SPINAL CORD TERMINATIONS OF AFFERENT NERVE FIBRES FROM CAT HIND LIMB MUSCLES

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UNIVERSITY OF EDINBURGH

1981

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ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr. Alan Brown, for his stimulating comment, advice and encouragement throughout this project and for helpful collaboration during some of the experiments.

My sincere thanks are also due to Mr. R. Hume for his excellent assistance in the laboratory, during histochemical procedures and in the preparation of many of the illustrations; Miss C. Dow for technical help; Mr. C. Warwick for production of the colour prints; Mr. R. Clark for building or modifying various mechanical instruments and Mrs. T. McCubbin for her kind secretarial help and for typing this thesis.

Drs. D. Ensor, V. Molony, K. Rose, P. Snow and Mr. R. Noble provided valued discussion and companionship.

I would like to thank my wife, Dorothy, for her support and encouragement while I completed this work.

The projects were supported by M.R.C. grants to Dr. A. G. Brown, and by a M.R.C. Research Studentship. Some results have been published (see Appendix).

The Thesis was composed by my own efforts

SUMMARY

Intra-axonal and intra-cellular ionophoretic injection of horseradish peroxidase was used to establish the anatomy of single, functionally identified, muscle afferent fibres and α - motoneurones in the lumbosacral spinal cord of the cat. The Ia, Ib and group II axons from triceps surae had distinctive and characteristic central branching patterns and terminal zones. Near the segment of entry into the cord, Ia afferent fibres project to the intermediate region, to lamina VII, and to the appropriate motor nuclei; Ib afferent fibres terminate predominantly in the intermediate region; group II afferent fibres project to the dorsal horn, intermediate region and also to the motor nuclei.

The dendritic trees of α - motoneurones are described: they are more extensive than earlier results had indicated. By combining injections of single Ia fibres and single α - motoneurones in the same preparation, the contacts made by Ia afferents upon their target motoneurones were observed. Each Ia afferent makes several synaptic contacts on each motoneurone; the terminals are grouped in varied arrangements and are distributed to widespread regions of the soma-dendritic membrane.

PART 1 : INTRODUCTION

Spinal α - motoneurones receive convergent excitatory and inhibitory effects from muscle nerves. Information from stretch receptors and tendon organs is relayed via the Ia, Ib and group II afferent fibres (see Matthews, 1972). Motor control has been intensively studied (e.g. Sherrington, 1906; Creed, Denny Brown, Eccles, Liddell & Sherrington, 1932; Granit, 1966). The muscle receptors and their innervation are now well defined (e.g. Barker, 1974; Hunt, 1974), as are many of the descending influences on the motoneurone (see e.g. Porter & Phillips, 1977).

This work will describe the anatomy and organization of the central parts of Ia, Ib and group II afferent fibres, the structure of α - motoneurones and the synaptic connexions between Ia afferents and α - motoneurones. The anatomical approach is designed to bridge gaps in our understanding of some of the spinal reflex pathways. Much of the currently acceptable information stems from electrophysiological approaches (Matthews, 1972; McIntyre, 1974; Burke & Rudomin, 1977). Ever more sophisticated and sensitive techniques divulge the patterns of convergence on to motoneurones, interneurones and tract cells (Eccles & Lundberg, 1959; Lundberg, 1966, 1971; Oscarsson, 1973; Mendell & Henneman, 1971; Kirkwood & Sears, 1975; Burke & Rudomin, 1977). Neurone modelling (Rall, 1959, 1962a, b, 1964, 1970, 1977; Jack, Noble & Tsien, 1975) permits interpretation and prediction

of synaptic and other active events (Jack, Miller, Porter & Redman, 1971; Edwards, Redman & Walmsley, 1976a, b; Dodge & Cooley, 1973; Dodge, 1977). And yet these advances have to be correlated with anatomical information which underwent rapid advances a century ago (Deiters, 1865; Held, 1897; Ramon y Cajal, 1909; see also Liddell, 1960), became acceptable (Ramon y Cajal, 1934), but has changed little (but note for example the recent ultrastructural investigations of Conradi, 1969) in the ensuing years whilst electrophysiological developments generated exciting new data.

In the last 12 years the introduction of intracellular staining techniques (see Kater & Nicholson, 1973)combined the power of the microelectrode with an ability to impregnate selected neurones with demonstrable markers. Tritiated amino acids and the fluorescent dye Procion Yellow were used as intraneuronal stains - they could be used in microelectrodes for conventional recording, ejected into cells from the electrode tip, and visualized in subsequent histological analysis.

Snow, Rose & Brown (1976) and Jankowska, Rastad & Westman (1976) used horseradish peroxidase (HRP), which is used extensively for retrograde neuroanatomical pathway tracing (see e.g. Jones & Hartman, 1978; Brown, Fyffe, Noble, Rose & Snow, 1980), as an intracellular dye. HRP stains dendritic trees more fully than previous dyes (Brown, Rose & Snow, 1977a) and, importantly, also stains axons and axon collaterals. Physiologically identified

neurones stained with HRP can also be studied at the ultrastructural level (e.g. Jankowska et al., 1976; Cullheim & Kellerth, 1976, 1978).

Next, Brown et al. (1977b) developed procedures to inject HRP into single primary afferent axons in the spinal cord. The anatomy and organization of primary afferent fibres can now be defined (Brown, Rose & Snow, 1978; Brown, Fyffe, Rose & Snow, 1981; Brown, Fyffe, Heavner & Noble, 1979; Brown, Fyffe & Noble, 1980; Light & Perl, 1979a; Brown, 1981). Muscle afferent fibres will be described in this thesis, although some of the results have been published (Brown & Fyffe, 1978a, b, c, 1979, 1981; Fyffe, 1979).

The results will be related to electrophysiological data concerning the possible central connexions of muscle afferent fibres. Microelectrode recording of focal synaptic potentials (FSP's) (Eccles, Fatt, Landgren & Winsbury, 1954; Fu, Santini & Schomburg, 1974) and of post synaptic responses (Brock, Coombs & Eccles, 1952; Burke, 1967; Lucas & Willis, 1974; Lundberg & Weight, 1971) determine the regions and cells synaptically activated by individual or multiple muscle afferent fibres. Further details of intraspinal projections, of single axons, are obtained by antidromic microstimulation (Fu & Santini, 1974; see Wall, McCulloch, Lettvin & Pitts, 1956, and Jankowska & Roberts, 1972a) and by spike triggered averaging (Mendell & Henneman, 1971; Brown & Tapper, 1971; Watt, Stauffer, Taylor, Reinking & Stuart,

1976; Munson & Sypert, 1979b; Kirkwood & Sears, 1975, 1976).

Anatomical material is difficult to interpret because the stained neurones or fibres are unidentified (Szentagothai, 1967; Scheibel & Scheibel, 1969; Rethelyi & Szentagothai, 1973; Sprague, 1958; Sprague & Ha, 1964) and thereby interpretation rests on various assumptions (e.g. Iles, 1976). Even the 'model neurone', the α - motoneurone, for which a great number of assumptions are made, is surrounded by problems because some structural features - dendritic length, tapering and even the number of dendrites - show variance depending on the staining method used (Lux, Schubert & Kreutzfeld, 1970; Aitken & Bridger, 1960; Barrett & Crill, 1974a). This must be resolved to aid interpretation of models (Rall, 1970) and other electrophysiological information (see Jack, Noble & Tsien, 1975). Intracellular staining of α - motoneurones with HRP will also help to answer questions regarding the spatial distribution of afferent synapses - revealed and identified following intra-axonal injection of single Ia afferents with HRP - on the motoneurone surface (cf. Wyckoff & Young, 1956; Conradi, 1969; Koziol & Tuckwell, 1978; Jack et al., 1971; Eccles, 1964; Redman, 1976).

PART 2 : METHODS

Preparation of the animal. The data on muscle afferent fibres and their central connexions were obtained from adult cats whose weights ranged from 1.9 - 3.2kg. Anaesthesia was induced with 2 - 4% halothane in an oxygen/nitrous oxide gaseous mixture. Chloralose (70mg/kg) was administered intravenously and halothane anaesthesia was discontinued as the chloralose took effect. All animals were paralysed with gallamine triethiodide (Flaxedil) and artificially respired. Throughout the experiment carotid arterial blood pressure and end-tidal CO_2 levels were monitored and maintained at physiological levels. Body temperature was measured with a rectal probe linked to a homeothermic blanket control and kept at 37 - 39°C. The level of anaesthesia was frequently checked by referral to the continuous blood pressure and end tidal CO_2 records and by checking the state of dilation of the pupils. Occasionally Flaxedil paralysis was allowed to wear off and responses to squeeze and pinch of the feet were observed. If necessary, additional chloralose was given (up to 100mg) but this requirement was rare in experiments lasting less than 15 - 18 hours, which was the usual duration.

The nerves to medial gastrocnemius (Mg) and lateral gastrocnemius with soleus (Lg - S) muscle and the posterior tibial (PT) nerve were exposed in both hind limbs. A lumbosacral laminectomy exposed spinal cord segments L_2 - S_2; the dura over these segments was

incised and reflected from the surface of the cord.

The animal was then transferred to an experimental frame and held rigidly by means of a stereotaxic head holder, spinal clamps on thoracic and L_1 - L_2 vertebrae and hip pins clamping the pelvic girdle.

Good stability was critical for the success of intracellular or intra-axonal staining attempts. As mentioned, Flaxedil immobilisation was used; a bilateral pneumothorax (tubes had inflatable bags on the ends to lessen moisture loss) was also performed. General stability was also increased by applying traction to the cord between the hip clamps and the L_1 - L_2 vertebral clamps. Finally, the bladder was catheterized and kept empty.

Electrophysiological methods. The triceps surae muscle nerves in both hind limbs were mounted in continuity on bipolar silver-silver chloride or platinum bipolar electrodes and linked to isolated stimulators for peripheral excitation of afferent or motor axons. A silver wire reference electrode inserted into the back muscles served to earth the preparation. The exposed spinal cord and back muscles and the peripheral nerves were covered in liquid paraffin at 37°C.

