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MORPHOLOGY OF INTERNEURONES MEDIATING Ia RECIPROCAL INHIBITION OF MOTONEURONES IN THE SPINAL CORD OF THE CAT

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SUMMARY

1. Interneurones identified by physiological criteria (Hultborn, Jankowska & Lindström, 1971*b*) to mediate Ia reciprocal inhibition of motoneurones in the spinal cord of the cat were stained by intracellular injection of a fluorescent dye (Procion Yellow).

2. The somas of the stained cells were found in Rexed's lamina VII, just dorsal or dorsomedial to the motor nuclei. Their size was about 30 × 20 μm. The cells had four to five slender, weakly branching dendrites. The total extension of their dendritic trees was about 600 μm dorsoventrally, 400 μm mediolaterally and 300 μm rostrocaudally.

3. The axons originated from a separate axon hillock or from the base of a dendrite. They were myelinated with external diameter of about 6–14 μm and projected to either the ipsilateral ventral or lateral funiculi. Early collaterals were found only exceptionally. Some axons bifurcated into an ascending and a descending branch within the funiculi.

4. The possibility of identifying the Ia inhibitory interneurones on purely morphological grounds is discussed.

INTRODUCTION

The present study is a continuation of a series of physiological studies on the pathway of reciprocal inhibition of motoneurones from muscle spindle Ia afferents and in particular on the interneurones interposed in it (Hultborn, Jankowska & Lindström, 1971*a*, *b*, *c*; Hultborn, Jankowska, Lindström & Roberts, 1971; Jankowska & Roberts, 1972*a*, *b*; Hultborn, 1972). Originally this pathway was supposed to be monosynaptic, hence the name 'direct inhibition' (Lloyd, 1941). However, later experiments demonstrated that the central latency of the Ia inhibitory post-synaptic potentials in motoneurones was too long to be compatible with a monosynaptic linkage, and it was concluded that an inhibitory interneurone is

interposed in the pathway (Eccles, Fatt & Landgren, 1956; Araki, Eccles & Ito, 1960). The demonstration of spatial summation in the Ia inhibitory pathway of effects from two nerve filaments supplying one muscle led to the same conclusion (R. M. Eccles & Lundberg, 1958). The technique of studying spatial and temporal facilitation in interneuronal chains terminating on motoneurones further developed by Lundberg & Voorhoeve (1962) revealed a convergence of excitation and/or inhibition on the interneurones in the Ia inhibitory path from different types of primary afferents, several descending systems, and motor axon collaterals (for references see Lundberg, 1969, 1970 and Hultborn, 1972). It appeared that the pattern of convergence on to them, and especially the inhibition from motor axon collaterals, clearly distinguishes these interneurones from other types of interneurones and allows individual cells to be identified as Ia inhibitory interneurones (Hultborn *et al.* 1971*a*, *b*). Direct evidence that such cells mediate Ia reciprocal inhibition was given by Jankowska & Roberts (1972*a*, *b*) who showed that interneurones with the properties defined by Hultborn, Jankowska & Lindström (1971*b*) do indeed evoke monosynaptic i.p.s.p.s in motoneurones.

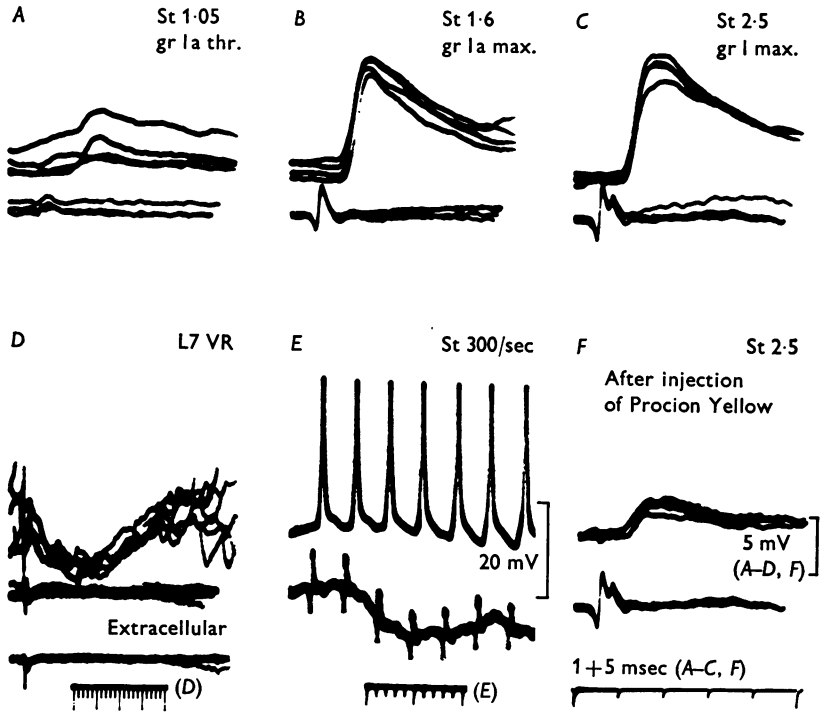
With this established, it became possible to identify the Ia inhibitory interneurones also morphologically, using the technique of intracellular staining. A number of Ia inhibitory interneurones were injected with Procion Yellow (Stretton & Kravitz, 1968), and their dendritic trees and axonal projections were reconstructed from serial sections. It was hoped that the morphological description of these neurones might be useful for their recognition in studies using conventional histological techniques, which cannot be combined with physiological identification of cells but are better suited for a full reconstruction of their axonal projections, and for investigation of their synaptic contacts with other cells. The results of this study fully support conclusions from the physiological experiments on the location (Hultborn *et al.* 1971*b*) and axonal projections (Jankowska & Roberts, 1972*a*) of Ia inhibitory interneurones.

METHODS

Identification of Ia inhibitory interneurones. Twenty-three Ia inhibitory interneurones located in spinal segments L6 and L7, were stained in seven adult cats (2·5–3·5 kg). They were identified on the basis of the following properties: (1) monosynaptic excitation from lowest threshold group I afferents, (2) inhibition following stimulation of motor axons in L6, L7 or S1 ventral roots (VRs), (3) lack of antidromic invasion upon stimulation of ventral roots or ascending tract fibres and (4) an ability to generate spike potentials at a rate of at least 300/sec.

