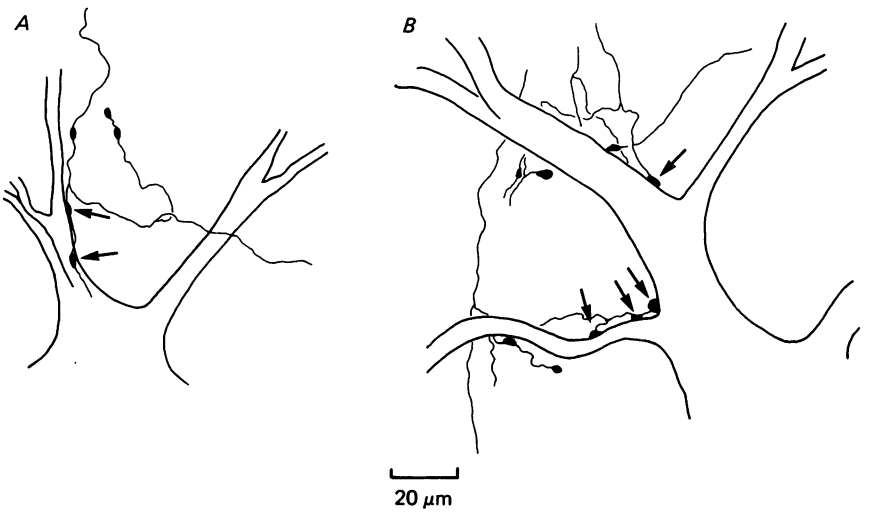

Fig. 7. Reconstructions, in the sagittal plane, showing juxtasomatic contacts made by Lg–S Ia afferents upon Lg–S α-motoneurones. In *A*, two climbing *en passant* contacts were made on a primary dendrite. The terminal axon extended over the soma of the motoneurone and may have made other contacts. Intense staining of the soma made differentiation of afferent and soma impossible. In *B*, the afferent collateral arborization generated four contacts within 70 μm of the soma.

Further examples of distal contacts are shown in Fig. 6 and Pl. 2. In Fig. 6 the collateral of a medial gastrocnemius Ia afferent fibre intersected the ventrally directed dendrites of a lateral gastrocnemius-soleus motoneurone and made two contacts de passage on a third order dendrite about 820 μm from the soma (Fig. 8*I*). Pl. 2, from a single 100 μm thick transverse section of spinal cord, shows contacts made by a lateral gastrocnemius-soleus Ia afferent fibre upon a medial gastrocnemius motoneurone (Fig. 8*H*). In Pl. 2*B* (right) two arrows indicate en passant contacts of the crossing over type made at about 620 μm from the soma by two collateral branches; at the top left of the micrograph a single contact is present about 550 μm from the soma.

Fig. 7 shows two examples of sets of contacts close to the motoneuronal soma, both between Ia afferent fibres and motoneurones of lateral gastrocemius-soleus. In the pair shown in Fig. 7*A* there were two en passant contacts on a primary dendrite (one near to its first bifurcation) at about 20 and 30 μm from the soma (see also Fig. 8*D*). The collateral branch giving rise to these contacts extended beyond the second one over the soma of the motoneurone but no further contacts were observed; it was impossible to differentiate the two elements due to the intense staining of the soma.

In the other example (Figs 7*B* and 8*B*) four contacts were observed on second and third order dendrites all within 70 μm of the soma. Finally, Pl. 3 shows juxtasomatic contacts between a medial gastrocnemius Ia afferent fibre and a medial gastrocnemius motoneurone (see also Fig. 8*A*). In the oil immersion photomicrograph of Pl. 2*B* the positions of five contacts are indicated; they were all within 40 μm of the soma and were distributed to three ventrally directed primary dendrites.