A monopolar silver ball electrode placed near the dorsal root entry zones of segments L_6 - S_1 monitored the ingoing afferent volleys and associated cord dorsum potentials. Normally the maximal Group I afferent volley was recorded in L_7 segment - initial microelectrode tracks

were always made in this area. Cord dorsum potentials were amplified via a Tektronix 122 preamplifier and displayed on a Telequipment DM64 oscilloscope. HRP filled microelectrodes were coupled to a WP1 M701 electrometer which allowed simultaneous signal recording and current passage and measurement. DC and high gain AC records from the microelectrode were displayed on a Tektronix 5113 oscilloscope. Microelectrodes were mounted on a stepping motor controlled manipulator (Clark & Ramsey, 1975) attached to an arc which allowed easy alteration of electrode tracking angles.

HRP electrodes for intra-axonal and intracellular injections. These were made from 1.5 or 2.0mm outside diameter capillary tubing which contained a single glass fibre. The tubing is now obtainable from Clark Electromedical Instruments (Reading, U.K.) but in the earlier stages of this work the fibres were inserted individually in the laboratory and glued in place at each end of a tube. Microelectrodes were pulled on a vertical electrode puller built by Mr. R. Clark.

HRP (Sigma type VI) was dissolved in 0.1M Tris/HCl containing 0.2M KCl with care being taken that no particulate matter remained in the solution. HRP concentrations ranged from 4 - 9%, and the volume available for each experiment was about 0.1ml. The HRP solution was prepared freshly for each experiment, and the small volume available required that the same solution be passed from electrode to electrode through

the experiment. Each electrode was filled via a very fine polythene tube inserted as far into the shank of the electrode as possible. The internal fibre meant that filling was complete in less than one minute or so although small bubbles frequently appeared near the tip. These were, if possible, removed by inserting a fine cat's whisker into the electrode or by gentle tapping.

The electrodes initially had tips of less than 1 μm diameter and resistances of 100 - 250MΩ. They were then bevelled - in early experiments using the method of Barrett & Whitlock (1973) and in the later series by the procedures described by Brown & Flaming (1974). The latter method was carried out on a Narishige EG - 5 microgrinder with aluminium oxide particles embedded on a polyurethane coated glass disc providing the grinding surface. This system allowed the electrode resistance to be measured during the bevelling process. For experimental use, electrodes with tip diameters of around 1 μm and resistances of 25 - 60MΩ were selected. It must be emphasised here that the selection of a particular electrode on the basis of preliminary observations on its resistance or tip characteristics often bore little relation to its performance in the spinal cord of the experimental animal. The test of a 'good' electrode for intra-axonal staining was its ability to impale single axons, cause minimal damage in doing so, maintain a stable penetration for up to 30 minutes and pass 10 - 20nA of current for that period without blocking or disrupting

the state of the penetration.

Recording and injection of primary afferent axons and α - motoneurones. Electrode tracks were made close to the region where dorsal root fibres entered the spinal cord. The location was selected, after recording the afferent volleys, to maximise the chances of obtaining triceps surae afferent fibres. Tracks were made to a depth of 1000 μm and usually with the electrode tip oriented at 10° laterally. The resistance of the electrode was monitored continuously during tracking by passing a 1V, 100 μsec pulse through the electrode test input of the electrometer once every sweep (600msec). This was essential to give indication of any blocking of the electrode tip, which would subsequently hinder or even prevent ionophoretic injection of the HRP. Once electrode resistance increased above about 60 - 80MΩ it was impossible to pass more than 3 - 4nA of current before blocking the amplifier.

Some records from single primary afferent fibres and spinal neurones are shown in Fig. 1. Impalement of an axon was signalled by abrupt appearance of 40 - 70mV membrane potential and action potentials of at least 50 - 80mV. Occasionally, extra-axonal recordings were made - these were characterized by 20 - 40mV positive action potentials, with little or no change in resting potential.

If the impaled axon innervated the triceps surae muscles, the peripheral conduction time and threshold to

electrical stimulation of the appropriate muscle nerve were recorded. The distance between the peripheral stimulating electrodes and the L_7 dorsal root entry zone was measured at the beginning of the experiment to permit estimation of axonal conduction velocity. The actual distance to the recording site was measured at the end of the experiment. Muscle afferent fibres were defined physiologically as follows: Ia fibres had conduction velocities greater than $75m.sec^{-1}$; they had a regular (8 - 20Hz) ongoing discharge (the hind limbs were extended after the feet were cemented to wooden blocks for stability and rigidity) when recorded, and were excited by very light stretch of the muscle. The stretch was elicited by pulling the tendon with forceps, or manually flexing the ankle joint. Ia fibres were also sensitive to light tapping on the muscle or tendon. The fibres were excited electrically from the muscle nerve and could not be orthodromically fired from any other muscle nerve. In a few experiments the muscle was directly stimulated via bipolar silver-silver chloride electrodes and in some cases this reduced the Ia firing rate by unloading the spindle. More often however the resultant twitch was sufficient to dislodge the intra-axonal electrode. Furthermore some fibres were firing at low rate and it was difficult to determine rapidly whether they exhibited a pause in firing.

Ib fibres had conduction velocities greater than $75m.sec^{-1}$ and responded at low threshold to electrical

stimulation of a muscle nerve. There was no ongoing activity in these fibres but they were excited by manual flexion of the ankle joint or by pulling on the Mg or Lg - S tendons. Direct electrical stimulation of muscle was an inconclusive aid because of the danger of directly exciting the Ib afferent fibre.

Muscle spindle Group II afferent fibres had conduction velocities less than 65m.sec^{-1} and higher threshold to stimulation of the muscle nerve than Group I fibres. They had regular ongoing discharges and were excited by stretching the muscle (pull on Lg - S tendon or flexion of the ankle joint).

Clearly then the basis for fibre identification is dependent mainly on conduction velocity measurements and the response to subjectively graded muscle stretch. These parameters, and the difficulties due to constraints on the time available and the inability to evoke nerve induced muscle contraction are discussed in Part 8.

Injection of HRP into an impaled single axon was only commenced after the tentative identification procedures outlined above, and providing that the resting potential was stable at more than 40 - 50 mV negative, and action potentials had amplitudes greater than 50mV. The current pulses were 10 - 20nA depolarizing (electrode tip positive) square wave pulses of 450msec applied once every 600msec. Current amplitudes of 10 - 20nA were preferable to give adequate staining, and injections lasted for 10 - 30 minutes. Resting membrane potential,

evoked action potentials and electrode resistance were continuously monitored during this period. Injections proceded so long as there was less than 20 - 30mV gradual shift in resting potential and action potential amplitude.

An additional indication of the intra-axonal placement of the current passing microelectrode was the presence of axon firing at the ON-phase of the depolarizing current pulse. The threshold to this direct stimulation was generally 0.5 - 2.0nA (450msec duration pulse every 600msec) and sometimes several spikes were evoked. Spikes could also be evoked after the end of a hyperpolarizing pulse passed through the electrode - however this current polarity is inneffectual for ionophoretically injecting HRP.

Penetration of α - motoneurones was signalled by large (60 - 80mV) resting potential and action potential amplitudes of 60 - 90mV. Orthodromic and antidromic action potentials could be evoked by nerve stimulation or sudden stretches of the appropriate muscle. Identification was based on axonal conduction velocity ($> 65 m.sec^{-1}$) and antidromic invasion from any one of the stimulated muscle nerves. Electrodes had resistances of 15 - 30MΩ when filled with HRP. Currents of 10 - 20nA for 5 - 10 minutes were usually sufficient to stain a motoneurone and its dendritic tree. The threshold of α - motoneurones to intracellularly injected current was usually higher than for axons, at about 5 - 10nA. At the HRP injection currents used (10 - 20nA) repetitive

firing usually resulted throughout the period of depolarization (see also Granit, Kernell & Shortess, 1963; Granit, Kernell & Smith, 1963). Action potential amplitudes during the current pulse were reduced compared to activity recorded during the off-phase between pulses, when resting potential and peripherally evoked action potentials were monitored.

At the end of the injection period (of both axons and α - motoneurones), current was switched off and the electrode left in situ for up to about 3 minutes. During this time the physiological features often recovered - action potentials sometimes doubled in size although seldom did they return to pre-injection levels. Further electrophysiological characterization could be done at this stage. The spike size and resting potential were measured as the electrode was withdrawn. Not every injection was terminated in a satisfactory fashion however, Problems arose when there was an abrupt change in resting potential or spike size. Some units were suddenly 'lost' or deteriorated very rapidly after penetration. In these cases the injection was terminated and the electrode withdrawn. The other major electrophysiological problem was electrode blocking. This seemed to occur due to a combination of tip clogging as the electrode penetrated through the nervous tissue, and blocking from within caused by particulate matter in the HRP solution. As resistance increased it became more and more difficult to pass sufficient current to eject enough HRP to stain

primary afferent axons or motoneurones. Occasionally electrodes could be cleared by reversing the current polarity, but if current could not be maintained above about 8 - 10nA the probability of successful injections was low. In fact these problems are related to an 'electrodes for courses' phenomenon which becomes apparent during intracellular recording and staining experiments. HRP can be injected into small neurones using very fine tipped, high impedance electrodes and low intensity (0.5 - 2.0nA) currents (see Light & Perl, 1979b). Thus the criterion for 'good' electrodes varies from preparation to preparation. To fill an axon and its collateral branches generally requires more HRP staining than to stain a small cell body and its dendrites. Larger neurones present fewer problems because stability is better, coarser electrodes can be used, and larger currents attained; staining quality can more reliably be predicted during the electrophysiological part of the experiment.