As shown previously (Hultborn *et al.* 1971*b*; Jankowska & Roberts, 1972*a*, *b*), these properties differentiate the Ia inhibitory interneurones from other categories of spinal neurones: α- and γ-motoneurones, ascending tract cells and other types of

interneurones, including the Ia activated interneurones in the intermediate region. The latter were previously supposed to mediate reciprocal Ia inhibition (Eccles, *et al.* 1956), but in view of their lack of recurrent inhibition from motor axon collaterals it was concluded that they have another function (Hultborn *et al.* 1971*b*, cf. also Hongo, Jankowska & Lundberg, 1972). Records from one of the stained cells are shown in Text-fig. 1 for illustration of the routine tests. In *A* through *C* are shown e.p.s.p.s evoked by successively increasing strengths of stimulation of the semitendinosus nerve. The threshold for evoking the e.p.s.p. was very close to the threshold for exciting group Ia fibres (*A*). The e.p.s.p. reached the maximum (*B*)



Text-fig. 1. Intracellular records from a Ia inhibitory interneurone activated from group Ia muscle spindle afferents in semitendinosus (St) nerve. Upper traces are intracellular potentials. Lower traces show corresponding nerve volleys recorded from the surface of the spinal cord at the dorsal root entry zone. Negativity is indicated downwards in intracellular recordings and upwards in surface recordings. *A–C*, excitatory post-synaptic potentials evoked at different strengths of stimulation of St nerve. *D*, an inhibitory post-synaptic potential following stimulation of L7 VR. This was clearly a post-synaptic potential as seen from a comparison with the lowermost record taken extracellularly just after withdrawal of the microelectrode from the cell. *E*, action potentials evoked by stimulation of St nerve before the spike mechanism of the cell deteriorated. *F*, as in *C* but at the end of injection of Procion Yellow. All time calibrations indicate 1 msec between small bars and 5 msec between the larger ones.

within the stimulus strength maximal for the first component of the afferent volley, attributed to group Ia afferents (Bradley & Eccles, 1953, cf. *B* and *C*). Records in *D* and *E* show that the interneurone was inhibited following stimulation of L7 VR and that it could fire at a rate of 300/sec.

Experimental procedure. The experiments were done under chloralose anaesthesia (50–70 mg/kg). The preparation and the general techniques of recording and stimulation were as described previously (Hultborn *et al.* 1971*a*). The micro-electrodes used for recording and dye injection were ordinary glass micropipettes with tips broken to 1·5–2·5 μm. They were filled with a freshly made 5–6 % solution of Procion Yellow M-4R (I.C.I.) in distilled water and had resistances of 15–25 MΩ.

The Ia inhibitory interneurones were sought mainly within an area dorsal and dorsomedial to the motor nuclei where they were found in the previous study (Hultborn *et al.* 1971*b*). The electrodes were introduced either from the cord dorsum (close to the dorsal-root entry zone) or from the lateral aspect of the cord and advanced slowly while stimulating a number of hind limb nerves. Usually the Ia inhibitory interneurones were identified from extracellular records after which a systematic tracking, guided by changes in the amplitude of their spike potentials, was performed until they were successfully penetrated. Intracellular records were then taken for final identification (see Text-fig. 1), and the staining proceeded. The dye was injected microelectrophoretically by passing a weak (5–10 nA), constant hyperpolarizing current through the micro-electrode (Jankowska & Lindström, 1970, 1971). If the cells seemed to tolerate the current well, after a few minutes its intensity was slowly increased to 20–25 nA. Currents stronger than 25 nA were not used because of an increased risk of losing the cells. The monosynaptic e.p.s.p.s were recorded during the whole period of dye injection in order to ascertain that the electrode remained inside the cell. The amplitude of these e.p.s.p.s gradually decreased during the dye injection (cf. *C* and *F* in Text-fig. 1) which was usually continued as long as any clear synaptic response was seen. The maximal amount of dye used for staining the cells corresponded to that ejected by 20 nA during 20 min (400 nA.min), but about one fourth of this proved to be sufficient for staining somas, dendrites and initial parts of the axons. Usually a few cells were stained in each experiment, and the time period between the dye injection and sacrificing the animal varied between 1 and 11 hr. In order to have longer times for diffusion of the dye along the axons the spinal cords were perfused *in situ* with cold Ringer solution and then kept for 16–32 hr at low temperature (about + 4° C) before fixation (Jankowska & Lindström, 1970).

Histological procedure and reconstruction of cells. The spinal cords were fixed in 10 % neutralized formalin, dehydrated in ethyl alcohol, and embedded in paraffin. Serial sections (15 μm) were cut either transversely or sagittally (cf. Table 1). The sections containing stained cells were deparaffinized and mounted in Entellan (Merck). The cells were viewed through a Leitz microscope with an incident light fluorescence illuminator of Ploem with a dichroic beam-splitting mirror, K 495, an exciting filter, BG 12 (3 or 5 mm), and an absorbing filter, K 510 or K 530. Photomicrographs were taken with Kodak Ektachrome high-speed (daylight) colour film or Ilford FP 4 black and white film. The dendritic tree and the axonal trajectory were reconstructed using a Leitz binocular tracing devise based on the split-image principle. Examples of such reconstructions are given in Text-fig. 3 and 4. For some cells a partial photomontage reconstruction was made from photomicrographs of a few neighbouring sections (Pl. 2).

All the dimensions are given without correction for shrinkage during the histological procedure and are therefore underestimated. The over-all shrinkage was about 10–15 %, as judged from changes in distance between two electrodes introduced into the spinal cord before sacrificing the animal.

Abbreviations and list of nerves used for stimulation. E.p.s.p., excitatory post-synaptic potential; i.p.s.p., inhibitory post-synaptic potential; VR, ventral root; ABSm, anterior biceps and semimembranosus; Add, adductor femoris; G-S, gastrocnemius and soleus; Grac, gracilis; PBSt, posterior biceps and semitendinosus; PB, posterior biceps; St, semitendinosus; Sart, sartorius; Q, quadriceps.

RESULTS

The majority of the stained Ia inhibitory interneurones were excited from either the Q (Table 1, cells nos. 1–9) or the PBSt (Table 1, cells nos. 15–23) nerves. These two subgroups were also most extensively studied previously (Hultborn *et al.* 1971*b*; Jankowska & Roberts, 1972*a*, *b*; Hultborn & Santini, 1972), and both are among the Ia inhibitory interneurones with longest axons. Q interneurones, which are located mainly in L5–L6 spinal segments, may inhibit PBSt and ABSm motoneurones within L7–S1 segments, Grac, ABSm and Add motoneurones within L6, and Sart motoneurones within L4–L5. PBSt interneurones, located mainly in L7–S1, inhibit Q motoneurones within L5–L6. Other subgroups of interneurones excited from either Grac, Add, ABSm or G-S (see Table 1, cells nos. 10–14), should inhibit motoneuronal groups within the same and neighbouring segments so their axons might also project over a distance of several millimetres.

1. *Location*

In the previous experiments (Hultborn *et al.* 1971*b*) the approximate location of the Ia inhibitory interneurones was estimated from the position of the recording electrode. In spite of the unavoidable inaccuracy of such a technique the results consistently indicated that the interneurones mediating the Ia reciprocal inhibition of motoneurones are located within a semilunar area just dorsal and dorsomedial to the motor nuclei.

This conclusion has now been fully confirmed since the location of the somas of all stained interneurones fell within the previously indicated area. This is illustrated in Text-fig. 2 and Pl. 1. Text-fig. 2 shows the location of all interneurones viewed in transverse sections. The location of these interneurones is indicated with respect to the borders of motor nuclei, the extent of which (dotted) was established as an area including somas of all the cells of a size of motoneurones. These areas were determined for each spinal level at which an interneurone was found (from three sections) and superimposed in such a way that their dorsomedial borders fitted. Small differences in the general outline of the grey matter were ignored, and the most typical contour of the latter was chosen for the diagrams. The location of one of the cells of Text-fig. 2, cell no. 21, is illustrated separately in Pl. 1*A*.