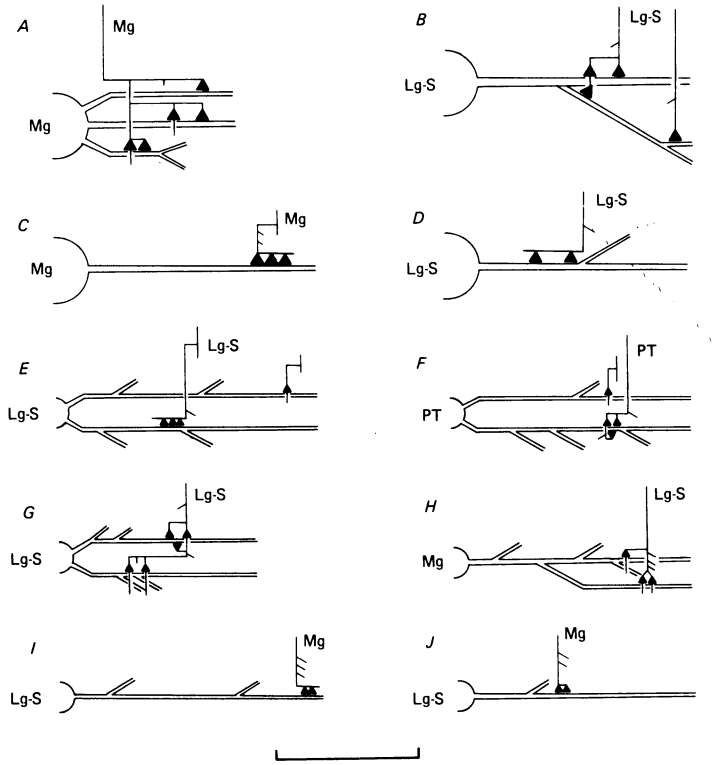

Fig. 8. Diagrammatic summary showing the locations and sometimes complex 'wiring' arrangements of all thirty-four contacts made upon the ten motoneurones. In each pair the identity of the motoneurone and of the Ia afferent fibre is indicated. Proximal contacts are shown in *A–D*; those predominantly on more distal dendrites are shown in *E–J*. The scale bar is 50 μm for *A–D* and 500 μm for *E–J* and refers to the geometric dendritic distances to branch points and synaptic locations (triangles). The afferent preterminal branching patterns are also shown, but not to scale. For further descriptions see text.

The summary diagrams of Fig. 8 show the locations and the diversity of preterminal arborization patterns for all ten of the Ia afferent fibre-motoneurone pairs we have stained with horseradish peroxidase. The scales in Fig. 8 refer to dendritic lengths, and therefore contact locations, but not to the terminal arborizations of the Ia afferent fibre collaterals. A few generalizations may be made from this small sample. All synaptic contacts were made by branches of the same collateral – even where one or two other collaterals from the same Ia afferent fibre passed through the dendritic tree of the motoneurone they did not form contacts with it. Usually all contacts made with a motoneurone by a single Ia afferent fibre were at about the same geometrical

distance from the cell soma, even when made upon branches of two or more dendrites (Fig. 8*A*, *C*, *D*, *F*, *G*, *H*, *I*, *J*). The terminal Ia arborizations giving rise to synaptic boutons contacting the motoneurones ranged from simple (fig. 8*C*, *D*, *H*, *I*, *J*) to relatively complex (Fig. 8*A*, *B*, *E*, *F*, *G*) and the latter arrangements lead to a number of possible ways in which the single fibre excitatory synaptic potentials might be fractionated.

(*b*) *Quantitative analysis.* A total of thirty-four contacts were observed between the ten pairs of Ia afferent fibres and motoneurones, with a range of two to five. It is perhaps worth pointing out that where the connexions were between afferent fibres and motoneurones from heteronymous muscles (Fig. 8*H*, *I*, *J*) then there were only two or three contacts whereas when both afferent fibre and motoneurone were from the same muscle (medial gastrocnemius, Fig. 8*A*, *C*) the number of contacts was three and five. Where the situation was equivocal (lateral gastrocnemius-soleus and posterior tibial pairs) the numbers ranged from two to five.

The sizes of the Ia boutons taking part in the thirty-four contacts were within the range previously described ($3{\cdot}5 \times 3$–$7 \times 3{\cdot}5\,\mu$m, Brown & Fyffe, 1978) and there were no obvious correlations between size and number of contacts per motoneurone nor between bouton size and contact location. Where distal contacts were made, however, the bouton usually had a greater diameter than the dendrite (see Pl. 4*E*) but ultrastructural examination would be required to determine the precise contact area.

Finally, no correlations were found between the conduction velocities of either the Ia afferent fibre or the motoneurone axons and the number of locations of the synaptic contacts. However, the sample is small and we did not classify motoneurones on the basis of twitch type (Burke, Levine, Tsairis & Zajac, 1973). These negative findings should not be taken to indicate that no such correlations exist.

DISCUSSION

In the present experiments we have used the intracellular injection of horseradish peroxidase to investigate the relationship between afferent fibres from primary endings in muscle spindles and their target motoneurones in the lumbosacral spinal cord. Intracellular horseradish peroxidase has provided a more complete view of the extent of motoneuronal dendritic trees than hitherto and intra-axonal injection of the enzyme has allowed firm lower estimates to be made of the distribution and density of Ia contacts upon motoneurones.

The α-motoneurone

Motoneurones innervating muscles of the triceps surae group and those innervated by the posterior tibial nerve have been shown to have between seven and eighteen primary dendrites (mean 11·6). Golgi impregnations in adult cats and dogs (Aitken & Bridger, 1961; Gelfan *et al.* 1970) demonstrate between two and fourteen primary dendrites with a mean of about seven, considerably fewer than reported here. Direct injection of either Procion Yellow (Barrett & Crill, 1974) or tritiated glycine (Lux *et al.* 1970) into α-motoneurones demonstrate the presence of more dendrites (eight to twenty-two) in line with our results. It must be concluded that, contrary to popular

belief, the silver staining methods do not demonstrate complete neurones but often fail to stain complete dendritic systems arising from primary dendrites. Recently Somogyi & Smith (1979) have indeed described some striatonigral neurones which were only partially impregnated by Golgi methods. Whether this unfortunate flaw in the method is restricted to material from adult animals or includes neonatal material is unknown. Obviously results purporting to show dendritic tree modelling during development (e.g. Conradi & Ronnevi, 1975) will need to be interpreted cautiously until there has been further investigation.

