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MORPHOLOGY OF SPINAL MOTONEURONES MEDIATING A CUTANEOUS SPINAL REFLEX IN THE CAT

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SUMMARY

1. Intracellular injections of horseradish peroxidase were made in a functionally identified population of motoneurones in spinal cords of cats. These motoneurones were activated by tactile stimulation of the hind-limb central foot pad.

2. Cell bodies of twenty-two such motoneurones were located in the dorsolateral portion of the ventral horn in the first sacral segment. The mean diameter of the major axis of transverse sections through twelve of these cell bodies was 68·2 μm, the mean diameter of the minor axis was 48·7 μm. The major axis tended to be oriented dorsomedially-ventrolaterally.

3. In the transverse plane, the dendrites had a characteristic configuration, with a prominent group of dendrites travelling from the cell body dorsomedially into the dorsal horn, entering Rexed's lamina VI. For seventeen motoneurones with well stained dendrites, the mean medial spread of the dendrites was 960 μm. Though the mean lateral spread was only 508 μm, all of these motoneurones sent dendritic projections into the lateral white matter. The mean dorsal spread of the dendrites was 693 μm, the mean ventral spread, 748 μm. In the rostrocaudal direction, the mean spread rostrally was 911 μm, the mean spread caudally was 998 μm. The maximum dendritic spread for a single motoneurone was 2,940 μm, in the rostrocaudal direction. The sum of dendritic lengths over an entire dendritic tree for the best-stained motoneurones exceeded 13,000 μm.

4. The mean diameter of the initial segment of axons of nineteen motoneurones was 4·3 μm. These axons were notable for the lack or paucity of axon collaterals. Only five of twenty-one axons possessed collaterals; of these, only one possessed more than a single collateral system. This sparseness of the collateral system was reflected in a low level of recurrent inhibition.

5. A possible relationship is discussed between the prominent dorsomedially oriented dendritic bundles of the motoneurones and the axon collaterals of dorsal horn cells mediating cutaneous stimulation which can activate these motoneurones.

INTRODUCTION

Firm pressure on the central foot pad of the hind limb of the cat results in reflex plantar flexion (extension) of the toes (Engberg, 1964; Egger & Wall, 1971) produced by the intrinsic plantar muscles, the flexors digitorum brevis and longus, and the

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plantaris muscle. Motoneurones innervating these muscles were found by Romanes (1951) to be located in the dorsolateral portion of the ventral horn in the first sacral segment of the spinal cord. We confirmed Romanes' localization electrophysiologically (Egger & Wall, 1971), but little has been known of the morphological characteristics of motoneurones mediating this reflex.

Functionally identified motoneurones can be studied in detail by means of the intracellular staining techniques based on horseradish peroxidase (HRP) histochemistry (Jankowska, Rastad & Westman, 1976; Snow, Rose & Brown, 1976). Cullheim & Kellerth (1978), using the intracellular HRP method to study axon collateral systems of α-motoneurones supplying different hind-limb muscles, stained ten motoneurones innervating the short plantar muscles in two cats. The axons of these motoneurones were remarkable for the absence of collaterals. We have confirmed and extended the observations of Cullheim & Kellerth (1978), finding a functional correlate of this lack of axon collaterals. Additionally, we have observed a characteristic dendritic pattern in these motoneurones.

Determination of dendritic configurations and of overall dendritic dimensions has important implications for motoneuronal functioning (e.g. Rall, 1959, 1970; Barrett & Crill, 1974*a*, *b*; Jack, Noble & Tsien, 1975 (chap. 7); Redman, 1976). We found that the motoneurones we studied have dendrites which extend farther from their parent cell bodies than revealed in previous studies of functionally identified motoneurones using [^{3}H]glycine labelling (Lux, Schubert & Kreutzberg, 1970) or Procion yellow dye (Barrett & Crill, 1974*a*), and greater overall length of the entire dendritic tree than typically measured from Golgi-stained spinal cord cells (e.g. Aitken & Bridger, 1961; Mannen, 1975). However, the sacral motoneurones we have studied did not have as great dendritic extents or individual dendritic lengths as the cervical motoneurones studied by Rose (1977) and Vanner & Rose (1979).

METHODS

Intracellular electrophoretic injections were made in spinal motoneurones of twenty-one adult mongrel cats, of both sexes, weighing 1·6–3·6 kg. The cats were anaesthetized with 1% α-chloralose, 80 mg/kg I.V., trachiotomized, and cannulated in both a cephalic vein and the left femoral artery. A bilateral pneumothorax was performed. Atropine sulphate, 0·15 mg I.V., and 6% dextran in saline were routinely administered. Before the recording, the cats were immobilized by I.V. infusion of gallamine triethiodide (Flaxedil), and artificially respirated. Flaxedil was administered in small doses that paralysed the cats for 1–2 hr. As the effects of the Flaxedil wore off, stimulation of the foot produced reflex contractions. As soon as any motor responses occurred, reactivity to cutaneous stimuli and to tactile stimulation of the cornea was investigated. If the cutaneous and, or, corneal reflexes began to reappear, additional α-chloralose was administered until these reflexes disappeared. Such supplementary doses of α-chloralose were rarely required.

The lumbosacral spinal cord was exposed and immersed in mineral oil maintained at 36–38 °C. Body temperature was maintained with a heating pad. The first sacral ventral root (VRS1) on the right was identified, cut intradurally near its exit, and mounted on bipolar platinum hook electrodes. The right hind limb was mounted rigidly with a pin driven through the ankle joint. Electrical stimuli were applied to the plantar cushion (central foot pad) through 27-gauge hypodermic needles inserted into the septa separating the medial and lateral lobes from the central lobe. The electrical stimuli delivered to the plantar cushion were monophasic, rectangular pulses of 0·5 msec duration, delivered through an isolation transformer. Electrical stimuli to VRS1 were delivered via a WPI PC-3 stimulus isolator.

To insure that physiological conditions were maintained, the blood pressure of the left femoral artery was monitored continuously, and repeated small samples of arterial blood were analysed using an IL Model 113 blood gas analysis system. Arterial blood pH, P_{CO_2} and $\%O_2$ saturation were monitored.

Reflex discharges elicited by stimulation of the plantar cushion were recorded from VRS1 (Egger & Wall, 1971; Egger, Bishop & Cone, 1976; Egger, 1978).