If an HRP filled microelectrode exhibited a marked decrease in resistance during tracking, as sometimes occurred when searching for motoneurones, it was immediately withdrawn from the cord. HRP leakage, in the white or grey matter resulted in extensive labelling of many neuronal and non-neuronal elements. The experiments were designed to allow staining of 1 - 2 afferent fibres on each side of the cord and/or up to about 5 motoneurones during that period. After the first injection (axon or

cell) about 8 - 10 hours was available to record and inject other neurones. This time limit arose from the early observations of A.G. Brown, P.K. Rose & P.J. Snow that staining of neurones was less intense if they were injected more than 15 - 18 hours before perfusion. Intra-axonal injections seem to be less affected by this possible metabolism of HRP than intracellular applications. A quantitative study of HRP labelling characteristics would of course be complicated by the many variables inherent in each injection routine.

At the end of the experiment, empty marker electrodes were inserted vertically into the mid-line at the level of each of the injection sites. Only one electrode would be inserted if injections were made bilaterally at any particular location. These markers aided identification of spinal cord blocks removed for HRP histochemistry, and in locating stained cells or axonal injection sites.

HRP Processing

Fixation of the spinal cord. At least one hour after the final injection of HRP, but no later than 12 hours, each cat was given 10,000 u. heparin intravenously. The preparation was perfused through the descending aorta with 200 - 500 mls warm (37°C) saline then with 450 - 500 mls cold (4°C) Karnovsky's fixative (Karnovsky, 1966; Graybiel & Devor, 1974; Snow et al., 1976). The fixative contained 1% paraformaldehyde and 2%

glutaraldehyde in 0.1M phosphate buffer pH7.2. The perfusion pressure was about 1m H_2O and perfusion lasted 5 - 10 minutes. The appropriate blocks of spinal cord were quickly excised and stored overnight at 4°C.

Histological methods. After overnight fixation the spinal cord blocks were rinsed for 2 - 4 hours in 0.1M phosphate buffer pH7.6 containing 5 - 10% sucrose. Blocks were cut at 100 μm on a freezing microtome after removal of all marker electrodes. Transverse or sagittal sections were collected serially in trays containing 0.1M phosphate buffer pH7.6.

In the present work two processes were used for the demonstration of HRP. Most of the axons described in Part 3 were processed according to Graybiel & Devor (1974) using diaminobenzidine (DAB) as enzyme substrate. This carcinogenic substances was used with extreme caution both in processing sections and when utensils were cleaned. DAB became virtually unobtainable for a while in mid-1977 and the Hanker-Yates method was adopted (see Hanker, Yates, Metz & Rustioni, 1977) for most of the material described in Parts 4 - 7.

A) The DAB method. Sections were incubated, with gentle agitation, for 20 - 30 minutes at 25°C in the following freshly made solution:

0.33% w/v DAB

0.1 % v/v H_2O_2 in 0.05M Tris/HCl buffer pH7.4.

The reaction was terminated by transferring the sections back to trays containing phosphate buffer. All

DAB containing vessels were deactivated by immersion in sodium hypochlorite solution.

B) The Hanker-Yates method. This method was originally used (Hanker et al., 1977) in the demonstration of retrogradely transported HRP. The reactants are supposedly less carcinogenic than DAB. Nevertheless, similar precautions in handling and cleaning utensils were observed. The method was rather capricious when initially used to demonstrate intracellularly or intra-axonally injected HRP. Freshness and reaction temperature were found to be critical for this method. Sections were incubated in a mixture of p-phenylenediamine (PPD) and p-catechol (PC) in the Tris buffer/H_2O_2 mixture used in the DAB technique. Substrate concentrations were 0.4% w/v PC and 0.2% w/v for PPD. The solution was filtered after these substances were dissolved, H_2O_2 was added and the incubation of sections carried out at 25^{o}C for 10 minutes. Reacted sections were transferred back to phosphate buffer.

Subsequently, in both methods, sections were rinsed in distilled water, mounted serially on gelatin coated slides and allowed to dry at room temperature. The slides were kept in formalin vapour overnight and dehydrated in an ascending series of alcohols and cleared in xylene. Coverslips were mounted over Gurr UV-inert mounting medium.

Interpretation and analysis of stained neurones.

Light microscope examination was performed on a microscope equipped with a Camera Lucida drawing tube. Profiles were drawn at magnifications of x100 - x1000. Serial sections were reconstructed in two dimensions by matching cut profiles and tissue landmarks on adjacent sections. Photomicrographs were produced from Agfa Ortho 40 film. Colour prints used Kodak ektachrome 50.

After initial light examination, some selected sections of interest were subsequently counterstained in order to define terminal zones and possible target neurones. Coverslips were removed under xylene, sections rehydrated and then stained in 0.1% cresyl violet or in 0.1% methylene green. These sections were re-coverslipped and re-examined. If anything, these procedures enhanced the appearance of HRP filled profiles by further clearing the background of the section.

It is difficult to compare the quality of one HRP labelled cell or axon with another because of injection and/or morphological variability. In the present work the Hanker-Yates substrate seems to be more effective although little difference in sensitivity was noted. The advantage of Hanker-Yates processing is mainly that it results in much less background staining of, for example, vascular elements containing endogenous peroxidase activity. Cobalt intensification (Adams, 1977) was rather ineffective when used occasionally in this study. Perhaps the intraneuronal concentration of HRP following

intracellular injection is already sufficient for maximal reaction. In retrograde tracing studies much less HRP is available at the soma and cobalt intensification increases the sensitivity of the method in detecting labelled cells. There are of course other recent sensitive and selective methods for demonstrating the presence of HRP including the methods of Mesulam (1978) using tetramethylbenzidine as enzyme substrate and the recent development of Hayes & Rustioni (1980) using ^{3}H - apo - HRP which can be demonstrated autoradiographically. Clearly all the procedures have value in some circumstances. The main requirement is for reasonable sensitivity and specificity, and the Hanker-Yates reagents meet these requirements adequately for intracellular demonstration purposes.

Some comments on the appearance of HRP stained neurones is given (see also Figs. 2 - 4), so that the Results sections of Parts 3 - 7 may concentrate on overall anatomical features of the relevant stained cells or axons.

The most important point is that a single intra-axonal or intrasomal injection of HRP labels only one neurone and its axonal or dendritic branches. There is thus no difficulty in identifying profiles on adjacent sections: the reconstruction problem lies in matching up cut branches in serial sections. When multiple injections are performed in an experiment the injection sites are separated so that there is no overlap between the stained

axons or cells. Of course in Part 7 the object was to demonstrate contacts between a primary afferent axon and a spinal cord cell so these neurones were injected at the same level of the spinal cord. Occasionally, following a single injection, more than one neuronal element was located (see Fig. 2). Even if one profile was evidently more densely stained than the other, the material was discarded. There should be little extracellular deposit observable around injected cells. If there was, provided there had been no obvious pick up by adjacent neurones and it did not obscure dendritic origins, etc., then that neurone could be analysed. The question of interneuronal transport of HRP did not arise in these studies except in circumstances where tissue damage and HRP spillage were apparent. Following intra-axonal injection, the site of HRP injection could sometimes be identified by a small amount of leakage around the penetration point. This was not sufficient to lable other fibres in the vicinity. If for some reason excess HRP leaked from the electrode tip, the results were devastating with respect to single neurone analysis. Such leakage produced a Golgi-like staining of many fibres, cell bodies, dendrites, glial and vascular elements. In summary, all the subsequent analyses refer to single physiologically identified fibres or cells stained by single injections of HRP.

Intracellularly injected HRP resulted in a dense uniform staining of cell body, dendrites, dendritic

appendages, axon and axon collaterals (see Part 6). Black and white photographic reproduction of stained neurones was complicated because the intense contrast between the soma and the surrounding tissue generated a haze effect around the cell. For this reason some colour micrographs are included in this work. Dendrites were easily distinguishable from axons by virtue of their branching patterns, orientation, surface variations and terminations. In all cases dendrites were reconstructable back to their origin at the soma although it is likely that some profiles were lost, or confused, on sections distant from the one containing the cell body. The intensity, and rapidity, with which HRP labels dendritic trees makes one confident that this method is capable of detecting the full extent of these dendrites. Perhaps electron microscopical investigations of the presumed dendritic terminations would provide an answer to this, and also throw some light on the nature of these end points. In some cases cell damage was evident - the soma was swollen and distorted as were the proximal dendrites. Generally however if the physiological features monitored during the injection (resting potential, action potential amplitude, response to depolarizing current pulses, etc.) maintained reasonable condition, then the neurone would appear in good shape. More common were problems associated with, for some reason, a failure to inject any (or enough) HRP or with complications arising from leakage of HRP or damage to blood vessels, particularly

likely if several tracks were made to impale any neurone. Blood cells were stained because of their endogenous peroxidase activity. If they were aggregated in the region of the HRP labelled neurone, they sometimes obscured the neuroanatomical features.

In the present study most of the stained cells were motoneurones whose axons exited the cord via the ventral roots. HRP can label funicular and projecting axons for considerable distances (up to 2.0cm, see Brown et al., 1977a) following intrasomal application, and infiltrate the collateral branches arising over that portion of the axon. HRP injected into axons within a few hundred μm from the soma (e.g. into motor axons coursing through the ventral horn towards the ventral roots) stains the axon and axon collaterals and also can infiltrate the cell body to provide fainter staining of the soma and proximal dendrites (see Fig. 3). Primary afferent axons were injected at a point in their course soon after entering the spinal cord. The injection sites are at a distance from the cell body (in the dorsal root ganglion) and from the eventual termination of the axon, so only a very small proportion of the injected axon is visualized. Axonal injection sites can sometimes be detected in the histological material; otherwise the injection location is indicated by the appropriate marker electrode. HRP migrates bi-directionally from the injection site. In many cases the axon can be traced back into the dorsal roots although the extent of filling of the peripheral

axon was not determined. Filled axons in the white matter have a smooth outline and show little evidence of tapering. Collaterals (see Fig. 4) arise at intervals, being issued almost at right angles from the parent axon. They are much thinner than the main branch and are constricted for their first few μm. The extent of myelination of the collateral branches cannot be definitely determined because HRP fills only the axon cylinder.