The most obvious difference between the motoneuronal dendritic trees demonstrated by intracellular injection of horseradish peroxidase and the previously used methods was the much greater dendritic lengths revealed by the former. Procion Yellow gives dendritic lengths of 330–800 μm (Barrett & Crill, 1974), Golgi staining and tritiated glycine give lengths of up to 1 mm. In our material most dendrites were at least 1 mm in total length from their origin at the soma to their final termination and lengths of 1500–1600 μm were common, giving tip-to-tip dendritic extents of 2·5–3 mm.

Most of the motoneurones in our sample had dendrites that radiated away from the cell body. Because of their length this led to dendrites extending into the white matter of the lateral and ventral funiculi and also dorsally into the base of the dorsal horn. This result is in agreement with the published reconstruction of a horseradish peroxidase injected motoneurone by Cullheim & Kellerth (1976; their Fig. 1). Rose & Richmond (1978) have shown that motoneurones in the cervical spinal cord also send dendrites out of the grey matter into the lateral and ventral funculi where they make synaptic contacts. Dendritic spines were sparsely distributed; most of those observed were located on the proximal half of the dendritic tree. These observations concur with the electronmicroscope study of Conradi (1969), and indicate that the spine-like structures common on neonatal cat motoneurone dendrites (Conradi & Skoglund, 1969) are lost during development.

Dendritic branching produced dendrites of the fourth or fifth order in the vast majority of dendritic trees and at branch points bifurcation was the general rule. There was, however, a surprisingly high incidence (5%) of trifurcations. At the dendritic branch points the 3/2 Power Rule (Rall, 1959) was preserved, in agreement with the observations on tritiated glycine labelled (Lux *et al.* 1970) and Procion Yellow labelled (Barrett & Crill, 1974) dendrites. Also in our material there was little or no tapering of dendrites within up to about 800 μm of the soma and the results are in closer agreement with those of Lux *et al.* (1970) than those of Barrett & Crill (1970). In our experience it is much more difficult to make measurements on Procion Yellow stained neurones than on those stained with horseradish peroxidase reaction product. Even the best-filled Procion material appears 'fuzzy' at the edges whereas there is always good contrast between horseradish peroxidase filled profiles and the surrounding tissue, making measurements much easier. One complicating factor with horseradish peroxidase material, however, is that distal dendrites may take on a beaded appearance (see also, Cullheim & Kellerth, 1976, 1978; Burke *et al.* 1979) making diameter measurements very difficult. The nature of these swellings (beads) is uncertain. Where beading was absent it could be seen that terminal dendrites tapered, often quite markedly. In summary, however, it seems reasonable to conclude that the equivalent cylinder model of Rall (1959, 1977) represents a good approxi-

mation for motoneuronal dendritic trees. Furthermore the much more extensive dendritic trees (geometric lengths) demonstrated in the present work emphasises the electrotonic shortness of such equivalent cylinders. Thus, measurements of the electrotonic length (of the equivalent cylinder) give values of less than two space constants (Rall *et al.* 1967; Nelson & Lux, 1970; Lux *et al.* Barrett & Crill, 1974; Jack *et al.* 1971). This, coupled with the relatively important contribution that dendrites make to input conductance measured at the soma (Iansek & Redman, 1973*a*; Barrett & Crill, 1974), means that synapses located even on distal dendrites will have an important role to play in the integrative activity of motoneurones.

The distribution of Ia synapses upon motoneurones

On the basis of the analysis of extracellular field potentials Fatt (1957) proposed that the excitatory post-synaptic potential evoked in α-motoneurones by a synchronous Ia afferent volley was generated primarily in the motoneuronal dendrites. In early studies of motoneuronal membrane properties it was assumed that, in electrical terms, dendrites were long and thin and this, coupled with an underestimate of membrane resistivity led to an underestimate of the dendritic contribution to input conductance (see Eccles, 1964; Rall, 1977). Subsequently, however, a large body of evidence has been built up to support the suggestion that Ia excitatory post-synaptic potentials are generated by synapses distributed over much of the motoneuronal surface including the distal parts of the dendrites.

Electrophysiological experiments have shown a wide range of Ia e.p.s.p. shapes in motoneurones, consistent with widespread distribution of Ia boutons (Kuno, 1964; Rall *et al.* 1967; Burke, 1967; Jack *et al.* 1971) and single Ia fibres can generate e.p.s.p.'s with vastly different time courses in different motoneurones (Mendell & Henneman, 1971). Some e.p.s.p. shapes indicate that boutons from a particular Ia afferent fibre are probably situated within a restricted electrotonic locus on the motoneurone (Jack *et al.* 1971; Mendell & Henneman, 1971; Munson & Sypert, 1979*b*) while others indicate boutons located at distinct and separate electrotonic positions (Rall *et al.* 1967; Munson & Sypert, 1979*b*). Jack *et al.* (1971) estimated that Ia synapses were situated for the most part at between 0·2 and 0·6 space constants from the soma (*on the assumption of a single locus for the Ia-motoneurone contact*), i.e. up to about half-way out on the dendrites. More recent estimates give values of 0–1·25 space constants (Iansek & Redman, 1973*b*) suggesting a wider spread over most of the motoneurone's receptive surface.