Recordings from motoneurones were made with glass micropipettes which were filled with 4–25% HRP (Sigma Type VI) in 0·1 M-NaOH (pH 8·3) or 0·2 M-KCl (pH 7·3). The tips of these electrodes were bevelled; the tip diameters ranged from 1·5 to 2·0 μm, with resistances of 25–60 MΩ.

The dura on the right side of the spinal cord was pinned back, rotating the spinal cord to the left. The seventh lumbar and first sacral roots were gently separated, facilitating entry of the micropipettes into the dorsolateral region of the first sacral (S1) ventral horn. The micropipettes were advanced into the spinal cord with a Transvertex stepping micromanipulator, 2·0 μm per step. The micropipettes were electrically coupled to a WPI M4A electrometer. VRS1 was stimulated at 1·0 Hz as the electrodes were driven into the ventral horn. Impaled cells were identified as S1 motoneurones by antidromic activation from VRS1. Then VRS1 stimulation was discontinued, and the plantar cushion stimulated, both electrically and tactilely. If the motoneurone under study was activated by low to moderate intensity tactile probing of the plantar cushion, sufficient to elicit a reflex (Engberg, 1964; Egger & Wall, 1971, Egger, 1978), but well below the threshold for a flexor withdrawal reflex (Egger *et al.* 1976), and if it was also driven by electrical stimulation at a threshold approximately equal to that of the plantar cushion reflex threshold, the motoneurone was then electrophoretically injected with HRP. Before these injections, however, seven of the motoneurones identified as mediating the plantar cushion reflex were examined for the presence of recurrent inhibition. Three were tested for recurrent inhibition using graded series of antidromic stimuli (Eccles, Fatt & Koketsu, 1954).

HRP was electrophoretically injected into the motoneurones by passing 20 nA for 10–30 min in 200 msec, positive pulses, delivered at 3·3 Hz. From one to three motoneurones were injected per cat. We attempted to make injections far enough apart in the spinal cord so that the dendritic arborizations of the injected motoneurones were completely distinct. For the data reported in this paper, no more than two cells were collected from a single animal. The duration between the beginning of injections until the perfusion of the spinal cord with fixative varied from 90 to 360 min.

At the end of the experiment, the cat was given I.V., 1·0 ml. heparin solution and an overdose of α-chloralose and perfused with 0·9% saline followed by 4% gluteraldehyde in 0·1 M-phosphate buffer at pH 7·3. The lumbosacral spinal cord was excised, placed in buffered 4% gluteraldehyde solution for 12–36 hr, then shifted to 5% sucrose in 0·1 M-phosphate buffer for 2–12 hr. The tissue was then cut into 90–160 μm sections on a freezing microtome, and placed in 0·1 M-phosphate buffer. Thirteen spinal cords were sectioned in the transverse plane, five in the sagittal plane, and three horizontally. Tissue from nine of the cats was reacted with diaminobenzidine (DAB), according to a method modified from Graham & Karnovsky (1966); tissue from twelve cats was reacted with Hanker-Yates reagent (Hanker, Yates, Metz & Rustioni, 1977; Data Sheet no. 204, Polysciences, Inc., 1977). The reacted tissue was conventionally dehydrated in alcohol, cleared in xylene, and mounted on slides. No corrections were made for tissue shrinkage (Lux *et al.* (1970) estimated that shrinkage was 12–16% in linear dimensions in alcohol dehydrated tissue).

The motoneurones were reconstructed using a Zeiss microscope equipped with a drawing tube. Reconstructions of entire motoneurones were made with a × 16 or × 25 objective. Measurements of dendritic spread (Proshansky & Egger, 1977) in two dimensions were made using an ocular micrometer and checked against measurements of drawings. Dendritic spread in the direction perpendicular to the plane of section was estimated from the number of sections containing dendrites. Cross sections of the cell bodies were drawn using a × 40 objective for a total magnification of × 730. For some of these drawings, rather arbitrary decisions had to be made about the position of the boundary between the cell body and the beginning of a primary dendrite.

A Zeiss MOP-3 system for quantitative digital image analysis was used to make measurements on the drawings. Measurements on the drawings of the cell body cross sections included the length of the major diameter, which was the longest straight line encompassed by the cross section; the minor diameter, which was the longest straight line at right angles to the major

diameter that was encompassed by the cross section; the circumference, and the area. Measurements of over-all length of dendritic trees were made from the reconstructions of entire motoneurones made with the × 25 objective. No corrections were made for loss of apparent length in projecting three-dimensional dendrites on to a two-dimensional surface.

Axons were usually discriminable from dendrites by tracing the course of an axon until it joined fibres exiting from the ventral horn with the ventral root. Measurements of axonal diameters were made using a × 100 oil-immersion objective and an ocular micrometer.

RESULTS

Cell bodies. We recovered cell bodies of twenty-two motoneurones that responded to tactile stimulation of the central foot pad (plantar cushion). (These cells were also driven by electrical stimulation of the plantar cushion. When the plantar cushion was

TABLE 1. Mean dimensions of cross-sections through motoneuronal cell bodies

Plane of section . . .	Transverse	Sagittal	Horizontal
Number of cells . . .	12	6	4
Major diameter (μm)	68·2 ± 14·5	67·4 ± 10·0	65·7 ± 17·0
Minor diameter (μm)	48·7 ± 11·8	49·6 ± 7·5	50·6 ± 10·1
Area (A) (μm^2)	2416 ± 976	2340 ± 681	2497 ± 1127
Perimeter (p) (μm)	189·5 ± 41·1	189·9 ± 27·4	186·2 ± 46·6
Form factor = $4\pi A/p^2$	0·82 ± 0·08	0·80 ± 0·06	0·87 ± 0·03

Means followed by sample standard deviations.

electrically stimulated at 3·5 × threshold, the mean latency of the intracellularly recorded motoneuronal action potentials was 9·8 msec). The motoneuronal cell bodies were concentrated in the dorsolateral region of the S1 segment of the ventral horn, in agreement with the localizations of Romanes (1951), based on retrograde degeneration; of Egger & Wall (1971), based on electrophysiology; and of Proshansky & Egger (1975) and Proshansky (1977), based on retrograde transport of HRP from the plantar muscles in kittens.