TABLE 1. List of stained cells

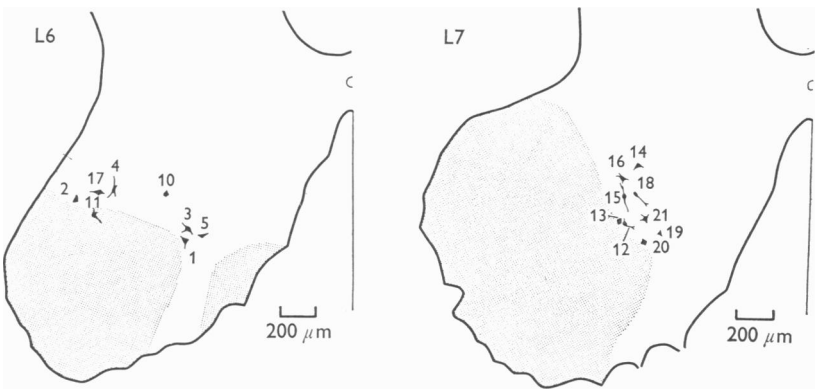
Unit no. 1	Gr. Ia input 2	Location segment 3	Plane of section 4	Soma size: long and short axes in μm* 5	No. of primary dendrites 6	Radius of dendritic projection in μm† 7	Diameter‡ of the axon in μm* 8	Axon length stained in μm† 9	Axonal projections to spinal funiculi 10	Axonal origin 11
1	Q	L5	Transverse	25 × 25	4	—	—	—		
2	Q	L6	Transverse	28 × 20	5	—	—	—		
3	Q	L6	Transverse	34 × 30	—	—	14·0	2350	Into ventral	Separate
4	Q	L6	Transverse	32 × 25	5	200	12·5	400	Towards ventral	Separate
5	Q	L6	Transverse	25 × 20	5	—	—	—	—	
6	Q	L6	Sagittal	33 × 25	5	240	9·5	390	Into lateral	Separate
7	Q	L6	Sagittal	25 × 20	4	—	—	—	—	
8	Q	L6	Sagittal	25 × 20	5	350	12·0	450	Towards lateral	?
9	Q	L7	Sagittal	30 × 15	5	350	12·0	300	Towards ventral	?
10	Grac	L6	Transverse	25 × 15	—	—	—	—	—	
11	Add	L6	Transverse	25 × 17	5	270	6·5	340	Into lateral	Base of dendr.
12	ABSm	L7	Transverse	30 × 30	3	255	7·5	200	Towards lateral	?
13	ABSm	L7	Transverse	25 × 17	—	—	—	—	—	
14	G-S	L7	Transverse	30 × 30	5	—	12·0	220	Towards ventral	Separate
15	St	L7	Transverse	25 × 20	4	310	8·0	540	Towards lateral	Base of dendr.
16	PB	L7	Transverse	25 × 25	5	320	7·5	660	Towards ventral	Separate
17	PBSt	L6	Transverse	33 × 20	5	350	—	—	—	
18	PBSt	L7	Transverse	30 × 20	—	—	—	—	—	
19	PBSt	L7	Transverse	20 × 20	—	—	—	—	—	
20	PBSt	L7	Transverse	30 × 20	5	—	—	—	—	
21	PBSt	L7	Transverse	30 × 17	4	375	9·0–12	1200	Towards lateral	Base of dendr.
22	PBSt	L7	Sagittal	27 × 20	5	250	9·5	820	Into ventral	Separate
23	PBSt	L7	Sagittal	40 × 20	5	550	10·0	1500	Into lateral	Separate

* Without correction for shrinkage.

† Without correction for shrinkage and only in a plane parallel or transverse to the plane of section.

‡ External diameter, judged from non-fluorescent space around the axons; means from several measurements.

The somas of the stained interneurones were found just outside the motor nuclei, generally some 100–300 μm from the nearest motoneurones in a given plane. Their location appears to be limited to a somewhat more narrow strip around the motor nuclei than indicated by Hultborn *et al.* (1971*b*). This may partly be due to the smaller sample of cells in the present study as compared with the previous one (seventeen neurones viewed in a transverse plane against fifty-one localized in L6 and L7 by Hultborn, Jankowska & Lindström, 1971*b*). However, the higher accuracy of determining the location of cells after staining is quite obvious, especially since the location of quite a number of cells in the previous study was estimated from extracellular records of spike potentials.

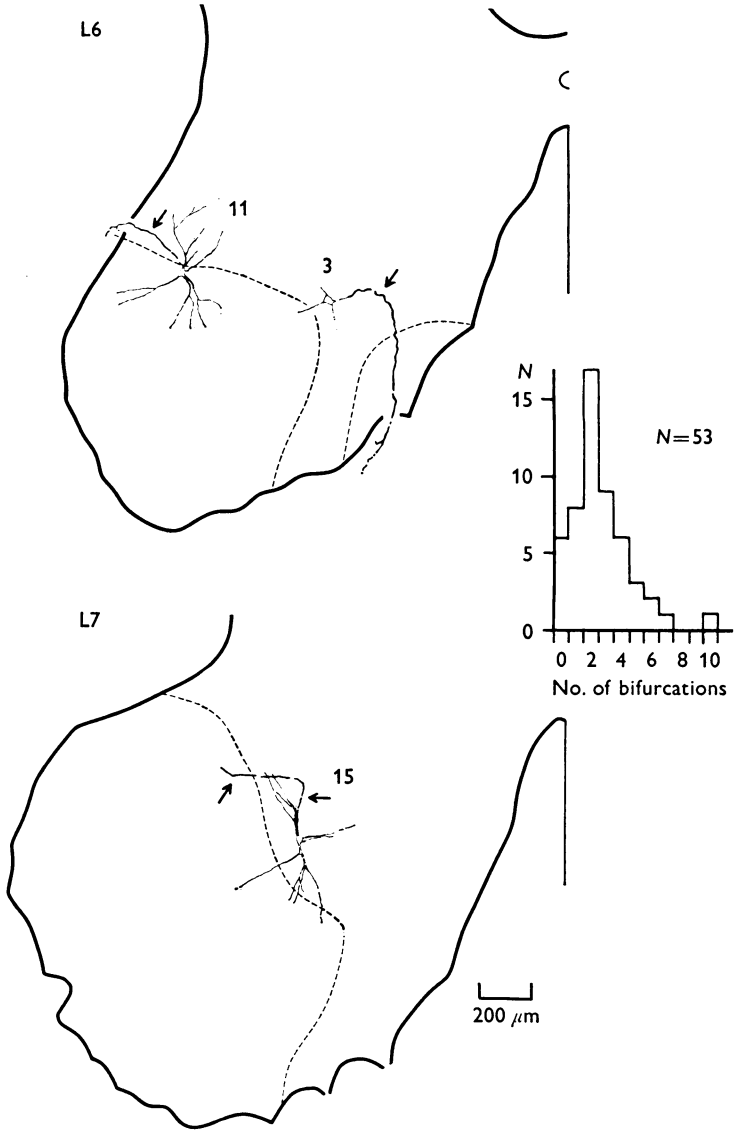


Text-fig. 2. Semi-schematic diagrams of the location of cell bodies of Ia inhibitory interneurones in L6 and L7 spinal segments. The dorsomedial borders of the motor nuclei (dotted) correspond to borders of the motor nuclei estimated for each interneurone. The contours of the grey matter correspond to the most typical ones in L6 and L7. The contours of the somas of the interneurones are traced from single sections. Numbers refer to the numbers of the cells in Table 1. Further details in the text.