Single Ia afferent fibres do not, however, terminate upon motoneurones at a single site. Electrophysiological evidence from studies in which single Ia fibres have been excited have shown that single fibre e.p.s.p.s can be fractionated (Burke, 1967; Kuno & Miyhara, 1969; Jack *et al.* 1971; Iansek & Redman, 1973*b*; Zucker, 1973; Mendell & Weiner, 1976; Edwards, Redman & Walmsley, 1976*a*, *b*) and that between one and fifteen unitary potentials make up a single fibre e.p.s.p., the usual number being less than five. The usual interpretation of this fractionation is that it is due to transmitter release from several sites.

In the experiments reported in the present paper two approaches were used to provide direct evidence on the location and numbers of Ia terminations upon motoneurones. In both, single identified Ia fibres were injected with horseradish

peroxidase to provide visualization of the Ia terminations. Assumptions made in the interpretation of the results were (1) that the method allowed complete visualization of the Ia terminals, and (2) that synapses were present at swellings (boutons) on the terminal axons. Without reconstruction of terminal arborizations from thin sections examined with the electron microscope we can never be sure that all the arborization is revealed nor that synapses are only formed at boutons. In order to reduce the risk of incomplete demonstration of terminal arborization we only examined Ia collaterals that appeared densely stained. Burke *et al.* (1979) have assumed that apparent contacts between Ia axons and motoneurones *that do not involve axonal swellings* may be considered to be contact sites. We have been more conservative than that since it is very difficult with thick light microscope sections to differentiate true contact from two profiles close together. It is still difficult where the axon has a swelling but we feel that such a restriction increases the probability of correct identification.

By combining horseradish peroxidase injection of Ia afferent fibres with counterstaining of cord sections it was shown that up to six (mean, 2·05) Ia boutons were located on the soma and up to about 30 μm of proximal dendrite. Iles (1976) examined counterstained material after filling dorsal roots with Co and obtained similar results (up to six boutons on motoneuronal somata and proximal dendrites with a mean value of 1·85) although he could not identify the afferent fibres and the demonstration that afferent fibres from spindle secondaries have monosynaptic connexions with motoneurones (Kirkwood & Sears, 1975; Stauffer *et al.* 1976) complicates interpretation of Iles's material. Ishizuka *et al.* (1979) have recently reported similar experiments to ours. Their data show rather more somatic and proximal dendritic contacts (3·3 per motoneurone) but they were able to examine the proximal dendrites out to a distance of 100 μm and this presumably accounts for their higher value.

Detailed examination of six collaterals from different lateral gastrocnemius-soleus Ia afferent fibres showed that only 9 % of their lamina IX boutons were in apparent contact with motoneurones (somata plus proximal dendrites). Ishizuka *et al.* (1979) observed figures of 21, 10, 20, 11 and 19 % for medial gastrocnemius, soleus, plantaris, flexor digitorum hallucis longus and hamstring Ia afferent fibres respectively. Obviously only a minority of Ia synapses on motoneurones are located on the cell body.

In order to determine the numbers and locations of Ia synaptic boutons upon motoneuronal dendrites we combined horseradish peroxidase injections of Ia afferent fibres with similar injection of motoneurones. This particular combination, although of no use for the determination of somatic contacts due to the lack of contrast between the terminal axons and the densely stained cell body, allowed the complete dendritic tree of the motoneurones to be examined. Thirty-four contacts were observed in ten pairs of Ia afferent-motoneurone combinations with a range of two to five. In only one of these pairs was there any possibility that somatic contacts were made in addition to the observed dendritic ones. It may be concluded, therefore, that Ia afferent fibres end on motoneurones with between two and six synaptic contacts and an average of between two and 3·5 contacts per motoneurone. These values are in remarkably good agreement with the electrophysiological data and the preliminary anatomical results of Burke *et al.* (1979). With 2–3·5 contacts per motoneurone a triceps surae Ia afferent fibre should distribute about 1000–2000 contacts to 550 of

the 725 motoneurones in the triceps motor pool (see Boyd & Davey, 1968; Iles, 1976; Mendell & Henneman, 1971; Scott & Mendell, 1976; Munson & Sypert, 1979*b*). Over the 10 mm or so length of the triceps motor cell column in an adult cat (Romanes, 1951; Sprague, 1958) a single Ia axon gives off about 10 collaterals (Brown & Fyffe, 1978; Ishizuka *et al.* 1979; Munson & Sypert, 1979*a*) and each should carry 100–200 boutons. Ishizuka *et al.* (1979) observed an average of 135 boutons per collateral in lamina IX, a value with which our own data agree.

In our material the contacts between Ia fibres and motoneurones were made on the soma and dendritic tree up to distances of 800 μm from the soma; the vast majority of contacts were within 600 μm of the soma and therefore in the proximal (geometrical) half of the dendritic tree. A striking feature of the results (see also Burke *et al.* 1979) was the tendency for contacts to be clustered at a particular distance on the dendritic tree, even where more than one primary dendrite and its branches were involved (see Fig. 8). When such clustering occurs on one dendritic branch then the effect will be similar to that produced by a single synaptic locus as is assumed in the electrophysiological analyses. Only rarely (Fig. 8*E*) were contacts widely separated on the dendritic tree in terms of distance from the soma. This restriction of synaptic location was suggested on the basis of anatomical data by Scheibel & Scheibel (1969) and from electrophysiological evidence by Kuno & Miyahara (1969), Mendell & Henneman (1971) and Jack *et al.* (1971).