The cell bodies of these motoneurones tended to be ellipsoidal: cross sections tended to be oval in the transverse and sagittal planes, slightly more circular in the horizontal plane (Table 1). In the transverse plane, the major diameter of the cell body cross section typically was oriented dorsomedially-ventrolaterally. That is, this axis was roughly parallel to a line the dorsal portion of which had been rotated about 45° medialward from dorsoventral. The orientations of the major diameters of the cell body cross sections in the sagittal or horizontal planes were inconstant.

For the twelve cells sectioned in the transverse plane, the major diameter of the cell bodies had a mean of 68·2 μm (Table 1), with a range of 46·9–96·4 μm. The minor diameter had a mean of 48·7 μm, with a range of 27·6–66·7 μm. This compares closely with a sample of 68 sciatic α-motoneurones from the cat studied by Cullheim (1978). Transverse cross-sections through the cell bodies of Cullheim's more heterogeneous sample of α-motoneurones had a mean major diameter of 57·5 μm, with a range of 44–77 μm; the minor diameters had a mean of 45 μm, with a range of 35–55 μm. Furthermore, the sizes of the cell bodies in our study were at least as great as those found for α-motoneurones by Bryan, Trevino & Willis (1972). However, there is a

possibility that some of our motoneurones may have been β-motoneurones (Bessou, Emonet-Dénand & Laporte, 1965; Emonet-Dénand, Jami & Laporte, 1975).

For the six cells sectioned in the sagittal plane, and the four cells sectioned in the horizontal plane, the major and minor axes of the cell body cross sections were of comparable magnitude to those in the transverse plane (Table 1). The mean lengths of the cell body perimeters were similar in all three planes of section: 189·5 μm for the transverse sections, 189·9 μm for the sagittally sectioned cells, and 186·2 μm

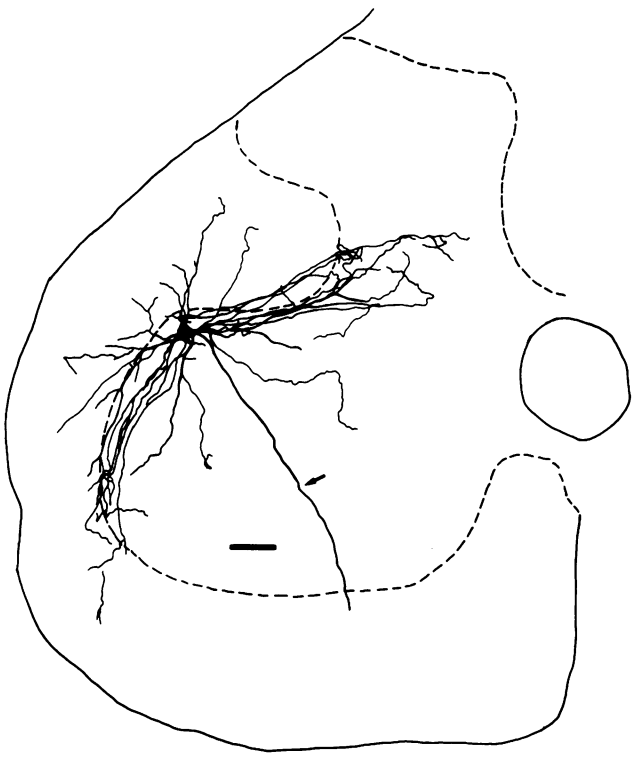

Fig. 1. Reconstruction from five transverse sections (each 120 μm thick) of an S1 motoneurone that responded to tactile stimulation of the central foot pad. Dashed lines indicate boundaries between gray and white matter. Arrow indicates axon. Note the prominent bundle of dendrites coursing dorsomedially from the cell body. Calibration = 200 μm.

for the horizontally sectioned cells. The mean areas of the cross sections through the cell bodies were also very similar in the three planes of section: 2416 μm^2 for the transverse sections, 2340 μm^2 for the sagittal sections, and 2497 μm^2 for the horizontally sectioned cells. A measure of the eccentricity of the cell body cross sections is given by a form factor, $4\pi A/p^2$, where A = the area and p = the perimeter of the cell body cross section. This form factor varies from 1·0 for a circle, to approach zero as the shape of the perimeter becomes more and more elongated. For the transversely sectioned cells, this form factor had a mean of 0·82; for the sagittally sectioned cells, 0·80; and for the horizontally sectioned cells, 0·87, suggesting that the cross sections in the horizontal plane were slightly less eccentric than in the other planes of section.

Lux *et al.* (1970) calculated the surface area of their motoneuronal cell bodies using the formula, $S = \pi d_{maj} \cdot d_{min}$, where d_{maj} and d_{min} were the major and minor diameters, respectively. They found a mean area of 7,300 μm^2 for seven motoneurones. Using this formula, the mean surface area of our twelve transversely sectioned cell bodies was 10,400 μm^2, 10,500 μm^2 for our six sagittally sectioned cell bodies, and 10,400 μm^2 for our four horizontally sectioned cell bodies.

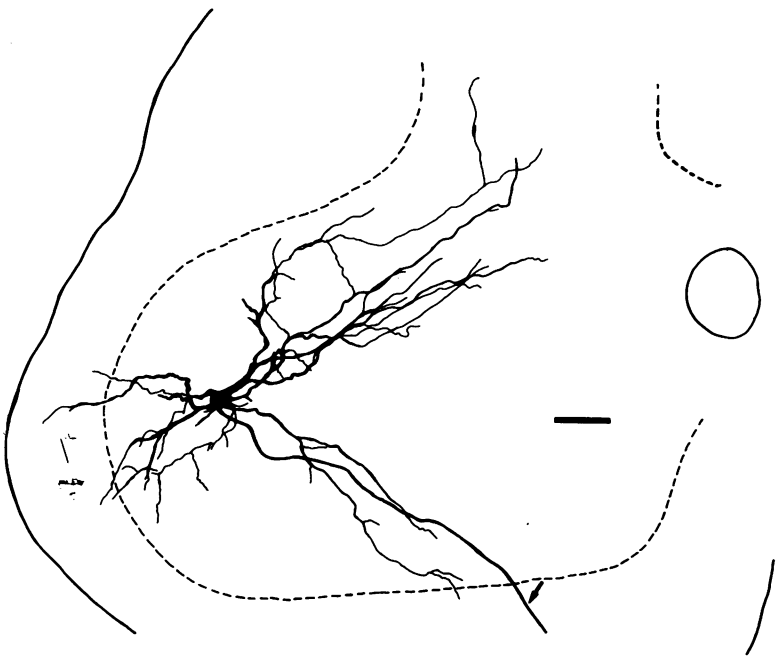

Fig. 2. Similar to Fig. 1. Reconstruction from five transverse sections (each 90 μm thick) of an S1 motoneurone that responded to tactile stimulation of the central foot pad. Arrow indicates axon. Calibration = 200 μm.