Minimal and maximal rostro-caudal extents of the stained parts of axons ranged from 5 - 12mm with, on average, about 1cm being traced. Staining gradually faded towards the extremes. Collaterals were generally better stained the nearer they were to the injection site. Consequently only these collaterals were suitable for detailed reconstruction and analysis of their terminal patterns. The well stained collaterals ramified in different fashions and generated very fine (< 1.0 μm diameter) terminal branches carrying boutons 'en passant' and 'terminaux'. These smooth contoured, 1 - 5 μm diameter swellings were not confused with axonal bending which under some circumstances presents a beaded appearance. Importantly, primary afferent 'boutons' as defined in light microscopic study of HRP labelled primary afferent axons have been recently confirmed by electron microscopy to contain synaptic specializations and make synaptic connexions with post-synaptic elements (Brown, Fyffe & Maxwell, 1981). Definition of

'contacts' between two HRP filled neurones is discussed more fully in Part 7.

It is perhaps worth emphasising that reliable staining of primary afferent axons to the level of well-defined boutons is more difficult technically than staining neuronal dendritic trees. The success rate for injections was not more than 20 - 50%, particularly when collateral branches were long (e.g. > 1mm). Even for well-stained axons, only 2 - 4 collaterals were usually adequately filled - collaterals given off further from the injection site showed only the initial part of their trajectory.

While, hopefully, the clarity of the histological material defends itself, some of the major advantages of HRP as an intraneuronal stain will be listed here:

i) Single physiologically identified neurones can be studied - at the light level and with the electron microscope for ultrastructural details.

ii) HRP labels soma, dendrites, axon, axon collaterals and boutons.

iii) The material can be analysed under ordinary light microscopy. Stained profiles have well defined edges making, for example, diameter measurements more reliable than for fluorescent dyes.

iv) Fading is not such an acute problem as with fluorescent dyes. Material kept for over 3 years after processing with Hanker - Yates reagent retains almost all

important detail. Cells and axons injected with HRP and processed with DAB have tended to fade although the time course is slow - several years.

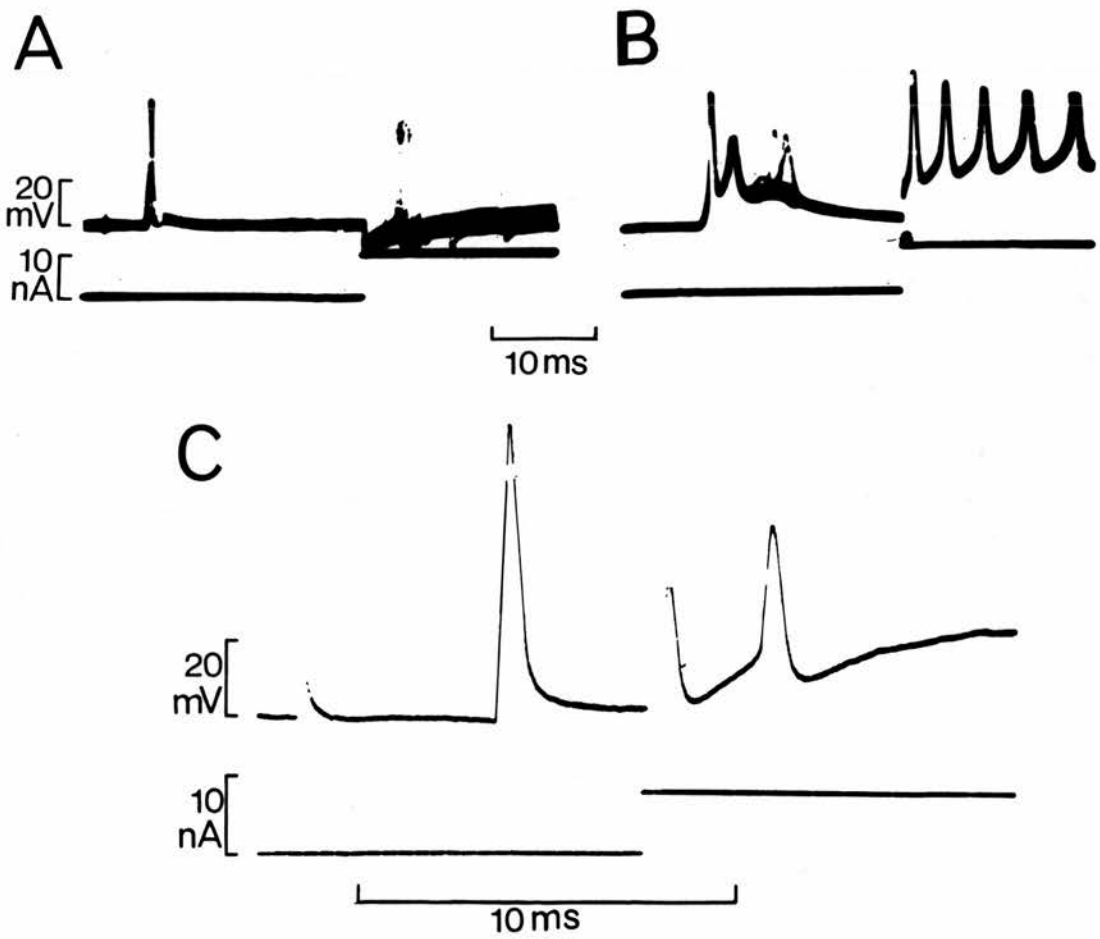
v) Cells and axons are impregnated rapidly. This indicates that migration of HRP within the neurone is not only by simple diffusion.

vi) Very fine neuronal processes, at the limits of light resolution, can be defined by the intraneuronal injection methods.

PART 2: Figure 1

The figures illustrate some of the electrophysiological features monitored during HRP ionophoresis into single axons or cells. A, B each show several superimposed sweeps during injection of a motoneurone; the records were taken 5 - 10 minutes after the start of the staining procedure. The cell was antidromically activated in A, and orthodromically excited in B. In each case a depolarizing current of approximately 10nA is being passed (see lower traces): this is sufficient to 'fire' the cell repetitively.

C is a record taken during injection of a Ia afferent fibre. The lower trace again shows the depolarizing current pulse. In the upper trace the first deflection at the beginning of the sweep was an electrode test pulse. Then there is the peripherally evoked action potential (slightly distorted because of electrode characteristics) and two spikes evoked at the start of the current pulse. The action potentials recorded during the current passing phase have greatly decreased amplitudes. In all three examples the electrodes were balanced during current passage.