Because single fibre Ia e.p.s.p.s usually have a simple time course it has been suggested that a motoneurone receives contacts from only a single terminal field (collateral) of an axon (Mendell & Henneman, 1971; Jack *et al.* 1971). This suggestion has been confirmed in the present work (see also Burke *et al.* 1979). Even though a motoneurone's dendrites stretch for 2–3 mm in the longitudinal axis and pass through the terminal arborizations of several (usually at least two) collaterals from a single Ia fibre contacts have only been observed between the motoneurone and terminal arborizations from one of the collaterals. This suggests that some selective process occurs during the initial formation of Ia motoneurone contacts, perhaps akin to the process of neuromuscular junction innervation (see Purves, 1976). Whether this pattern of connectivity has a functional correlate is unknown but Ishizuka *et al.* (1979) suggest it might help to link inhibitory effects from the lamina VI interneurones onto those motoneurones excited by the same fibres.

In conclusion it may be stated that the direct observations on the Ia motoneurone system presented in this paper have been in remarkably close agreement with previous indirect evidence about the system. This agreement includes both the number and the location of Ia synapses upon motoneurones. Furthermore, additional evidence has been provided on the extent of motoneuronal dendritic trees. Since Ia synapses are located within the proximal half of the tree, it may be assumed that the remaining, distal, half (including those parts of the dendrites in the white matter) receives inputs from other sources.

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EXPLANATION OF PLATES

All photomicrographs are of single, 100 μm thick transverse or sagittal sections of spinal cord.

PLATE 1

Relationships between Ia afferent fibre collaterals and ventral horn neurones. Sections counterstained lightly with methylene green.

A, view of a single transverse section of cord showing a Mg Ia collateral coursing ventrally through the dorsal horn then turning ventrolaterally to approach the motor cell column. In this section none of the Ia terminals in lamina IX contacted the observed motoneurones. A small neurone (arrow) lying dorso-medial to the motor nuclei received a single somatic contact.

B, lamina IX terminals of a Lg–S Ia afferent collateral. Two motoneuronal somata (arrows) received contacts (three each).

C, oil immersion micrograph showing a Lg–S Ia collateral branch making three *en passant* and one terminal contact on a motoneurone soma.

D, Mg Ia afferent terminations: contacts (arrows) were made on soma and proximal dendrites of two cells. Note that where multiple contacts were made (at right) these were on different primary dendrites and from different branches of the collateral.

Scale bars represent 500 μm in *A*, 200 μm in *B*, 20 μm in *C*, 100 μm in *D*.

PLATE 2

A, in this example, distal contacts were made by a Lg–S Ia afferent on two different dendritic branches (arrows) of a Mg α-motoneurone. Afferent terminal branches intersect the dorsolaterally directed dendrites of the motoneurone and also contact some of the counterstained neurones in that region of the ventral horn. (Montage constructed from a single 100 μm thick transverse section of cord).

B, the enclosed area of *A* is shown in more detail. Arrows indicate the three contacts. One crossing over *en passant* contact is made on a 5th order dendrite (top left) whilst the other two contacts are on a third order branch of the same dendritic trunk. The three contacts are at similar geometric distances from the soma (approx. 600 μm). See text.

PLATE 3

Micrographs from a single 100 μm thick sagittal section of cord. A Mg Ia afferent and homonymous (Mg) α-motoneurone were labelled.

A, a low-power view illustrating the dendrite branching pattern (note trifurcations). Ventral to the soma the afferent fibre makes five juxtasomatic contacts.

B, an oil-immersion photomicrograph showing the locations of these five contacts (arrows and open circles). All were on primary dendrites within 45 μm of the dendrite origin at the soma. The circles emphasise the position of three of the boutons which overlay densely stained areas and could not be easily photographed. Many other afferent branches and boutons lie close to the soma and its proximal dendrites.

PLATE 4

A–C, spine like structures (arrows) at isolated locations on 2nd–4th order dendritic branches. Note also that there is little if any tapering of these HRP stained branches.