Dendrites. In seventeen cells, the dendritic arborizations were well filled with HRP reaction product, ten sectioned in the transverse plane (e.g. Figs. 1 and 2), five in the sagittal plane (e.g. Fig. 3), and two horizontally (e.g. Fig. 4). Photomicrographs of a representative cell sectioned in each of these three planes are presented in Pl. 1*A* and Pl. 2*A*, *B*. The number of primary dendrites ranged from nine to eighteen with a mean of 11·9. This is similar to the number reported (eight to twenty-two) for the functionally more heterogeneous sample of Barrett & Crill (1974*a*) in cells stained intracellularly with Procion yellow and to the nine to fifteen primary dendrites reported by Lux *et al.* (1970) for motoneurones labelled with [^{3}H]glycine. In γ-motoneurones stained intracellularly with HRP, Westbury (1979) found four to six primary dendrites.

A striking characteristic readily apparent in the motoneurones sectioned transversely was a prominent bundle of dendrites travelling dorsomedially from their cell bodies, typically continuing in the direction of the major axis of the cell body in the transverse plane and traversing the intermediate gray region to enter the dorsal

horn (Figs. 1, 2, Pl. 1*A*). Once this characteristic had been noticed, it was possible to pick out similar cells comparably located in Golgi-stained material (Pl. 1*B*).

In the transversely sectioned cells, more primary dendrites tended to emerge from the dorsomedial and ventrolateral quadrants of the cell bodies, than from the ventromedial quadrant, with the dorsolateral quadrant giving rise to fewer still primary dendrites. Furthermore, the largest primary dendrites, some approaching

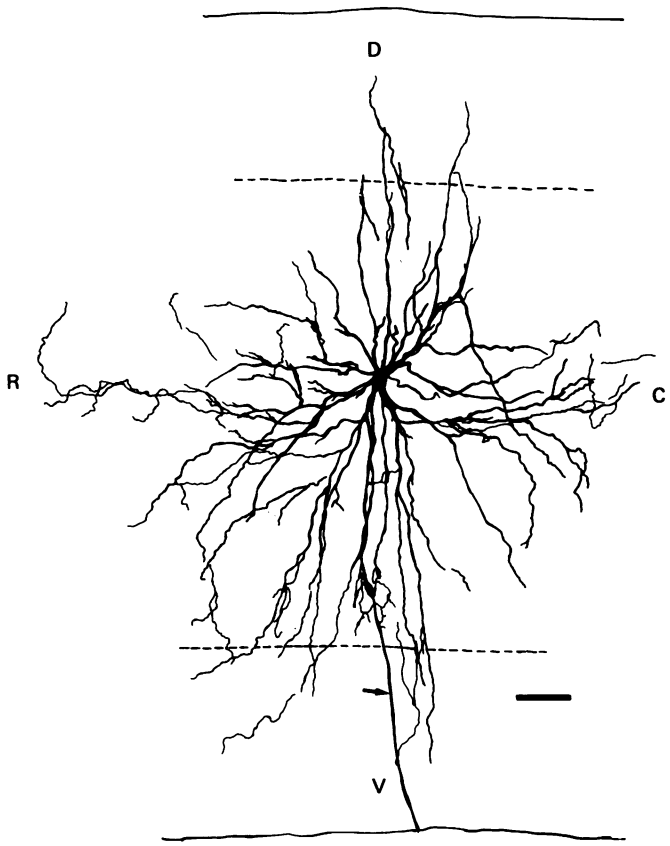

Fig. 3. Similar to Fig. 1, but depicting an S1 motoneurone reconstructed from five sagittal sections (each 120 μm thick). Dashed lines correspond to dorsal and ventral limits of the ventral horn. R, rostral; C, caudal; D, dorsal; V, ventral. Arrow indicates axon. Calibration = 200 μm.

20 μm in diameter, usually emerged from the dorsomedial quadrant. Of the four quadrants, the dorsolateral tended to give rise to primary dendrites with the narrowest maximum diameter.

Of the ten transversely sectioned cells, seven sent dendrites into dorsal horn lamina VI (Rexed, 1954), entering the lateral portion of lamina VI and continuing into the central portion of this lamina. None of the motoneuronal dendrites continued to the medial edge of this lamina. Of the seven motoneurones with dendrites in lamina VI, two also sent dendrites that continued into dorsal horn lamina V as well.

All five of the sagittally sectioned, and both of the horizontally sectioned cells with well stained dendrites also sent dendrites into the dorsal horn.

In several cells another prominent group of dendrites travelled ventrally or ventromedially through the ventral horn gray matter. In addition, all seventeen motoneurones with well stained dendrites sent substantial dendritic projections into the white matter of the lateral column (e.g. Fig. 1). While all cells possessed