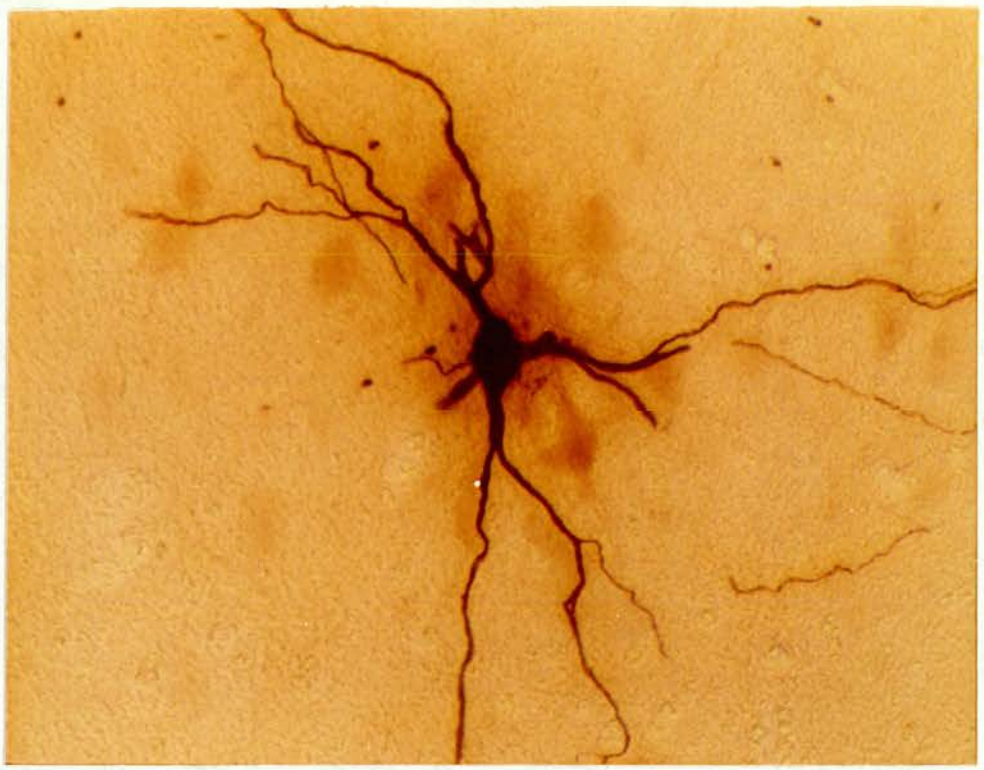

A
B
20
mV
10
nA
10 ms
C
20
mV
10
nA
10 ms

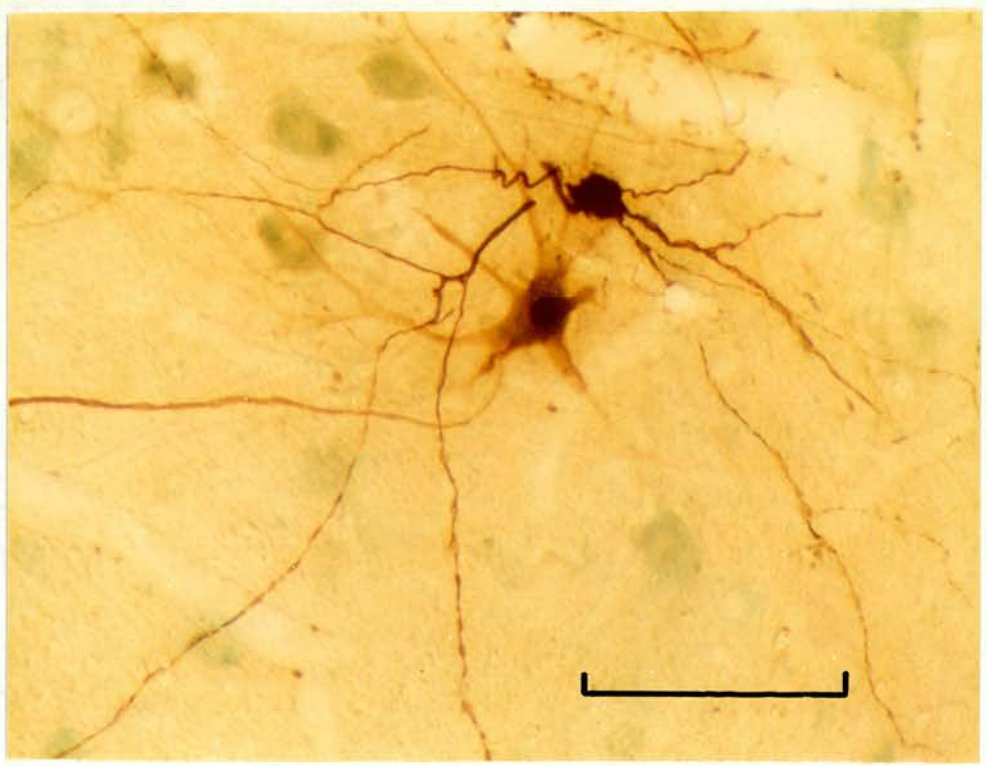
PART 2: Figure 2

Accidental HRP labelling.

These figures illustrate unfortunate problems which may affect HRP injection studies. Extreme leakage produces a dense network of stained cells and fibres; the examples shown here are less severe in degree, with fairly restricted unintentional labelling, but would not be selected for description or discussion in the subsequent results sections. In one case (above) the injected neurone is densely stained; some HRP spilled around the soma and has been taken up by neighbouring neurones which are lightly stained. In the second example two motoneurones are stained. Again, although one cell is more densely stained, there must be doubt as to the identity of the initially recorded and identified unit.

Scale Bar: 200 μm.



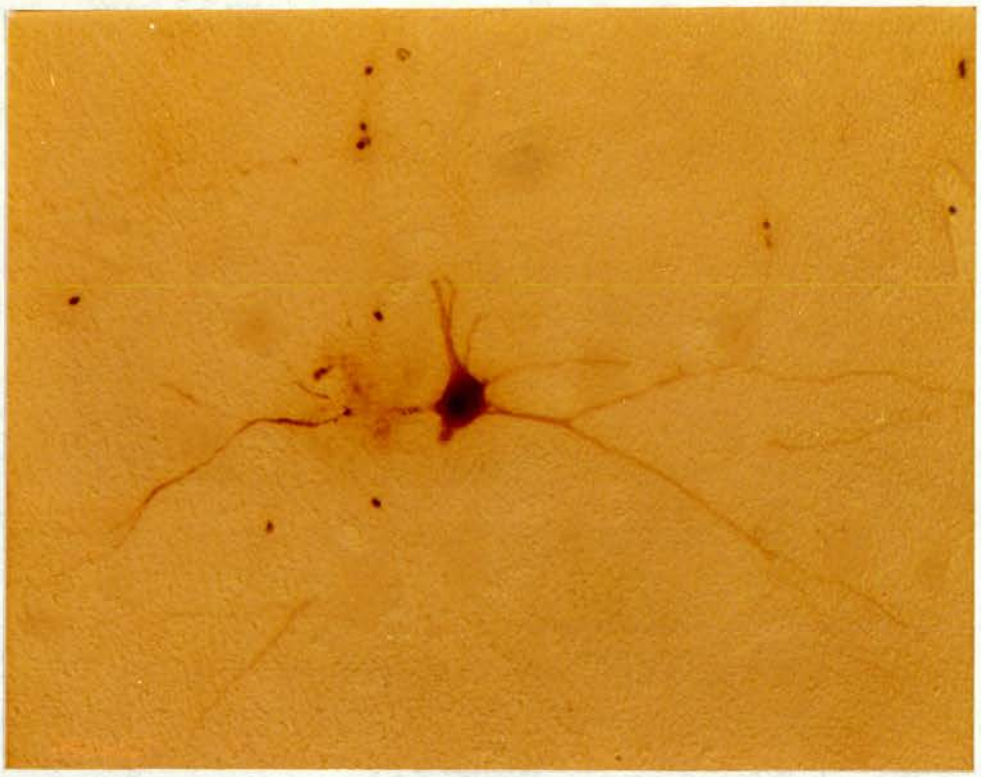


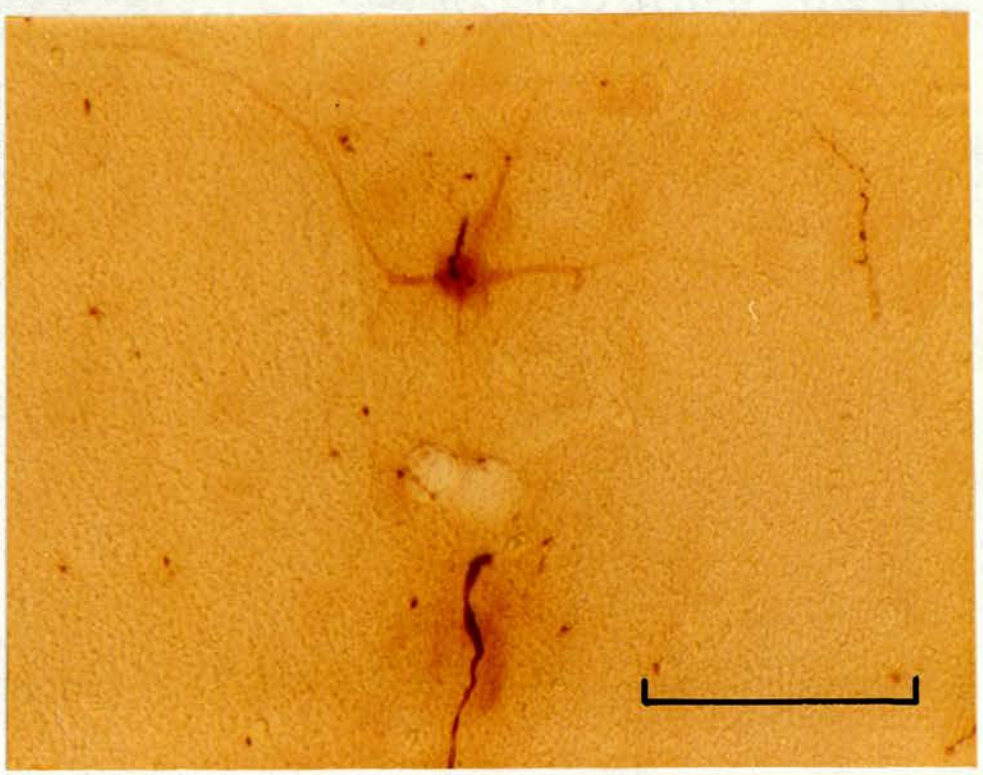
PART 2: Figure 3

Faintly stained neurones.

These two motoneurones are poorly labelled for different reasons. The upper cell received only a small injection (30nA.min) and only the nucleus is densely stained. The lower micrograph demonstrates the effect following intra-axonal injection relatively close to the soma. The axon and its collaterals are well stained. The axon can be traced to its initial segment as a darkly stained profile; the motoneurone soma and dendrites are diffusely infiltrated and appear lightly stained. For intra-axonal labelling of primary afferents the stained axon was never traced as far as the cell body: intra-somal injection of spinal neurones reveals both soma-dendritic and axonal profiles as well stained entities.