The interneurones excited from a given nerve were found, as a rule, in the same spinal segment at which the afferents from this nerve entered the spinal cord (Hultborn *et al.* 1971*b*). This is indicated in column 2, Table 1. On the other hand there was no indication of a somatotopic organization in the transverse plane. Interneurones with the same Ia input were found in lateral as well as medial parts of the total area where they are located.

2. *Soma and dendrites*

Soma. Text-figs. 3, 4 and Pl. 2 show examples of cell bodies of stained interneurones after a reconstruction from all the sections containing their fragments. From these examples it can be seen that the size of the somas



Text-fig. 3. Reconstruction of axons and dendrites of two interneurones in L6 (nos. 3 and 11 in Table 1) and one interneurone in L7 (no. 15 in Table 1). Axons are indicated by arrows. In the case of cell no. 3 the dye was injected into the axon 1·5 mm caudal to the soma and diffused back to the cell body, which explains the poor staining of the dendrites. This axon ran in the white matter just ventral to the medial border of the ventral horn (as shown in Pl. 1*B*). The borders of the motor nuclei (dashed lines) were defined as for Text-fig. 2. Note the bifurcations of the axons just after entering the white matter. The histogram gives the number of bifurcations of fifty-three dendrites of well stained cells.

of the Ia interneurones is fairly uniform. The dimensions of individual cells are given in Table 1 (column 5), expressed as the length of two axes passing at right angles between the dendrites and through the nucleus or the centre of a cell body (cf. Schadé, 1964). On the average they were about 30×20 μm. In several of the cells, especially those lightly stained, the nucleus could be distinguished easily since it stained most heavily. The nucleus was round or slightly elongated with a diameter of about 10 μm.

Dendrites. In well stained cells the dendrites were fairly uniformly stained, and they could be traced until they tapered to a diameter of less than 1·0 μm. They seemed to end with a series of drop-like expansions connected with very thin bands (Pl. 2*C* and Pl. 3*A*). Whether this was really the end of the dendrites is impossible to decide with light microscopy. The dendrites, as well as the soma and the axon hillock, had an irregular granular contour but the resolution was not sufficient to reveal eventual spines.

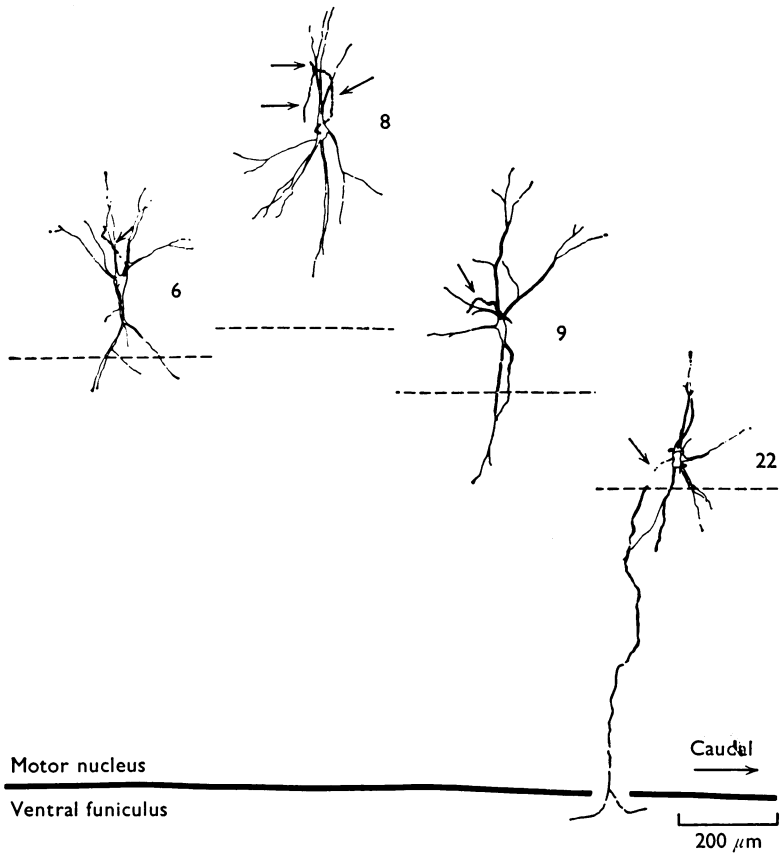
The general outline of the dendritic trees of a few interneurones is shown in Text-figs. 3, 4. They were reconstructed as a projection of all the dendrites on to one plane, transverse in Text-fig. 3 and sagittal in Text-fig. 4, the dendrites being traced over their whole visible length. These reconstructions are supplemented by photographic reconstructions of the proximal parts of the dendrites in Pl. 2. As seen from these examples and specified in column 6, Table 1, most of the cells had four or five primary dendrites which gave rise to only relatively small numbers of secondary and higher order dendrites. Taking into account the most satisfactorily stained cells the number of bifurcations has been calculated for fifty-three dendrites and is summarized in the histogram of Text-fig. 3. The total number of bifurcations per dendrite was between 0 and 10, but more than half of the dendrites dichotomized twice at most.

The dendrites extended over a considerable distance (cf. column 7 in Table 1). In the transverse plane they radiated without preferred directions, although the extension of the dendritic tree was usually larger dorsoventrally (about 600 μm) than mediolaterally (about 400 μm). The extension of dendrites was shortest (about 300 μm) in the rostrocaudal direction as was best seen from sagittal sections (Text-fig. 4). In nearly all cases the dendrites of the stained cells extended ventrally over the dorsal part of the motor nuclei, cell no. 8 in Text-fig. 4 being an exception. Dorsally they projected to the ventral part of the intermediate region.

3. *Axons*

The axons were differentiated from dendrites on the basis of the following properties: (1) a uniform diameter in contrast to the tapering of dendrites, (2) an initial narrow segment, (3) an envelope of nonfluorescent space

corresponding to the myelin sheath (see below), (4) a gradual weakening of the intensity of the fluorescence with the distance from the soma (Jankowska & Lindström, 1970, 1971), (5) a projection over considerably longer distances than of the dendrites and (6) a smooth contour and a winding trajectory (cf. Jankowska & Lindström, 1970). The latter feature was very characteristic for axons, though probably an artifact due to shrinkage during the histological procedures.