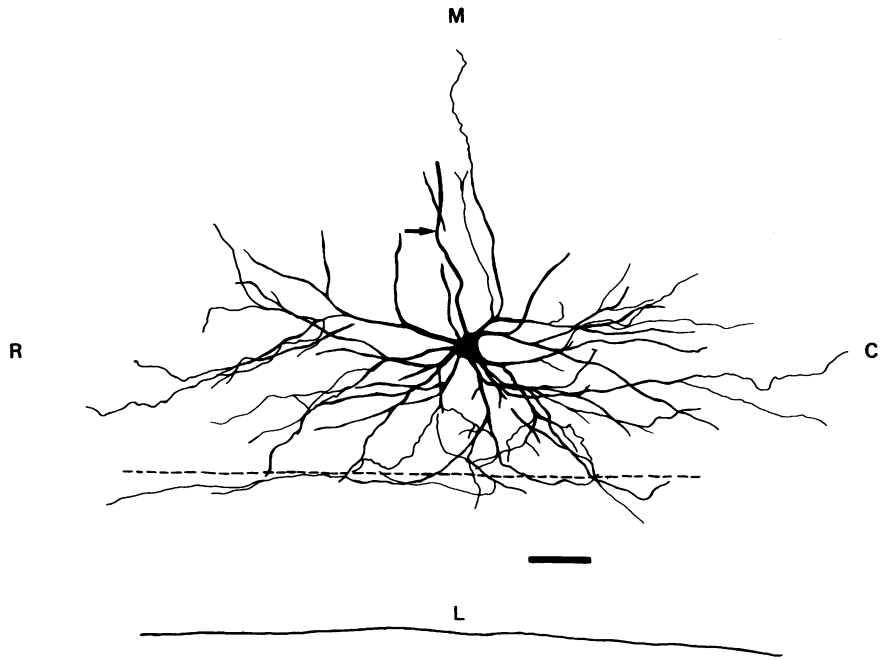

Fig. 4. Similar to Fig. 1, but depicting an S1 motoneurone reconstructed from five horizontal sections (each 120 μm thick). Dashed line corresponds to lateral border of ventral horn. R, rostral; C, caudal; M, medial; L, lateral. Arrow indicates axon. Calibration = 200 μm.

dendrites which entered the white matter, the extent of these projections was variable from cell to cell. Some cells had dendrites which penetrated quite deeply into the white matter, nearly reaching the surface of the spinal cord, while others showed only shallow penetration (e.g. Fig. 4) (cf. Rose & Richmond, 1978). The total number of dendrites entering the white matter was also quite variable from cell to cell. Furthermore, the length of the dendrites entering the white matter was significantly less than the dorsomedially directed dendrites confined to the gray matter. None of the cells possessed the prominent contralaterally projecting dendrites described for some coccygeal motoneurones by Light & Metz (1978).

Cells sectioned in the sagittal plane revealed dendrites which appeared to radiate out more or less symmetrically in the rostrocaudal and dorsoventral directions (Fig. 3).

That the characteristic pattern of dendritic polarization seen in the transverse plane in motoneurones excited by tactile stimulation of the plantar cushion (Figs. 1,

2, Pl. 1*A*) is not a universal characteristic of motoneurones in this region of the spinal cord is illustrated in Fig. 5, a reconstruction of an S1 motoneurone in which plantar cushion stimulation elicited an inhibitory post-synaptic potential (i.p.s.p.). Cells inhibited by plantar cushion stimulation did not show the prominent bundle of dorsomedially directed dendrites, but demonstrated a rather symmetrical appearance in transverse section.

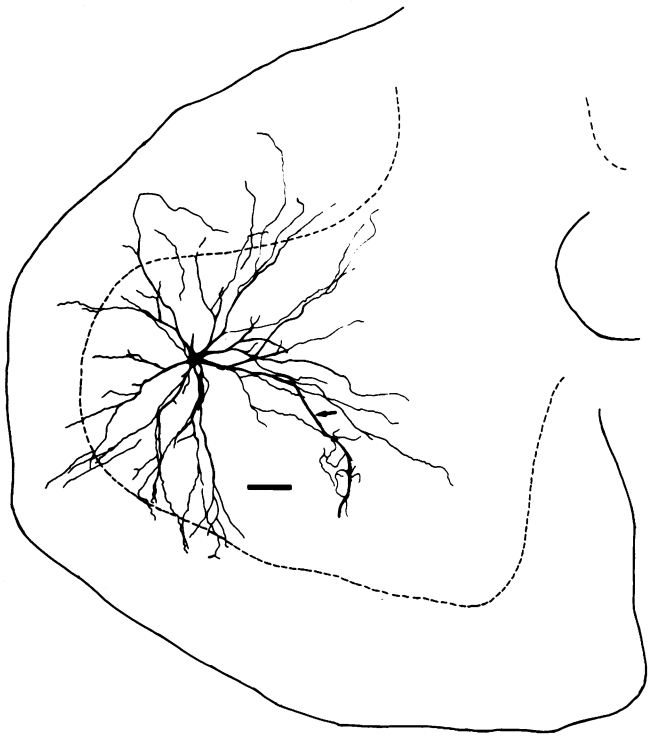

Fig. 5. Reconstruction from five transverse sections (each 160 μm thick) of an S1 motoneurone in which stimulation of the central foot pad produced i.p.s.p.s. Arrow indicates axon. Calibration = 200 μm.

TABLE 2. Mean dendritic spread (μm) of seventeen motoneurones activated by tactile stimulation of the central foot pad

Medial:	960 ± 255	Dorsal:	693 ± 209	Rostral:	911 ± 300
Lateral:	508 ± 228	Ventral:	748 ± 284	Caudal:	998 ± 357

Means followed by sample standard deviations.

We measured the maximum distances that dendrites could be found from their cell bodies (Proshansky & Egger, 1977), referring to this quantity as dendritic spread (Table 2), and analyzed these data by means of an analysis of variance. There was no effect of the plane of section on over-all dendritic spread, or any significant interaction of plane of section with dendritic spread in any of the six directions: medial, lateral, dorsal, ventral, rostral, caudal. There was a highly significant ($P < 0{\cdot}001$) over-all effect of length of dendrites in the various orientations, however.

This was the result of three statistically significant effects (all of $P < 0{\cdot}001$): (1) the medial spread of dendrites was greater than the lateral spread, (2) the over-all rostrocaudal dendritic spread was greater than the overall mediolateral dendritic spread, or (3) than the over-all dorsoventral dendritic spread.

The mean dendritic spread of these seventeen motoneurones in the rostrocaudal direction was almost 2·0 mm (Table 2). This is longer than expected from the data of Lux *et al.* (1970) or Barrett & Crill (1974*a*) but less than the 3·0–4·0 mm dendritic spread Vanner & Rose (1979) found for trapezius motoneurones in the cat. The range of dendritic lengths we found, from 245 μm for some lateral-going dendrites to 1650 μm for a caudally directed dendrite, was greater than the 640–1150 μm range reported by Lux *et al.* (1970) or the 300–800 μm range reported by Barrett & Crill (1974*a*), but in trapezius motoneurones Vanner & Rose (1979) found many dendrites from 1000 to 2000 μm in length.