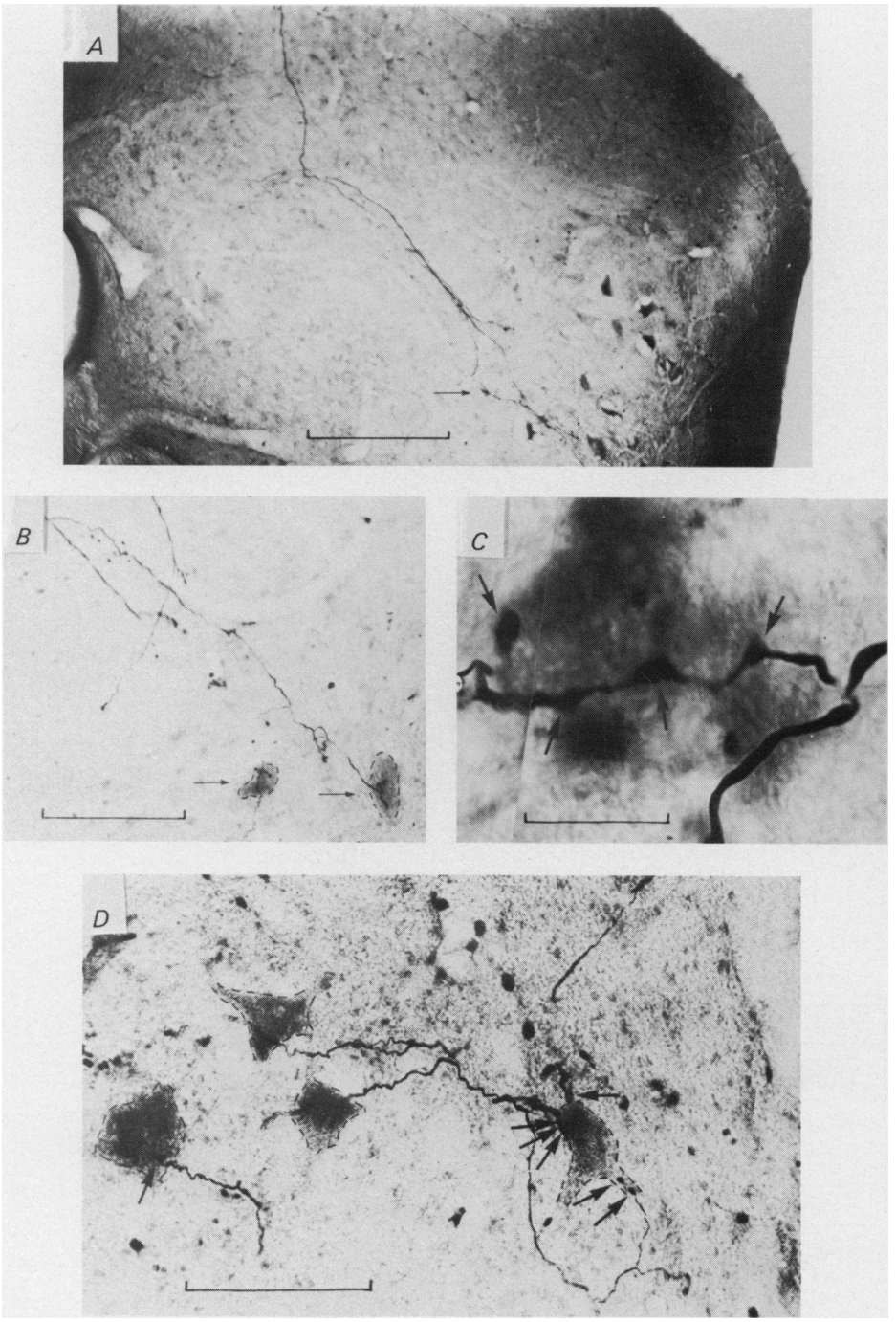
D, a terminal dendritic branch in the lateral white matter (note surface of cord at right). This dendrite had conspicuous varicosities or 'beading' towards its termination.

E, a crossing over contact (arrow) made on a fine distal dendrite more than 800 μm from the soma. The Ia bouton is much larger in size than the dendritic diameter.

F, three contacts on a beaded third order dendrite (see also Fig. 3*B*).

G, three *en passant* climing type contacts on a second order dendrite. Not all the boutons *de passage* on this collateral branch contacted this particular dendrite although they lie very close to it.