Text-fig. 4. Reconstruction in the sagittal plane of axons and dendrites of four interneurones, all stained in the same experiment. Continuous line indicates the border between the motor nuclei and the ventral funiculus, and the dashed lines indicate the dorsal border of the motor nuclei, defined as for Text-fig. 2. Other indications as in Text-fig. 3.

The axons were recognized in thirteen of twenty-three cells stained in the present series (column 9 in Table 1). Examples of the proximal parts of the axons are shown in Pl. 2. These were selected to show that the axons originated either directly from the perikaryon (*C*) or from one of the

primary dendrites (*A*, cf. Ramon y Cajal, 1909). Intermediate cases seem to be represented in *B* and *D*.

The narrow initial segment of the axon (less than 1·0 μm) and a subsequent increase in the diameter of the axis cylinder can best be seen in cell *D* of Pl. 2, in which the proximal part of the axon was found undamaged in one section. The axons seemed to attain their final diameter and to acquire the myelin sheath some 50–100 μm from the soma. The presence of the myelin was indicated by counterstaining with Luxol fast blue in some cases and by a nonfluorescent space around the fibres (Pl. 3*C*, *D*, *E*). A similar space can be seen around undoubtedly myelinated axons of motoneurones within ventral root filaments (Pl. 3 *F*) or of VSCT cells (Jankowska & Lindström, 1970). The diameters of the non-fluorescent space around the axis cylinders were taken as an approximate measure of external diameters of the fibres (cf. column 8, Table 1). The range of these values (6·5–14·0 μm, without correction for shrinkage) is in reasonably good agreement with the diameters expected from the conduction velocity of these fibres. This was estimated to be of the order of 80 m/sec (Jankowska & Roberts, 1972), which with the Hursh (1939) factor of 6 would correspond to a diameter of about 13 μm.

Funicular projections. Previous physiological studies (Hultborn *et al.* 1971*b*; Jankowska & Roberts, 1972*a*) have shown that the Ia inhibitory interneurones must be provided with relatively long axons, extending over one to three segments, and suggested that these axons may travel along the spinal cord within either the ventral or the lateral funiculi (Jankowska & Roberts, 1972*a*). In the present study axons could be followed for distances of 300–1500 μm (column 9 in Table 1), though in one exceptional case (cell no. 3), in which the dye was injected into the axon in the white matter, it was followed over 2 mm. All axons were found to travel in the direction of either the ventral or the lateral funiculi (column 10 in Table 1). The axons of five cells were traced to the white matter and even some distance within it. Interneurones located more laterally seemed to send their axons preferably to the lateral funiculus, while those located more medially aimed towards the ventral funiculus (Text-fig. 3). This was, however, not an absolute rule since interneurones located in close proximity (and with the same input) happened to send their axons in opposite directions, e.g. cells nos. 6 and 9 or 22 and 23 (column 10 in Table 1).

The axons projecting either laterally or ventrally crossed the grey matter just outside the motor nuclei or within their dorsal or medial boundaries. Those directed ventrally travelled parallel to a bundle of motor axons and reached the ventral funiculus where the motor axons crossed it. The axons did not always take the shortest way to the funiculi but sometimes made loops in different directions in their initial course. The five

axons which could be traced within the white matter ran rostro-caudally, parallel to the border of the gray matter. In all cases they were located in the deepest layers (cf. Pl. 1*B*), not more than 50–100 μm from the border of the gray matter. A funicular trajectory similar to that of Pl. 1*B* was also indicated for a number of Q interneurones by histological verification of sites from which they could be activated antidromically (E. Jankowska & W. J. Roberts, unpublished).

Axon collaterals. Axonal branching within the grey matter was observed only twice. One case is illustrated in Pl. 3*B* which shows the stem axon of a stained cell cut transversely on its way to the lateral funiculus and a thin collateral given off at a right angle. This collateral also seemed to be myelinated. As far as it could be followed it was directed caudally, dorsal to the motor nuclei. In the other case (cell no. 8 in Text-fig. 4), the two secondary branches had similar diameters and both were directed laterally. Unfortunately they could not be traced long after the bifurcation. Of the five axons which could be traced to the white matter, three bifurcated within the funiculus (cell nos. 3 and 11, Text-fig. 3 and cell no. 22, Pl. 2). One branch projected rostrally and the other caudally, and both were of about the same size as the parent axon.

DISCUSSION

The present study provides for the first time a morphological description of a functionally identified and homogeneous group of interneurones in the spinal cord, namely those mediating disynaptic inhibition of motoneurones from large muscle spindle Ia afferents (similar studies on Renshaw cells are now in progress (cf. Jankowska & Lindström, 1971 & van Keulen, 1971)). It is therefore of great interest to compare their morphology with the description of unidentified samples of spinal interneurones given in earlier anatomical studies (Ramon y Cajal, 1909; Ramon-Moliner, 1962; Testa, 1964; Scheibel & Scheibel, 1966, 1969, 1971; Szentágothai, 1967; Mannen, 1969; Gelfan, Kao & Ruchkin, 1970; Matsushita, 1969, 1970*a*, *b*). Of special importance is a consideration of the uniqueness of the features of the Ia inhibitory interneurones and of the extent to which these cells can be differentiated from other spinal interneurones on morphological grounds. The comparison is, however, complicated by the use in the different studies of various histological techniques and of animals of different ages. Furthermore the sampling of interneurones in these studies might have been biased by different criteria used to define interneurones and by the difficulty of impregnating smaller cells and their axons with the Golgi technique.

The Ia inhibitory interneurones stained in the present study were all fairly uniform in size. Their somas were of medium size (about 30×20 μm) like most interneurones in Rexed's lamina VII (Rexed, 1954). Compared with other functionally identified neurones in the ventral horn they were clearly smaller than the α-motoneurones, the cells of origin of the ventral spinocerebellar tract (Cooper & Sherrington, 1940; Jankowska & Lindström, 1970) and some of the commissural cells (unpublished observations), but somewhat larger than the Renshaw cells (Jankowska & Lindström, 1971). The γ-motoneurones appear to be of about the same size (Bryan, Trevino & Willis, 1972).

The long slender dendrites of the Ia inhibitory interneurones project in all directions in the transverse plane with extensions somewhat larger dorsoventrally than medio-laterally. Their extension along the longitudinal axis of the cord is only about half of that along the dorsoventral. According to the classification of Ramon-Moliner (1962), the Ia inhibitory interneurones would thus belong to cells with dendrites of the radiate type with a planar orientation. Scheibel & Scheibel (1966, 1969) claimed that most of the interneurones in lamina V through IX have dendritic systems characterized by maximal extension in the transverse and dorsoventral axis and little or none along the rostrocaudal axis, so their input might be limited to a mediolateral strip only 10–30 μm wide across the total cross-section of the cord. The rostro-caudal extension (over some 300 μm) of dendrites of the Ia inhibitory interneurones is clearly larger. Nor can the dendritic trees of these interneurones be compared with dendrites of cells, considered by Scheibels as exceptions, which had a longitudinal or a symmetrical orientation (cf. cells *a* and *b*, Fig. 4 in Scheibel & Scheibel, 1966). Only those characterized as 'with some degree of rostro-caudal dendrite orientation' (Scheibels, 1969, p. 176), for example, the dendritic tree of cell no. 2 in Fig. 9 in Scheibel & Scheibel (1966), might correspond to dendrites of the Ia inhibitory interneurones. In these comparisons, however, it must be kept in mind that the dendrites of cells described by Scheibels were drawn from single sections and that some of their branches might have extended outside the sections.