For the three best-stained motoneurones mediating the plantar cushion reflex, the over-all length of the entire dendritic tree, measured branch by branch, was in excess of 13,000 μm. The total summed dendritic length for the cell depicted in Fig. 1 and Pl. 1*A* was 16,350 μm (sum of first-order through sixth-order dendrites). The total dendritic length for the cell depicted in Fig. 3 and Pl. 2*A* was in excess of 14,700 μm (sum of first-order through sixth-order dendrites). The cell depicted in Fig. 4 had a total dendritic length in excess of 13,090 μm (sum of first-order through seventh-order dendrites). This is approximately an order of magnitude greater than the over-all dendritic lengths reported by Mannen (1975) from measurements of Golgi-stained cells from the spinal cords of kittens. Note that these values for the over-all dendritic lengths are *minimum* estimates for the following reasons: (1) no corrections were made for shrinkage during histological preparation; (2) no corrections were made for losses as a result of projecting three-dimensional structures in two dimensions; (3) it is possible that not all dendritic branches filled sufficiently with HRP reaction product to render them visible in the light microscope; (4) some dendritic branches were possibly lost in sections remote from the cell body; (5) large segments of what appeared to be dendritic branches, which were stained, but which could not be unambiguously connected to the continuous dendritic tree, were not included in computing the over-all dendritic lengths. Inclusion of these doubtful pieces of dendrite in the sums of over-all dendritic lengths for the cells described above brings their total dendritic lengths to greater than 20,000 μm each.

By measuring the widths and lengths of dendritic segments, using values of specific resistance of the membrane, R_m, and of resistivity of the cytoplasm, R_a, from Barrett & Crill (1974*a*), and making the usual assumptions about the uniformity of these values throughout the entire motoneurone, it was possible to estimate roughly the electrotonic lengths of some dendritic branches (see, e.g., Rall, 1970; Jack *et al.* 1975). (The measurement of dendritic widths was made doubtful in some cases because some dendrites appeared to be in the shape of flattened, twisted ribbons. In these cases, the maximum diameters were taken as the widths.) The electrotonic length of many dendrites extended to 2–3 space constants. This is longer than electrotonic lengths of motoneurones reported in earlier studies (e.g. Lux *et al.* 1970; Barrett & Crill, 1974*a*). These greater electrotonic lengths are consistent with the greater geometric lengths of dendrites found in this study.

Some of the dendrites of our motoneurones showed prominent beading (Pl. 2*A*: inset). Whether or not this beading is normally present, or is an artefact of the HRP injection or the histological procedures, is unresolved (cf. Burke, Walmsley & Hodgson, 1979). Only one of the seventeen motoneurones with well-stained dendrites possessed discernable dendritic spines.

Axons and axon collaterals. Main-stem axonal diameters were measured in the region of the initial segment for nineteen motoneurones. The mean diameter was $4{\cdot}3 \pm 0{\cdot}6$ μm, which agrees well with the values reported for initial segment diameters of sciatic α-motoneurones by Cullheim (1978). At greater distances from the cell

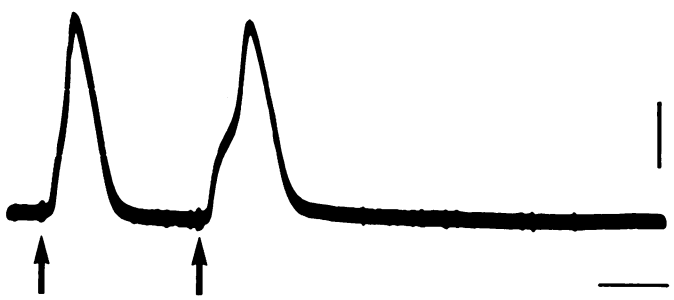

Fig. 6. Intracellular recording from a motoneurone activated by tactile stimulation of the central foot pad. Antidromic responses to a pair of stimuli delivered to the S1 ventral root. Arrows indicate location of stimulus artifacts. No recurrent inhibition was observed at this or slower horizontal sweep speeds. Calibration: horizontal = 2·0 msec; vertical = 20 mV.

body, within the gray matter and into the white matter, we found the axonal diameters to have increased to a mean of $4{\cdot}8 \pm 0{\cdot}7$ μm, which is smaller than the mean axonal diameter of 6·7 μm which Cullheim & Kellerth (1978) found for ten motoneurones innervating the short plantar muscles.

Cullheim & Kellerth (1978) did not find any collaterals arising from the axons of the ten motoneurones innervating the short plantar muscles, in marked contrast to their finding of abundant collaterals arising from motoneurones innervating the following muscles: gastrocnemius-soleus, anterior tibial, quadriceps, and posterior biceps (the mean number of first-order collaterals per axon for forty main-stem axons innervating these motoneurones was 2·2). In our sample of axons of nineteen motoneurones activated by stimulation of the plantar cushion, fourteen had no collaterals, four gave off a single collateral, and one axon gave rise to four collaterals. Typically, the single collaterals which were seen were short, poorly ramified, and sparsely supplied with terminal knobs. The terminations of these collaterals occurred ventrally, ramifying between their points of exit from the main-stem axon and the ventral border of the gray matter.

In contrast to the axons of motoneurones activated by stimulation of the plantar cushion, the axons of two motoneurones inhibited by plantar cushion stimulation (e.g. Fig. 5) each had four collaterals with an abundance of secondary branches and terminal knobs.

Cullheim & Kellerth (1978) speculated that, due to the paucity of collaterals, the

α-motoneurones of the short plantar muscles should produce negligible recurrent effects. We have confirmed this hypothesis: in seven preparations, we found no appreciable evidence of recurrent inhibition in motoneurones activated by stimulation of the plantar cushion. In particular, in three preparations investigated intensively with graded antidromic stimulation to differentiate recurrent inhibition from after-hyperpolarization (Eccles *et al.* 1954), we observed little or no recurrent inhibition (Fig. 6).