Scale Bar: 200 μm



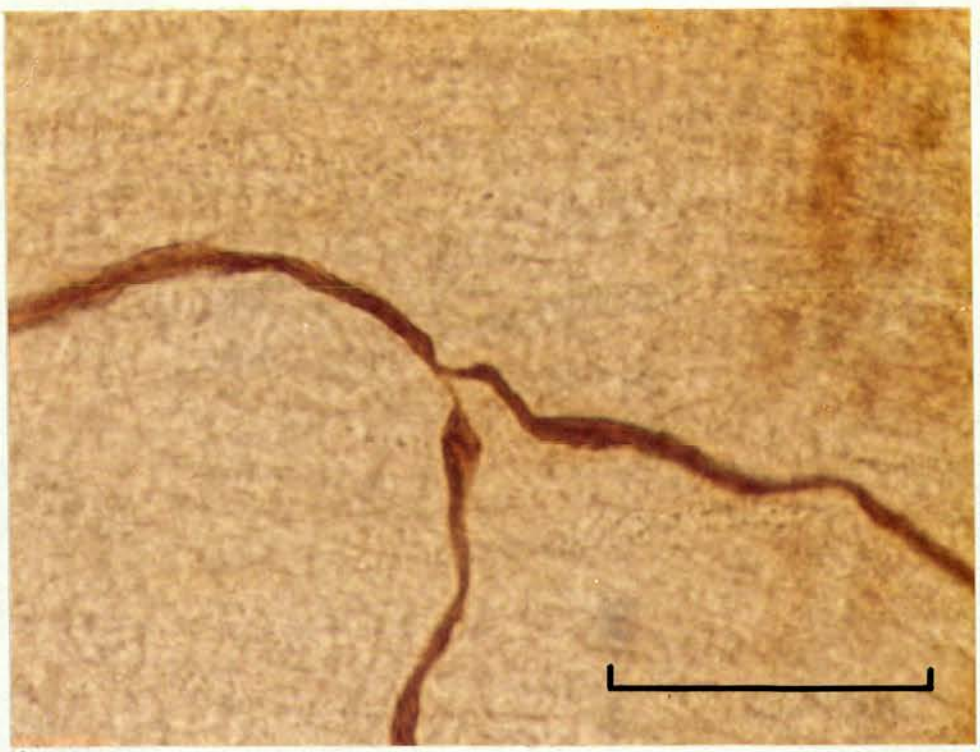


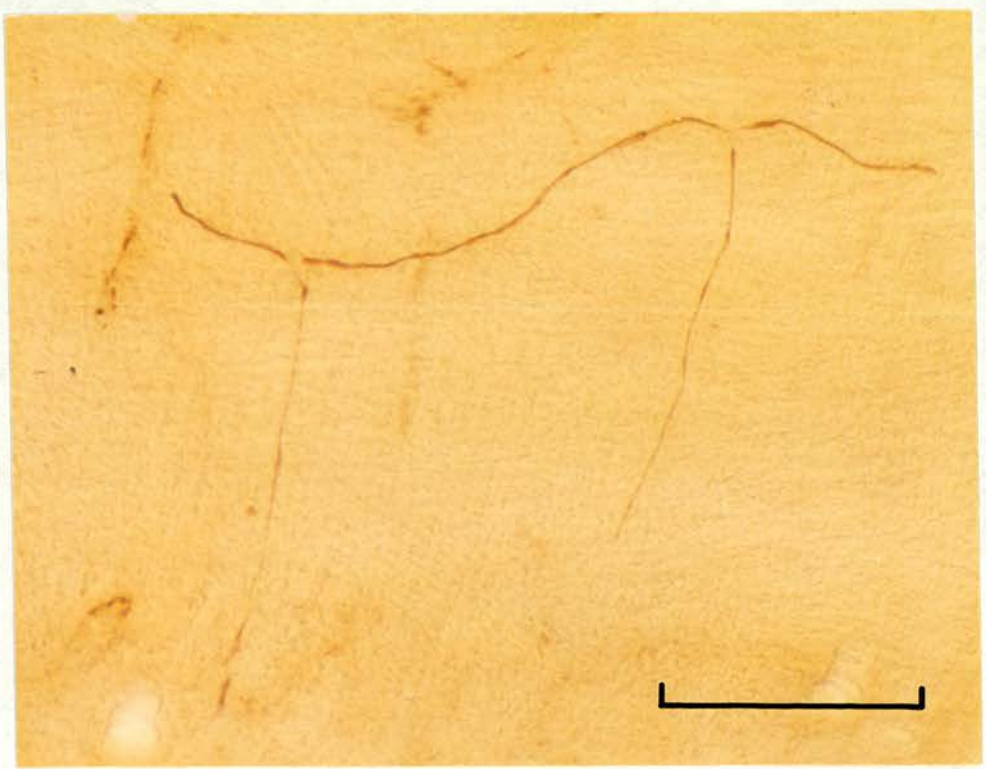
PART 2: Figure 4

Afferent fibre collateral origins.

Collaterals arise from the main axon in the dorsal columns. There is usually a constriction of the main axon, probably at a node of Ranvier, and the collateral which branches off at this point is also constricted for the first few μm (see upper figure). The lower power micrograph ahows adjacent collaterals from a Ia axon. The collaterals often take a rostrally directed trajectory towards their terminal zones.

Scale Bars: Upper 25 μm
Lower 200 μm.





PART 3: THE MORPHOLOGY OF Ia AFFERENT FIBRES

Introduction

The central connections of Ia afferent fibres have been well defined by electrophysiological approaches. And yet there are many deficiencies in our knowledge of the morphology of the Ia system even at the level of the light microscope. Using the techniques described by Brown et al. (1977) the present series of experiments was aimed at resolving, by direct anatomical visualization, the central projection patterns of these fibres.

Results

The experiments were performed on 15 cats, anaesthetized with chloralose (70mg/Kg) and paralysed with gallamine triethiodide.

The initial results were disappointing! Similar electrode and injection parameters as were highly successful for labelling hair follicle axon collaterals (Brown et al., 1977) only produced a rather faint staining of Ia axons. Clearly, although the axons were larger and penetrations were more stable than for cutaneous axons, the transport distances in Ia collaterals reaching the ventral horn were much greater. To stain Ia fibres successfully, 8% HRP was used, and injections increased to over 200nA.min, with the emphasis on passing at least 10 - 15nA through the microelectrode.

Twenty three Ia afferent fibres were intra-axonally stained with HRP: 7 from medial gastrocnemius (Mg),

9 from lateral gastrocnemius-soleus (Lg-S) and 7 from muscles with axons running in the posterior (PT) nerve. All axons had peripheral conduction velocities greater than 80m.sec^{-1} (80 - 110m.sec^{-1}; mean 94m.sec^{-1}) and therefore the sample was not contaminated with Group II fibres (Matthews, 1972). Every fibre had a regular ongoing discharge when isolated; the extended hind limb was held rigidly and in a stretched position. The afferent fibres were excited by brief stretches or lightly tapping the muscle, and, in the few cases where direct electrical stimulation of the muscle was applied, showed a pause in activity during the muscle twitch. This procedure was performed carefully so as to avoid direct activation of the nerve fibres themselves. A general discussion of the identification of these and the Ib and Group II muscle afferents is presented in Part 8.

Ia afferent fibre collaterals

The most intensely stained collaterals were near the site of injection - towards the extremes of the labelled part of axon the collaterals (and main axon) became progressively fainter. Faint collaterals were not interposed between more densely stained ones. There is no evidence that collaterals between the most rostral and most caudal ones stained were not revealed by the method. Thus the distribution of collaterals illustrated in fig. 1 represents a complete description over the stained portions of the Ia axons. Fig. 1 also shows, where possible, the site of injection with HRP and whether the

axon could be traced into the dorsal roots (most of these axons entered the cord about L7 segment: the dorsal root entry positions have simply been lined up for clarity). The axons which were not traced back into the dorsal roots were probably ascending branches labelled by injections at about L6 segment i.e. still only about one segment away from the root entry.

All 20 of the axons which were traced to their entry into the cord bifurcated into rostral and caudal branches. These branches moved medially and generally took up their ascending or descending positions in the dorsal columns within 1 - 2mm of their bifurcation. The axons assumed wavy and gradually deeper courses in the dorsal columns, often close to the medial edge of the dorsal horn. None of the main axon branches terminated in a collateral; presumable they extended for longer distances than indicated and gave off further collaterals.

In these experiments HRP stained total lengths of main axon (rostral plus caudal branches) of 4 - 11mm (7.8 $\pm$ 2.0mm; mean $\pm$ S.D.). (Caudal branches 0.7 - 7.0mm; rostral branches 2.5 - 8.5mm; 3 (rostral) branches not traced into roots 6.0 - 8.0mm.) These figures are comparable with other muscle and cutaneous axons injected with HRP. A total of 156 collaterals arose from the stained axons with a range of 3 - 12 per axon, average 6.8 per axon. Obviously only a fraction of all the collaterals from any axon are stained. The distance between the most rostral and the most caudal

collateral on an axon ranged from 1.3 to 8.2mm.

Collaterals were spaced at intervals of 35 - 2600 μm (mean 1040 μm). There were no statistical differences between collateral spacings on caudal or rostral branches or between collaterals arising close to or distant from the dorsal root entrance of the axon.

Ia collateral anatomy

The gross morphology or branching patterns of Ia collaterals from several different Ia axons are illustrated in figs. 2-12 . The similarities between collaterals are striking, and apply to all the main features.

Triceps surae Ia collaterals leave the parent axon as it courses medially over the top of the dorsal horn or from superficial or deep locations in the dorsal columns. All the collaterals pass through the upper layers of the dorsal horn to lamina V or VI before they begin to branch. Collaterals running down through the dorsal horn are already considerably narrower than the parent axon (see e.g. figs.4,5,10).

In lamina VI, the collaterals gave off several fine branches which generated terminal arborizations in the medial half of lamina VI (intermediate region) and occasionally in the middle third of that lamina. As viewed from sagittal reconstructions (e.g. figs. 7, 8) the main collaterals in the dorsal horn ran rostrally as they descended through the grey matter. This shifting

of the fibres generally meant that lamina VI terminations were slightly rostral to the level of collateral origin; the cranialward tilt often continued as the collateral entered the ventral horn.

From lamina VI, the main collaterals changed direction and ran ventrolaterally at about 45° towards the region of the triceps surae motor nucleus. Before reaching the nucleus the collaterals subdivided, giving off a few branches in lamina VII, to produce terminal arborizations in lamina IX.

At more rostral levels (see Fig. 6) collaterals entered the dorsal horn from deep positions. Again terminals were generated in the intermediate region but in the ventral horn branching patterns differed with less marked penetration of the motor nucleus.

Collaterals from PT nerve Ia axons had greater transverse spread of terminations in lamina VI. The stained axons projected to target motor nuclei in lamina IX.

Terminal arborizations and synaptic boutons in the intermediate region

Boutons were located in areas some 500 μm wide in the transverse plane and up to 600 μm in rostro-caudal extent. There were usually gaps between the terminations of adjacent collaterals although almost continuous sagittal columns of boutons were formed when collaterals were close together. Each collateral gave rise to 29 - 140 boutons

boutons in lamina VI (mean 97). The boutons (see figs.13,16) were frequently arranged 'en passant' along a 20 - 100 μm terminal axon. Branches carried up to nine (usually 3-4) boutons along their course plus one terminal bouton. Single terminal boutons were often offset from fine axons on their own relatively short thin stalk. In small volumes of cord, the density of boutons was quite high, with small clusters of boutons from 2 - 3 terminal branches. Bouton sizes were irregular, ranging from 2.5 x 2.5 μm to 6.5 x 3.5 μm.

The terminal, bouton carrying axons were usually oriented within the transverse plane of the cord. In counterstained material up to 6 contacts were observed on small lamina VI neurones. Contacts often came from boutons on separate terminal branches of the axon. Presumably the remaining Ia boutons in lamina VI contact the dendrites of their target cells, which in this region will probably have their dendrites oriented also within the transverse plane (see Fyffe, 1981).