Matsushita (1970*a*) has classified interneurones in the spinal cord into four groups according to cell form and different features of their dendritic trees. The Ia inhibitory interneurones are rather difficult to fit with any of these groups. Most of them seem to have the characteristics of Matsushita's type I cells (with few loosely branched dendrites extending over about 300 μm), but some also resemble cells of other types.

Generally it appears that cells with dendritic trees similar to those of the Ia inhibitory interneurones can be found in different parts of the spinal cord. This is indicated not only by the above mentioned studies (Scheibel

& Scheibel, 1966, 1969; Matsushita, 1970*a*) but also by a comparison of a number of primary, secondary, and high-order dendrites and their length in a large sample of small and medium size interneurones in both the ventral and the dorsal horn (Gelfan *et al.* 1970). Thus even if the overall picture of the dendritic trees of the Ia inhibitory interneurones is similar, it does not differentiate them from other groups of interneurones.

The axons of properly stained cells were found to project to either the ventral or the lateral funiculi; if the sample was representative the Ia inhibitory interneurones can thus be classified as funicular neurones. Their funicular character was rather to be expected on the basis of both physiological and anatomical studies. The analysed types of Ia inhibitory interneurones should send their axons one to three segments rostrally or caudally to reach their target motoneurones (Hultborn *et al.* 1971*b*), and the axons of a majority of investigated Ia inhibitory interneurones could be stimulated in the white matter (Jankowska & Roberts, 1972*a*). Furthermore no short-axoned interneurones of Golgi type II have been found in the ventral horn (Ramon y Cajal, 1909; Testa, 1964; Scheibel & Scheibel, 1966, 1969, 1971).

All the projections were exclusively ipsilateral in agreement with the physiological studies which failed to disclose any contralateral effects of Ia inhibitory interneurones (Holmqvist, 1961). Interneurones in lamina VII, centrally in the ventral horn where most Ia inhibitory interneurones are localized have been reported by several authors to project to the ipsilateral ventral or lateral funiculi (Ramon y Cajal, 1909; Szentágothai, 1967; Scheibel & Scheibel, 1969; Matsushita, 1970*b*) although a considerable proportion of neurones with this location send their main axon or a significant branch across the mid line (Scheibel & Scheibel, 1971; Matsushita, 1970*b*). About one half of the stained cells sent their axons towards the lateral funiculus, and the other half to the ventral funiculus, showing that their trajectory may vary considerably. The different axonal projections seem not to be related to the functional subdivisions (the input and the destination) of Ia inhibitory interneurones as both PBST and Q activated interneurones were found to project to either the ventral or the lateral funiculi. More important seems to be a more medial or more lateral location of the somas of these interneurones according to the principle that the interneurones send their axons radially to the nearest funiculus (Ramon y Cajal, 1909). Thus the present results do not give positive support to the conclusion of Sterling & Kuypers (1968) and Rustioni, Kuypers & Holstege (1971) that the soma location and the axonal projection of proprioneurones terminating on different groups of motoneurones are related to the location of their target motoneurones. An overlap of fibres from the lateral and the ventral funiculi in a number of motor nuclei, including biceps and vasti, which are most relevant for the

comparison with the present results, was found also by Rustioni *et al.* (1971). However, this was not considered to invalidate their general postulate that interneurones aiming to the laterally situated motor nuclei send their axons via the lateral funiculus, while those terminating on medially situated motor nuclei project in the ventral funiculus.

Most funicular neurones in lamina VII of the ventral horn stained in Golgi studies were claimed to give rise to a large number of profusely branching initial collaterals, before joining the white matter (Szentágothai, 1967; Scheibel & Scheibel, 1969). In contrast, the Ia inhibitory interneurones only occasionally were found to give off a collateral or a branch within the ventral horn. Most of them did not bifurcate until they reached the white matter where they gave rise to one ascending and one descending branch. The paucity of early collaterals may be due partly to the weak staining of the axons. However, since some collaterals were stained it is quite possible that these interneurones usually send their axons directly to the white matter without giving off early collaterals.

The stem axons seem to run in the deepest layers of the funiculi. The results of physiological studies showed that they can project within a distance of some 20 mm and that at a level of the target motor nuclei the axon collaterals are given off at several levels, innervating a few millimetres' length of the nuclei (Jankowska & Roberts, 1972*a*). A cell of this type has been described by Mannen (1969, cell *a* in Figs. 3 and 4). According to Jankowska & Roberts (1972*a*) the collaterals might run first in parallel with the stem axons and only later change direction and enter the gray matter; within a proper motor nucleus the axon collaterals would be expected to branch profusely and to terminate on up to 20 % of the given population of motoneurones. In addition to motoneurones, some Ia interneurones should terminate on ventral spinocerebellar tract neurones (Gustafsson & Lindström, 1970) and on other Ia inhibitory interneurones (H. Hultborn & M. Santini, personal communication). The terminations on motoneurones would be mainly on the soma and the proximal parts of the dendrites (Burke, Fedina & Lundberg, 1971; Jankowska & Roberts, 1972*b*), though possibly more distally on some kinds of motoneurones (unpublished observation). A few terminal axon collaterals of slightly different length would be likely to terminate on a single motoneurone (Jankowska & Roberts, 1972*b*). This type of termination might correspond to the terminations of relatively large calibre collaterals given off by axons of short propriospinal neurones, which terminate in a dense, grape-like manner on the soma of a single or of a few closely neighbouring neurones (Szentágothai, 1964). Unfortunately the experiments in which these collaterals were disclosed were not designed for identification of their stem axons and cells of origin.

All the stained Ia inhibitory interneurones were found within a rather narrow zone in Rexed's lamina VII just dorsal and dorsomedial to the motor nuclei, only part of the region in which Sprague (1951) found most of the propriospinal neurones of the ventral horn. This region corresponds to one of the two areas outside the motor nuclei where massive termination of Ia afferent collaterals has been found (Szentágothai, 1967). The other area in the intermediate region corresponds to the location of another group of Ia activated interneurones which originally were supposed to mediate the Ia reciprocal inhibition (Eccles *et al.* 1956). In view of the arguments discussed by Hultborn *et al.* (1971*b*) it is unlikely, however, that they should participate in this reflex, and their function remains to be established (cf. Hongo *et al.* 1972).