DISCUSSION

We have described the characteristic morphology of a functionally defined motoneuronal population. A prominent feature of these cells was a dendritic bundle coursing dorsomedially from the cell body across the intermediate gray region and ending in the dorsal horn. This group of dendrites appears to be characteristic of the functionally defined group of motoneurones receiving cutaneous excitation from the plantar cushion, since it was not present on adjacent, inhibited cells. While relatively distinct morphological differences are known to exist among the major functional classes of motoneurones (e.g. α *vs.* γ), the more subtle differences in dendritic orientation which we have observed may also be a feature of different motoneurone populations. Differences in dendritic orientation may reflect differences in the organization of the major sources of input. For example motoneurones, in the coccygeal segments project dendrites contralaterally, a feature which may be related to bilateral sensory innervation from midline structures. In this respect, it is of particular interest to note that the prominent dorsomedial course of dendrites of motoneurones mediating the plantar cushion reflex very closely parallels the route of collaterals of Ia afferents from the posterior tibial nerve (Brown & Fyffe, 1978; Ishizuka, Mannen, Hongo & Sasaki, 1979). This dendritic orientation could provide maximal *en passant* contact between the dendrites and a major primary afferent input. In addition, dorsal horn cells in laminae IV and V which respond to cutaneous stimulation of the central foot pad have axon collaterals which ramify in the deep laminae of the dorsal horn, including lamina VI (Egger, Freeman & Proshansky, 1979). The dorsomedial course of our motoneuronal dendrites into lamina VI would thus place them in a region where they could receive contacts from those cells, in accord with the polysynaptic nature of the plantar cushion reflex (Egger & Wall, 1971).

Although the muscles activated during the plantar cushion reflex tend to be smaller than the more proximal muscles of the hind limb, the cell body sizes of the motoneurones innervating these smaller muscles are as large or larger than those of α-motoneurones innervating some of the larger muscles (Cullheim, 1978). Furthermore, the dendritic spread of the plantar cushion reflex motoneurones in the transverse plane is comparable to that of the α-motoneurone innervating the lateral gastrocnemius muscle depicted by Cullheim & Kellerth (1976).

The great length of some of the individual dendrites of our motoneurones, continuing in some cases for over 1600 μm from the cell body, may pose a challenge for understanding the detailed physiology of these cells. These dendritic lengths correspond to electrotonic lengths of about 2–3 space constants. Barrett & Crill (1974*a*)

found dendritic electrotonic lengths from 0·8 to 2·2 space constants, with a mean of 1·4, corresponding to dendritic terminals 300–800 μm from the cell body. However, some trapezius motoneurones studied by Rose and his collaborators possess dendrites which terminate as far as 8 space constants from the cell body, with a mean length of about 3–4 space constants (P. K. Rose, personal communication). The effectiveness of synaptic contacts on dendrites 2, 3 or more space constants from the cell body, relative to synaptic contacts on or near the cell body, has been the subject of considerable experimental and theoretical investigation (see, e.g. Barrett & Crill, 1974*b*; Jack *et al.* 1975; Redman, 1976).

Although the functional significance of the substantial projection of dendrites of our sacral motoneurones into the lateral columns is unclear, the penetration of the dendrites of ventral horn cells into white matter has been frequently noted, especially in lower vertebrates (e.g. Niewenhuys, 1964). Rose & Richmond (1978) have recently called attention to this dendritic projection into the white matter in cervical motoneurones.

The dimensions of the motoneuronal cell bodies were similar in all three planes of section (Table 1). In particular, the means of the perimeter measurements agreed with each other within 2%. Furthermore, the standard deviations of the means of the form factor, the measure of cross section eccentricity, were each less than 10% of the means, indicating that the form factor was a stable parameter from cell to cell, for each of the three planes of section.

Cullheim & Kellerth (1978) found that the motoneurones innervating the plantar muscles had a paucity of collaterals, a finding we have essentially confirmed. We observed further that this paucity of collaterals is accompanied by a low level or absence of recurrent inhibition, as Cullheim & Kellerth (1978) suggested.

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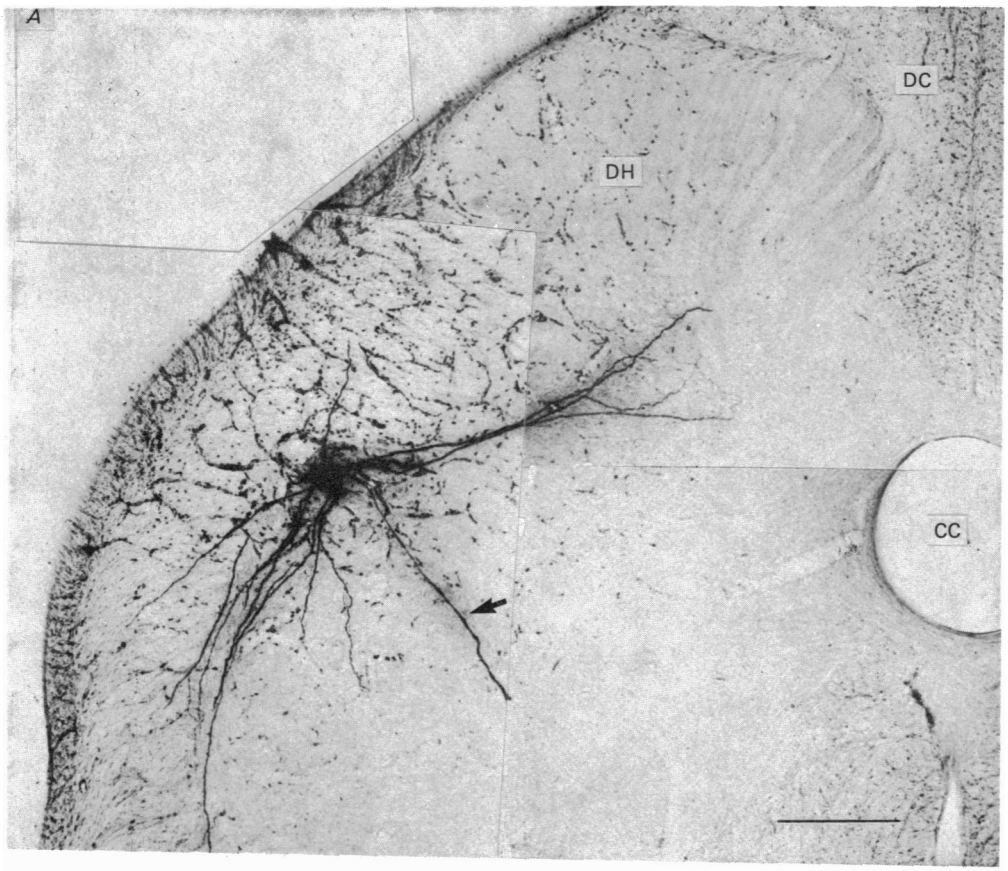