Scale bars: 100 μm in *D*, 20 μm in all other micrographs.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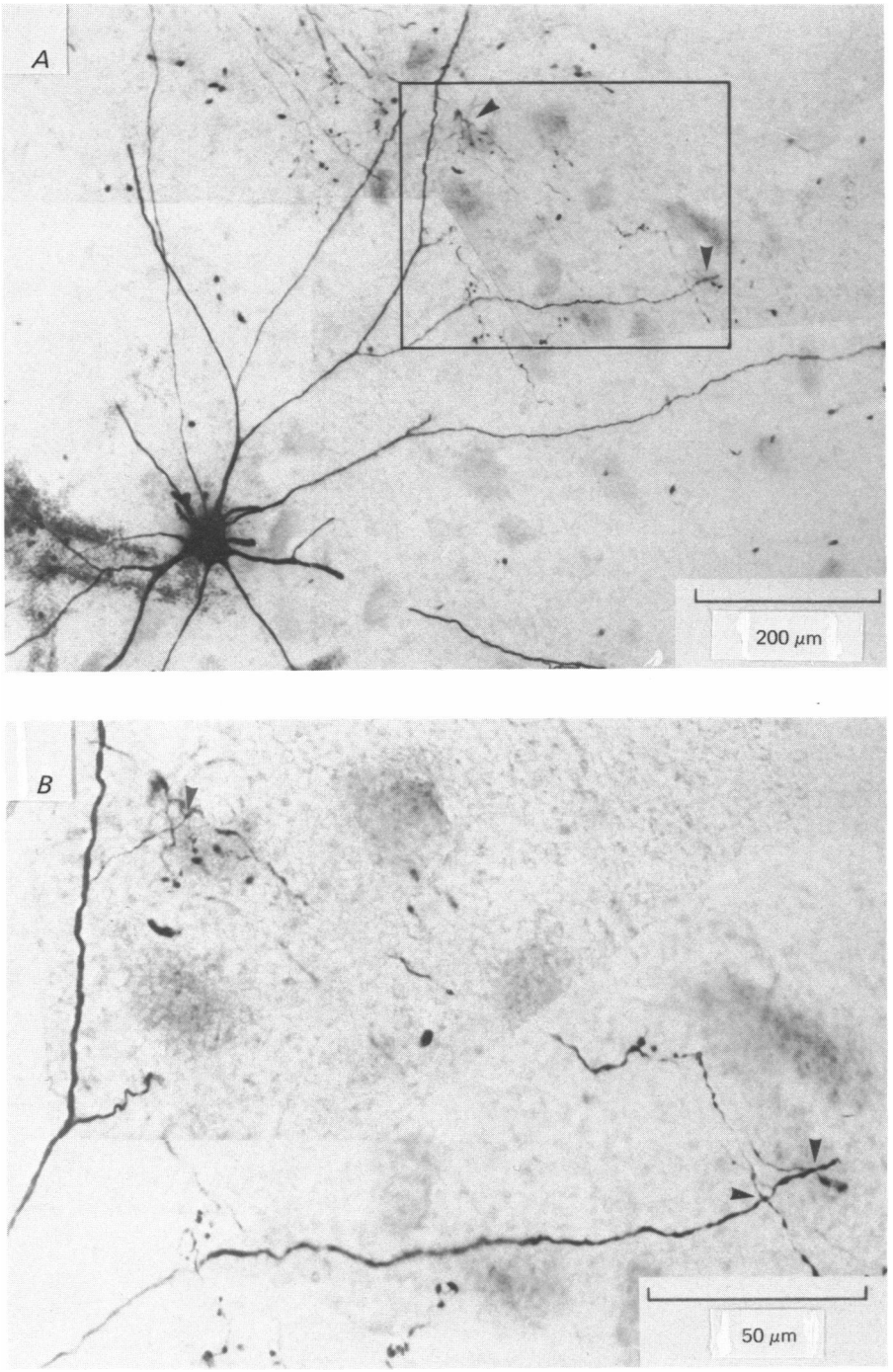

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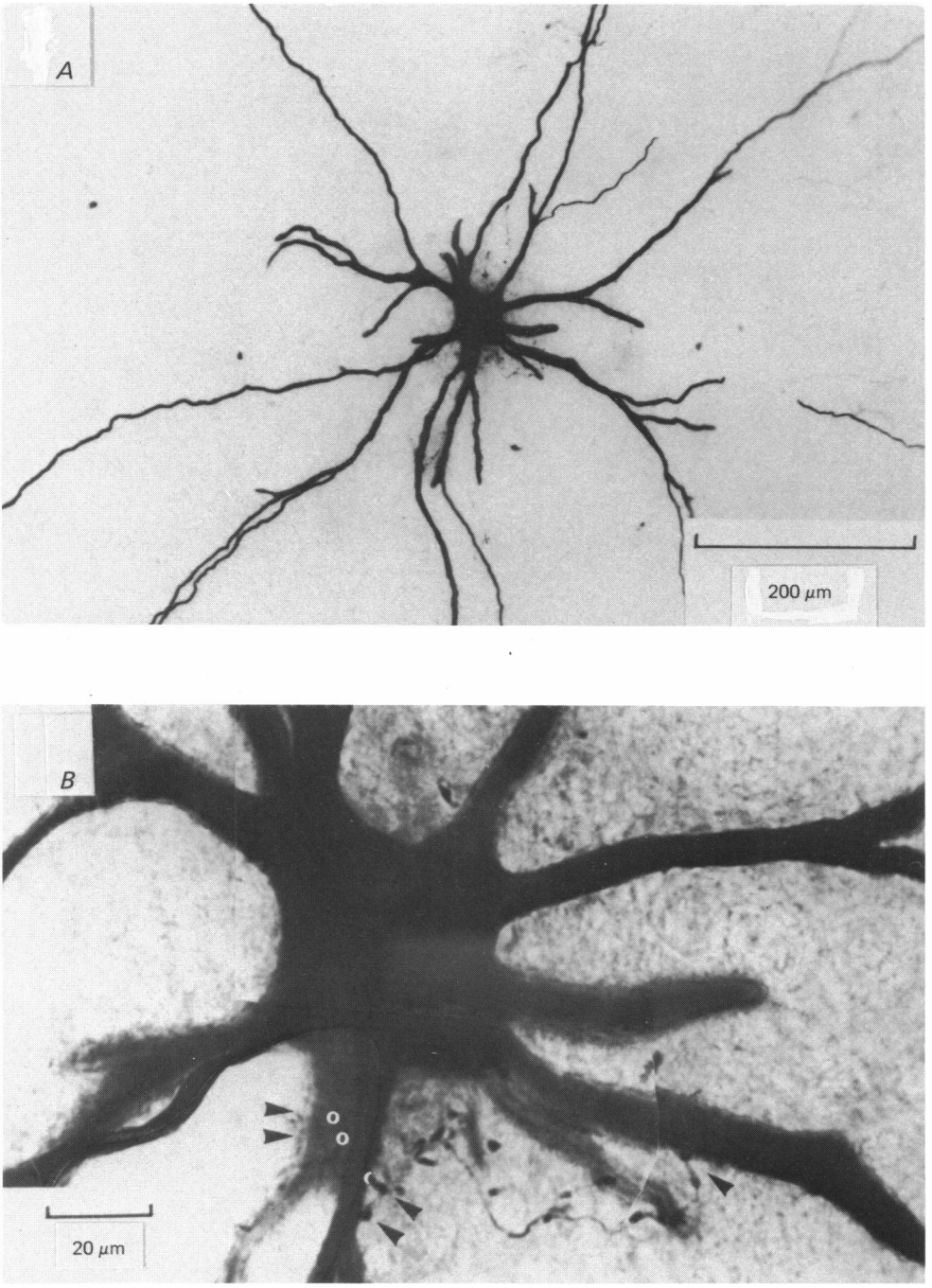