The finding that the Ia inhibitory interneurones and the Renshaw cells (Thomas & Wilson, 1965; Jankowska & Lindström, 1971; van Keulen, 1971) are localized in two restricted zones in the ventral horn indicates that also for interneurones there is a geographical organization of the spinal cord. However very little is known to which extent other groups of cells are located within the same region as the Ia inhibitory interneurones. There seems to be hardly any overlap between the localization of these neurones and the Ia activated interneurones in the intermediate region (Hultborn *et al.* 1971*b*) or the Renshaw cells (Jankowska & Lindström, 1971). The latter are generally found more ventrally in lamina VII just where the motor axons leave the gray matter. Occasionally extracellular spike potentials of a Ia inhibitory interneurone and a Renshaw cell have been recorded simultaneously at the same electrode position, but the optimal position for recording spike activity of the Renshaw cells has always been at least 100 μm ventral to that of Ia inhibitory interneurones. It seems further well established that γ-motoneurones are localized in the motor nuclei (Eccles, Eccles, Iggo & Lundberg, 1960; Bryan *et al.* 1972). Some ventral spinocerebellar tract cells have been found in L5 within about the same region as the Ia inhibitory interneurones (S. Lindström & E. Schomburg, unpublished), although the majority of these cells are found more laterally in the ventral horn (Cooper & Sherrington, 1940; Burke, Lundberg & Weight, 1971; Jankowska & Lindström, 1970). However, the location of other kinds of interneurones, commissural cells, or ascending tract cells in lamina VII is not known well enough to exclude that some of them are intermixed with the Ia inhibitory interneurones.

In conclusion the Ia inhibitory interneurones seem to constitute a rather homogeneous group from the point of view of their location, size, and type of dendritic tree, while the axonal projections of individual cells may differ considerably. It appears that so far none of their features separately differentiates them from other types of interneurones, but, taken together,

these features might allow a tentative recognition of the Ia inhibitory interneurones on morphological grounds. Nevertheless, for a proper identification of this group of cells the physiological criteria still would be indispensable.