Terminal arborizations and synaptic boutons in lamina VII

Figs.13, 14 show some Ia boutons in lamina VII. Terminal branches carried from 1 - 6 boutons, and did not subdivide very often. Each collateral had 10 - 42 boutons distributed to lamina VI; the boutons of adjacent collaterals were usually separated by gaps of up to 700 μm. The terminals in lamina VII were restricted to the area dorso-medial to the motor nucleus, i.e. the site of the interneurones on the direct inhibitory

pathway from Ia afferent fibres to antagonistic motoneurones (Jankowska & Lindström, 1972). In this region contacts were observed on the somas of small - medium (20 - 40 μm) sized neurones. Bouton sizes were similar to those in the other terminal regions (4.71 ± 1.53 x 3.03 ± 0.77 μm; means ± S.D.).

As a digression from Ia anatomy, Figs. 17, 18 show details of a lamina VII interneurone labelled by intracellular HRP injection (115nA.min). It was not fully identified as a Ia inhibitory interneurone (see criteria of Hultborn, Jankowska & Lindström, 1971a, b, c). It was tonically active, and monosynaptically excited by MG group I afferent volleys. Since soma size, location and dendritic tree morphology concur with the descriptions of Jankowska & Lindström (1972), it is presumed to be a Ia inhibitory interneurone. In fact HRP has stained the dendrites and axon more fully than Jankowska and Lindström's (1972) Procion Yellow injections. The soma (30 x 35 μm) had seven main dendrites. They were up to 1mm long and fairly straight with very few bifurcations; few dendritic spines were seen, but dendritic varicosities were apparent on the more distal dendrites. Within the transverse plane, dorso-ventral dendritic spread was about 1.7mm; medio-lateral extent was 1.3mm. Some dendrites penetrated into the motor nuclei regions but in the main the dendritic tree was restricted within lamina VII. One dendrite ran longitudinally (caudally) from the soma for

about 900 μm but the major part of the dendritic tree was contained within a 400 μm rostro-caudal extent.

The cell's axon ran dorsally then laterally into the lateral funiculus where it ascended the cord for at least 1.6mm (although the axon was well stained this was the limit of the tissue block which was processed). There were two collaterals, at 200 μm and 800 μm rostral to the soma. They re-entered the ventral horn and terminated extensively amongst the motoneurones of Romanes' (1951) cell columns 2 and 3. Many axo-somatic and axo-dendritic contacts were seen, with up to 11 (mean 6.5) terminals per motoneurone. While remembering that this thesis emphasises the need for precise physiological identification of stained neurones, this circumstantial evidence is included because it supports the conclusions of Jankowska & Lindström (1972) on the funicular axonal trajectory and synaptic terminations (in antagonistic motoneurone pools) of these interneurones. Indeed the finding of predominantly juxta-somatic terminations of these cells upon the target motoneurones also supports the evidence of Jankowska & Roberts (1972b); Coombs, Eccles & Fatt, (1955); Curtis & Eccles (1959); Smith, Wuerker & Frank (1967); and Burke, Fedina & Lundberg (1971) that Ia inhibitory interneurones terminate close to and on the motoneurone soma.

Terminal arborizations and synaptic boutons in lamina IX. Contacts upon motoneurones are considered

in detail in Part 7. The arborizations in the motor nucleus have rostro-caudal extents of some 500 - 700 μm. Boutons from adjacent collaterals may overlap; gaps between terminal arborizations are usually small so that an almost uninterrupted column of endings is formed. The boutons were largely restricted to the motor nucleus innervating the same muscle group. Collaterals gave rise to more boutons in lamina IX (63-192, mean 127) than in laminae VI or VII. Bouton sizes in lamina IX ($5.01 \pm 1.06 \times 2.76 \pm 0.63$ μm; means ± S.D.) were not significantly different from the sizes measured in laminae VI or VII.

Discussion

It was important to exclude Group II fibres from the present sample since they have monosynaptic connexions to motoneurones (Kirkwood & Sears, 1974, 1975; and see part 5). Of course some value may be lost because 'borderline' axons are not described. They will have to await future study. Separation of Ia and Ib afferents was tentatively carried out on the basis of presence or absence of 'resting' discharge under the experimental conditions, the regularity of the response and subjective thresholds to muscle stretch. The tentative classification was always confirmed by the anatomy; no "Ib collaterals" ever projected into the motor nuclei.

The anatomical results in fact confirm some of the

Golgi-study observations on 'presumed' Ia collateral morphology, and now provide a firm basis for the interpretation of electrophysiological data.

The Ia fibres bifurcate on entering the spinal cord. This contrasts with the observation that 66% of hair follicle afferents do not bifurcate but simply turn and ascend the cord (Brown et al., 1977a). Ia fibres thus distribute their information to spinal segments above and below that of their dorsal root entry zone (also Sprague, 1958). Collaterals arise approximately every mm along the main axon as it courses from its entry zone to a position in the dorsal columns. A significant feature was that collaterals ran rostrally as they entered and passed down through the grey matter. The sagittally cut, Golgi stained section of kitten spinal cord of Scheibel & Scheibel (1969) showed large collaterals dropping vertically "with plumbline precision" to the ventral horn. It is possible that the rostral (or caudal, Ishizuka, Hongo, Mannen & Sasaki, 1979) shifting of collaterals is a developmental consequence as the kitten grows.

A further comment of Scheibel and Scheibel (1969) and other users of Golgi stains was that primary afferents penetrated the grey matter in microbundles. Obviously since only single axons were labelled in the present study no comparison can be made except to say that where HRP leakage occurred in the white matter, several fibres emanating from the area of HRP deposition

were observed to run together. It would be interesting to investigate this possible organizational feature more closely, particularly to see if the 'gaps' between collaterals of one axon are or are not 'filled in' by collaterals from other axons.

There are several other features revealed by single fibre staining which seem to be at variance with the earlier anatomical descriptions of primary afferent organization.

Firstly, with regard to the frequency of collaterals (the significance of the collateral distribution is discussed in Part 7), Scheibel & Scheibel (1969) state that collaterals arise at intervals of 100 - 200 μm. Iles (1976), following cobalt loading of dorsal root fibres in adult cats, agreed with this figure. But the present data shows collaterals arising approximately every mm along the axon (see also Ishizuka et al., 1979; Burke, Walmsley & Hodgson, 1979). The HRP data is emphatically confirmed by independent electroanatomical analysis by Munson & Sypert (1979a) who demonstrated mean collateral spacing of 1071 μm, a figure remarkably close to that reported here. It therefore seems unlikely that HRP misses any collaterals. With regard to the Golgi results, differences in cord length in kittens and adult cats probably accounts for the observations; also the difficulty in tracing single fibres in Golgi preparations may impart some error. Each Ia axon

probably has about 10 collaterals over the length of the triceps surae motor nucleus.

Secondly, although Ia collaterals have strikingly similar morphology in relation to one another and resemble the well known collaterals seen in Golgi material (see Rethelyi & Szentagothai, 1973), the sagittal view seems to differ from that of Scheibel & Scheibel (1969). They state that terminal arborizations are severely restricted in the sagittal plane (see also Iles, 1976). We observe boutons from single collaterals spread over 400 - 800 μm in the rostro-caudal direction. This does not imply that the boutons or terminal branches are all oriented longitudinally as such - it simply means that the branching pattern of axons entering lamina IX results in an extensive distribution. Terminal branches do in fact run longitudinally as well as vertically or horizontally. Munson & Sypert (1979a) have also presented evidence for some considerable spread (up to 800 μm) of lamina IX boutons in the rostro-caudal direction. We cannot then think of Ia terminal fields, in any region of the cord, as being two dimensional. Each collateral arborization occupies a finite volume of cord and the spacing of collaterals, together with rostro-caudal spread of terminations, often results in essentially uninterrupted columns of boutons. It is possibly worthwhile to mention here the different perspective the anatomist gains from simply cutting sections in different planes. With transverse sections

the longitudinal extent and possible overlap of collateral arborizations in different regions was difficult to interpret. Conversely, although sagittal sections illustrate the cranial tilt of collaterals and the rostro-caudal spread of their terminations, it is more difficult to estimate laminar location and the trajectories in the medio-lateral plane.

Projections of Ia afferents. In this part of the discussion the more familiar transverse views of collaterals will be considered, to give easier referral to Rexed's (1952) laminar scheme for describing locations in the cord, and to the locations of possible target neurones. Single Ia collaterals had 3 main areas of terminal arborization and these agreed with expectations from both anatomical and electrophysiological results gathered by many workers over many years (review, Rethelyi & Szentagothai, 1973).

The three terminal zones can be considered in terms of functional connexions. In lamina IX, synapses are made upon the appropriate α - motoneurones (Bradley & Eccles, 1953); these connexions are described in Part 7. But the projections of Ia collaterals to the ventral horn may also serve to activate the cells of origin of the VSCT, which probably include the spinal border cells (Cooper & Sherrington, 1940; Burke, Lundberg & Weight, 1971) in the dorso-lateral parts of the ventral horn. Other types of VSCT cells, located in the intermediate region (Ha & Liu, 1968; Hubbard & Oscarsson, 1962) are

probably excited, in part, by the more dorsal Ia terminal arborizations in laminae VI and VII. The most dorsal terminations, correlating with the dorsal FSP's of Eccles et al. (1954) were in lamina VI, predominantly in medial locations. There is high security of synaptic excitation of lamina VI interneurones by Ia afferents (Eccles, Fatt & Landgren, 1956; Eccles, Eccles & Lundberg, 1960) and the anatomical substrate for this may be the common clustering of 4 - 6 boutons close to the soma of small lamina VI neurones. It is now known that these neurones do not contribute to the Ia reciprocal inhibitory pathway (see later). Some cells in this region do project to the motor nuclei (Czarkowska, Jankowska & Sybirska, 1976) and may subserve polysynaptic actions of Ia afferents on motoneurones (Alvord & Fuortes, 1953; Granit, Phillips, Skoglund & Steg, 1957; Pacheco & Guzman, 1969). The evidence for these polysynaptic autogenetic excitatory pathways was the long latency of response under certain circumstances. Latency fluctuations, as described by many authors (see Kuno, 1964a, b; Burke, 1967; Edwards et al., 1976a, b; Collatos et al., 1979), have relevance to the nature of the synaptic transmission rather than to the number of synapses involved in the reflex pathway; such fluctuations will be discussed in Part 7.