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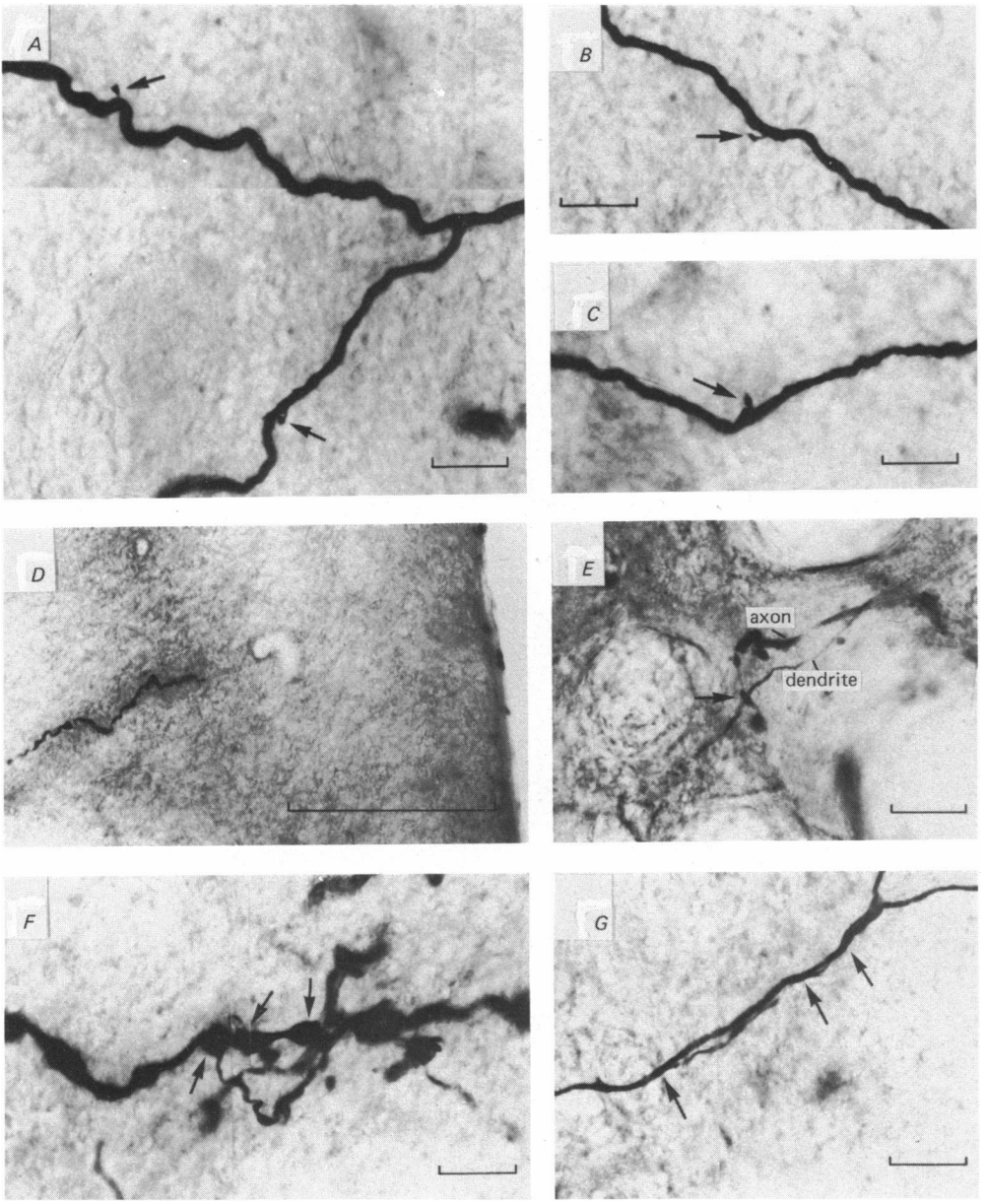

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