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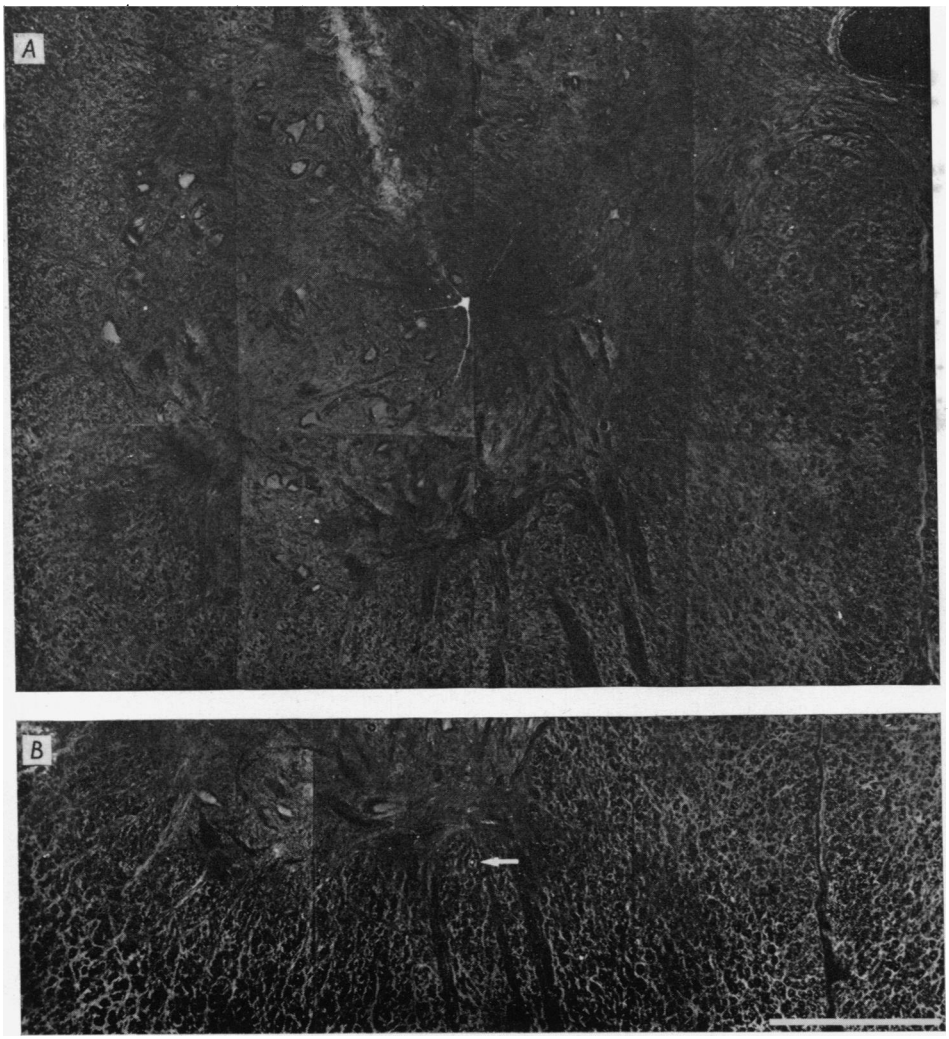
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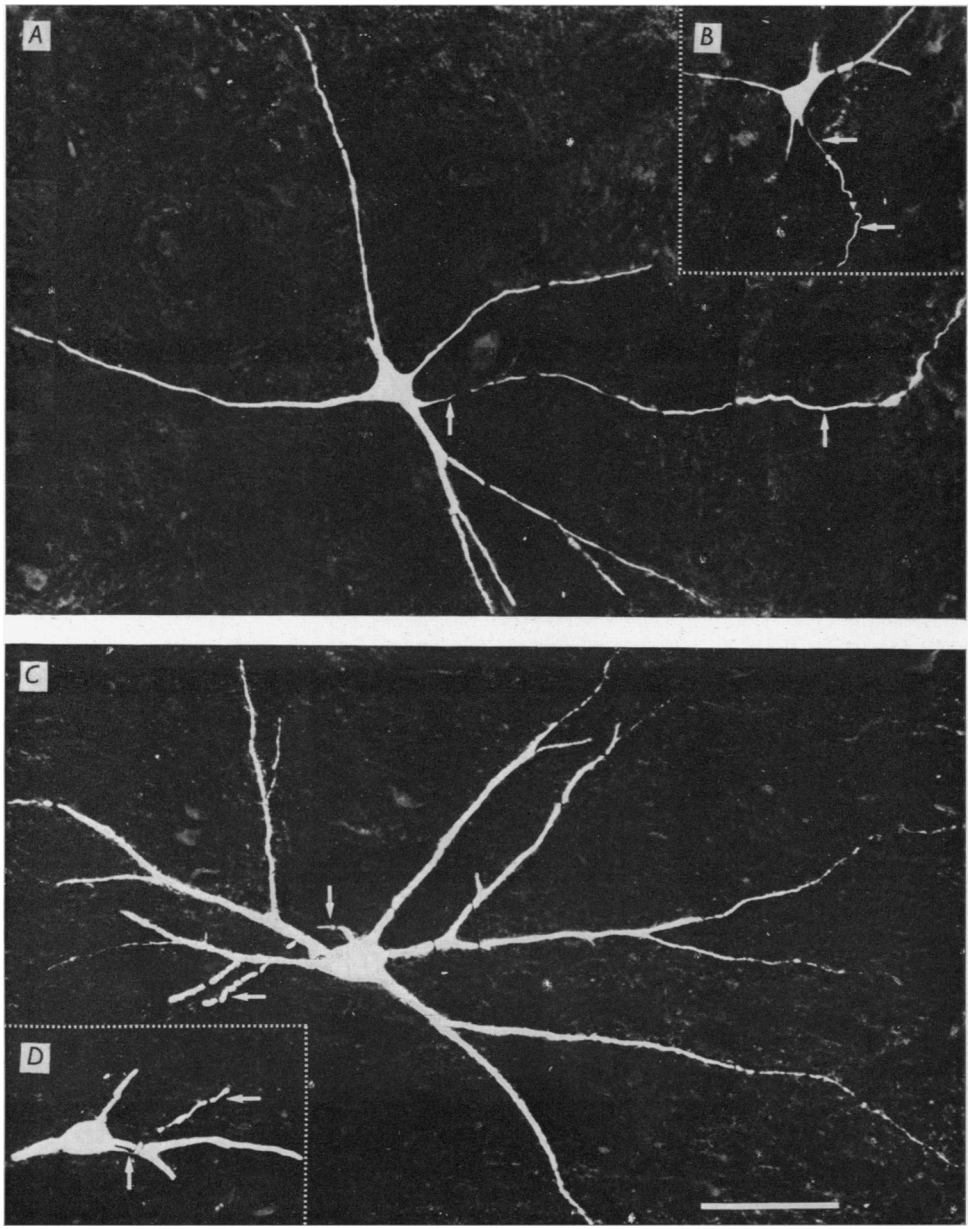
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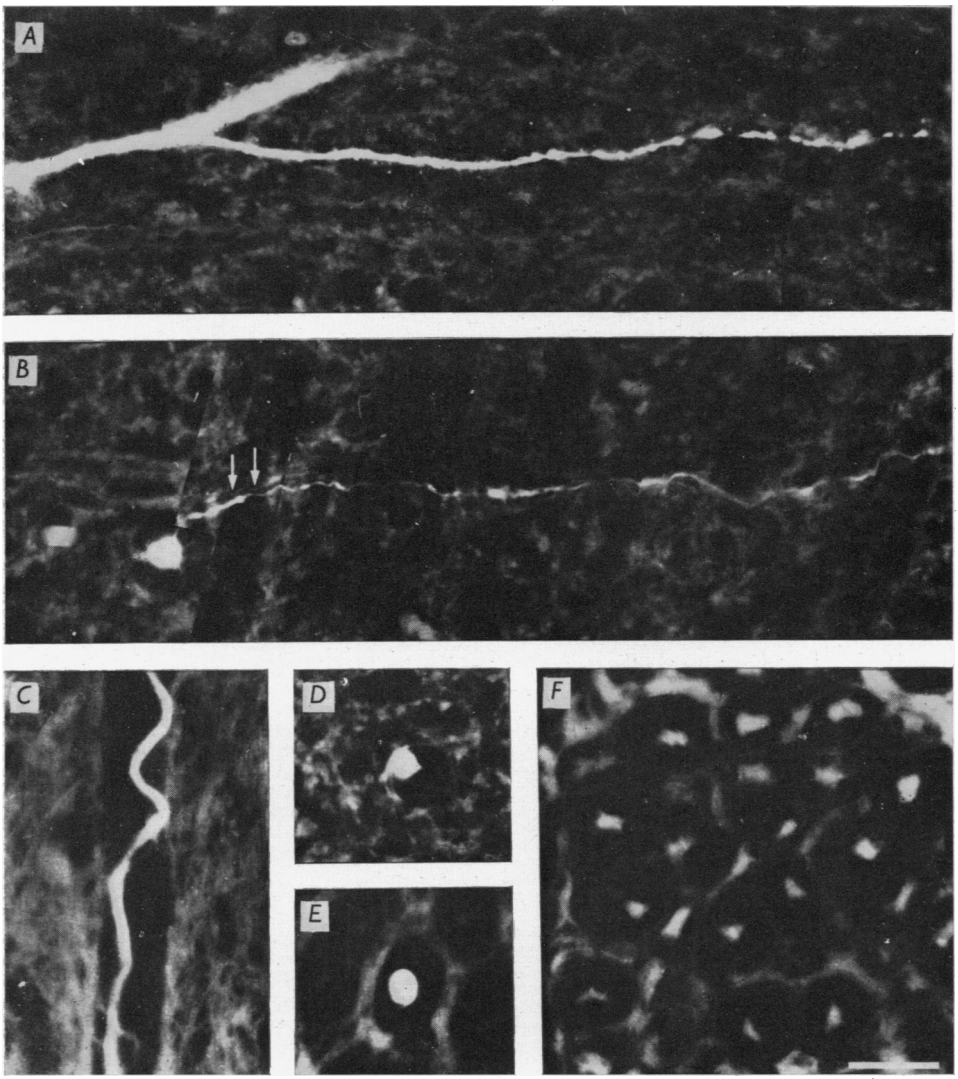
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EXPLANATION OF PLATES

PLATE 1

Photo-reconstructions of single transverse sections of the spinal cord. *A*, soma and initial part of dendrites of cell no. 21 (see Table 1) in the L7 segment. *B*, transversely cut axon (arrow) of cell no. 3 (Table 1) running in the ventral funiculus in L6. Compare the size of the interneurone with the size of the unstained motoneurones. Calibration bar in *B* is 500 μm for both *A* and *B*.

PLATE 2

Photo-reconstructions of somas, initial parts of axons, and dendrites of four cells viewed in either transverse (*A*, *B*) or sagittal (*C*, *D*) planes. A montage of photomicrographs from three (*B*, *D*), ten (*A*), and twenty (*C*) neighbouring sections of 15 μm. Axons are indicated by arrows. The more proximal arrows are at levels of the narrow initial segment. *A–D* are cells nos. 21, 16, 23 and 6, respectively. The pictures are oriented so that the right margins are dorsal and the lower margins are medial for *A* and *B* and caudal for *C* and *D*. Calibration bar in *C* is 100 μm for all cells.

PLATE 3

Photomicrographs of dendrites and axons. *A*, a segment of a dendrite of cell no. 23. *B*, transverse section of the axon of cell no. 23 with an axon collateral (arrows) given off within the gray matter. *C*, a segment of the axon of cell no. 3 in a section cut parallel to its trajectory. *D* and *E*, transverse sections through axons of cells nos. 23 and 3. *F*, transverse section through a ventral root filament (axis cylinders are seen due to their auto-fluorescence). Note the winding trajectory of the axon in *C* and the non-fluorescent space around the axis cylinders in *C–F*. Calibration bar in *F* is 20 μm for all pictures.