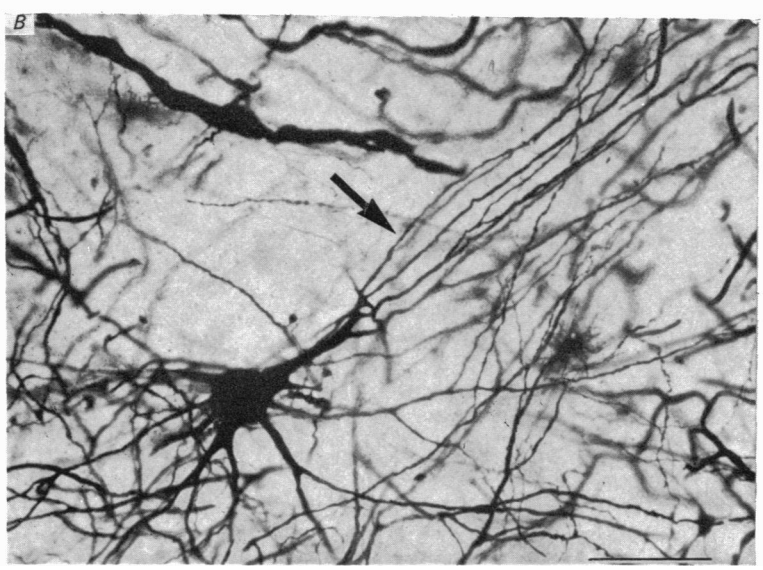

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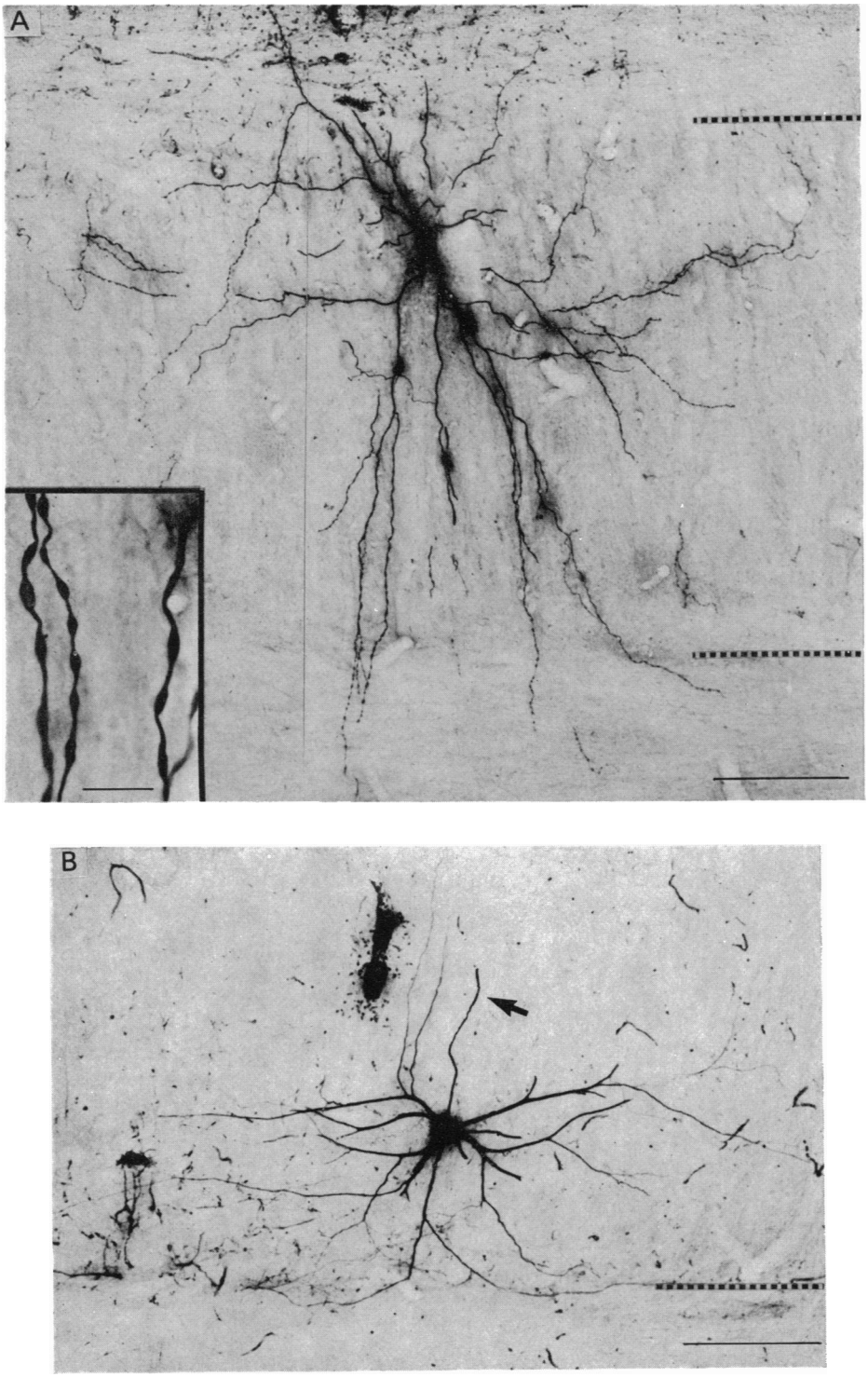

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EXPLANATION OF PLATES

PLATE 1

A, microphotomontage of 120 μm thick transverse section through the motoneurone reconstructed in Fig. 1. DH, dorsal horn; DC, dorsal column; CC, central canal. Arrow indicates axon. Calibration = 340 μm. *B*, microphotograph of a neurone in the dorsolateral portion of the ventral horn, modified Golgi stain (Proshansky & Egger, 1977). Arrow indicates band of dendrites coursing dorsomedially from the cell body. Calibration = 115 μm.

PLATE 2

A, microphotomontage of 120 μm thick sagittal section through the motoneurone reconstructed in Fig. 3, but with the caudal direction to the left and the rostral direction to the right. Interrupted lines indicate dorsal (upper) and ventral (lower) boundaries of the gray matter. Calibration = 320 μm.

Inset: beading in the dendrites of this motoneurone. Calibration = 30 μm.

B, microphotograph of 120 μm thick horizontal section through the motoneurone reconstructed in Fig. 4, but with the caudal direction to the left and the rostral direction to the right. Interrupted line indicates the lateral border of the ventral horn. Arrow indicates axon. Calibration = 340 μm.