The lamina VI terminals may be involved in synaptic connexions with neurones of the dorsal column system in

the cat (Jankowska, Rastaad & Zarzecki, 1979), since Brown & Fyffe (1980, 1981) have demonstrated extensive branching of some of these neurones in and around the intermediate region. This pathway should now be investigated further as a mode of relaying muscle spindle activity to higher centres. The convergence on to the dorsal column system neurones includes low and high threshold cutaneous mechanoreceptive components. Few of the other identified target neurones of Ia afferents (motoneurones, Ia inhibitory interneurones, VSCT cells) receive this type of convergence pattern. Some Ia and Ib activated DSCT cells also receive monosynaptic cutaneous inputs (Lundberg & Oscarsson, 1960; Kuno, Munoz-Martinez & Randic, 1973).

Unfortunately the present information does not describe Ia collaterals at the level of Clarke's column (but see Rethelyi & Szentagothai, 1973) which begins caudally at about L_4 segment. Ia fibres provide a prominent input to the DSCT (see Oscarsson, 1967; Lundberg & Winsbury, 1960). Certainly at the level of L_6, Ia collaterals still have arborizations in the intermediate region - if this pattern is maintained at higher levels it would account for Ia activation of DSCT neurones.

There are however some neurones at lower lumbar levels which project to supraspinal levels (Aoyama, Hongo & Kudo, 1973) and are monosynaptically activated by Group I muscle afferent fibres. The cells were

predominantly located in the medial half of the intermediate region, corresponding to the dorsal arborization region of Ia (and Ib) afferent fibre collaterals.

Dendritic trees of laminae VI and VII neurones are predominantly oriented within the transverse plane (Ramon-Moliner, 1962; Fyffe, 1981). Ia terminal arborizations in the intermediate region thus overlap a neuropil composed of many different neuronal elements. It is not known yet whether there is selective formation of synapses, that is contacts close to or distant from the soma of particular neurones, dependent upon the functional identity of the various cells. No Golgi type II cells (with short axons) have been recognised in the recent intracellular staining investigations of neurones in the intermediate region (see Brown, 1981). Some lamina VI neurones (Czarkowska et al., 1976a; A.G. Brown & R.E.W. Fyffe, unpublished observations) send their axons via the ventral commissure to ascend contralaterally. Since the afferents converging on these cells were not identified the significance of these neurones is unclear.

One other pathway which should be activated by Ia collaterals is that leading to presynaptic inhibition at Ia synapses (see Burke & Rudomin, 1977). There is mounting evidence (ten Bruggencate, Lux & Lieble, 1974; Eide, Jurna & Lundberg, 1968; Rudomin, Nunez, Madrid & Glusman, 1974; Rudomin, 1980) to support the models

using axo-axonic synaptic interactions to explain primary afferent depolarization (PAD) and presynaptic inhibition in the spinal cord. Conradi (1969) described a necessary corollary of this model - axo-axonic synapses on M boutons (presumed Ia origin) contacting ventral horn neurones (but cf. McLaughlin, 1972a, b). This observation is not of course final proof of the existence of the postulated mechanisms. PAD may also be generated by non-specific mechanisms, namely an increase in extracellular K^+ concentration due to activity in nearby neurones. Ia collateral arborizations do overlap with terminal zones of, for example, descending fibres and may converge on to the same spinal neurones. K^+ accumulation is greatest in the intermediate region. (ten Bruggencate et al., 1974) where Ia fibres terminate extensively. But transmission in Ia pathways can be presynaptically inhibited without affecting transmission in other fibres ending on the same neurone, which indicates only a minor role, if any, for K^+ levels in presynaptic inhibition of Ia terminals. Furthermore, a specific synaptic mechanism might be able to reduce selectively transmission in particular parts of the complex Ia terminal arborizations. 'Normal' action potential propagation throughout the preterminal axon branches is discussed in Part 7. Neurones responsible for PAD generation might be located amongst the varied array of Ia excited neurones in lamina VI.

Boutons given off in lamina VII, just before the

collaterals enter the motor nuclei, probably synapse on Ia inhibitory interneurones (Hultborn et al., 1971a,b,c; Jankowska & Roberts, 1972a, b; Jankowska & Lindström, 1972, 1974).

The concept of an interneurone interpolated on the pathway mediating reciprocal (or direct) Ia inhibitory action on antagonistic motoneurones (Eccles et al., 1956; but cf. Lloyd, 1941) proved controversial but gained general acceptance (e.g. R.M. Eccles & Lundberg, 1958 a, b; Araki, Eccles & Ito, 1960; Eide, Lundberg & Voorhoeve, 1961). The characteristics and locations of the neurones involved remained in doubt until the elegant series of experiments by Jankowska, Hultborn and their co-workers (see refs. above) which led to identification, characterization and eventual morphological demonstration of the appropriate cells. They are located in lamina VII, dorsomedial to the motoneurone pools, in a region distinct from the areas occupied by Renshaw cells (Renshaw, 1941; Thomas & Wilson, 1965; Jankowska & Lindström, 1974) or cells of the intermediate region (Eccles et al., 1954) and have funicular axonal projections (cf. Eccles et al., 1956).

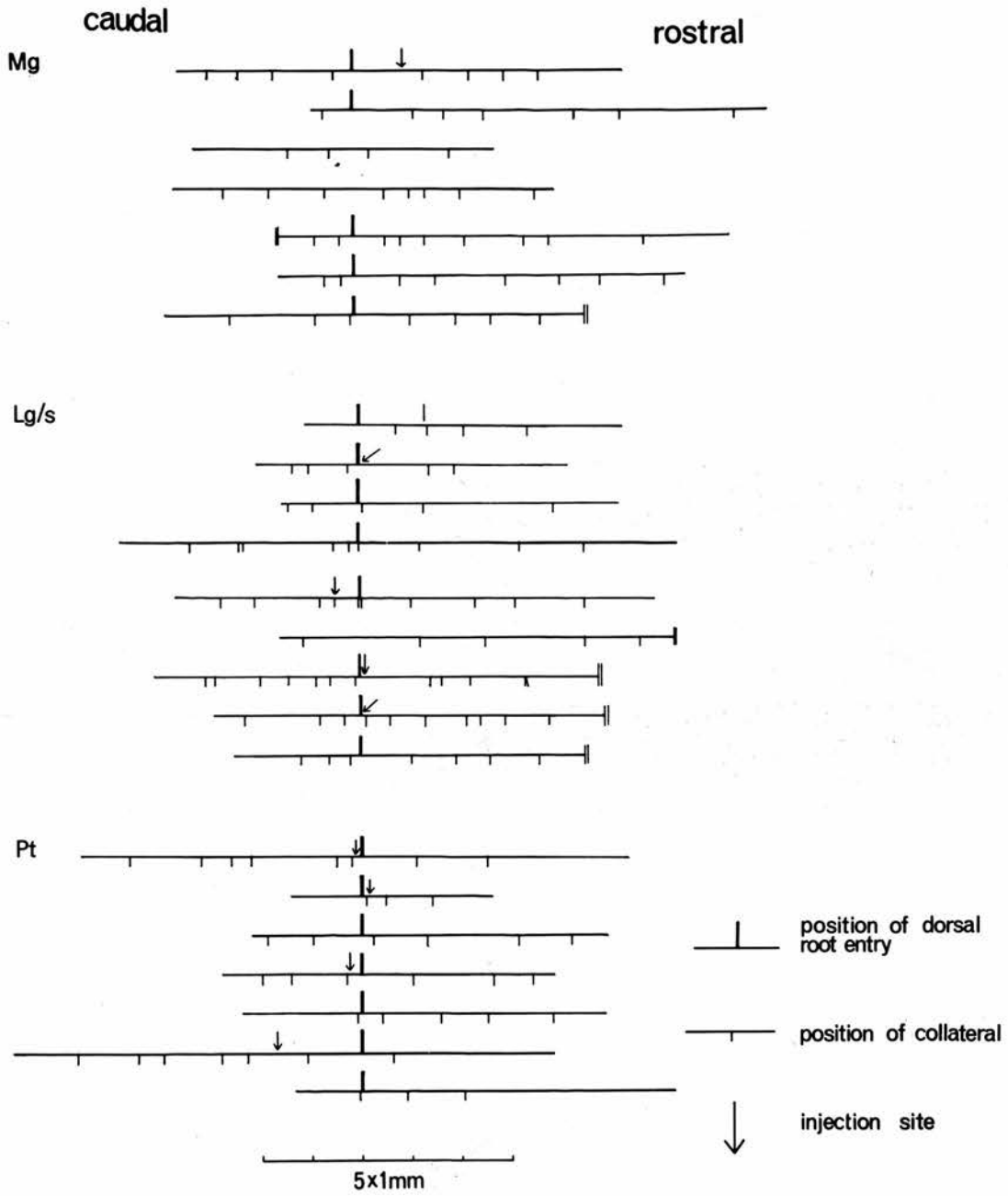
Ia boutons often end close to the somas of cells in this region (up to five possible contacts on some cells); this juxtasomatic mode of termination may form the basis of the secure transmission in the inhibitory pathway (Hultborn et al., 1971b).

PART 3 : Figure 1

Intraspinal branching of Ia afferent fibres.

The stained parts of 23 HRP labelled Ia axons are shown (7 innervating Mg; 9 innervating Lg-S and 7 from the PT nerve). Represented here are the lengths of axon stained, number and spacing of central collaterals and where possible the site of injection and the position of afferent fibre entry into the cord. Cut ends of some fibres (because of tissue blocking) are shown by double vertical lines. In most cases the ends represent the traceable limits.

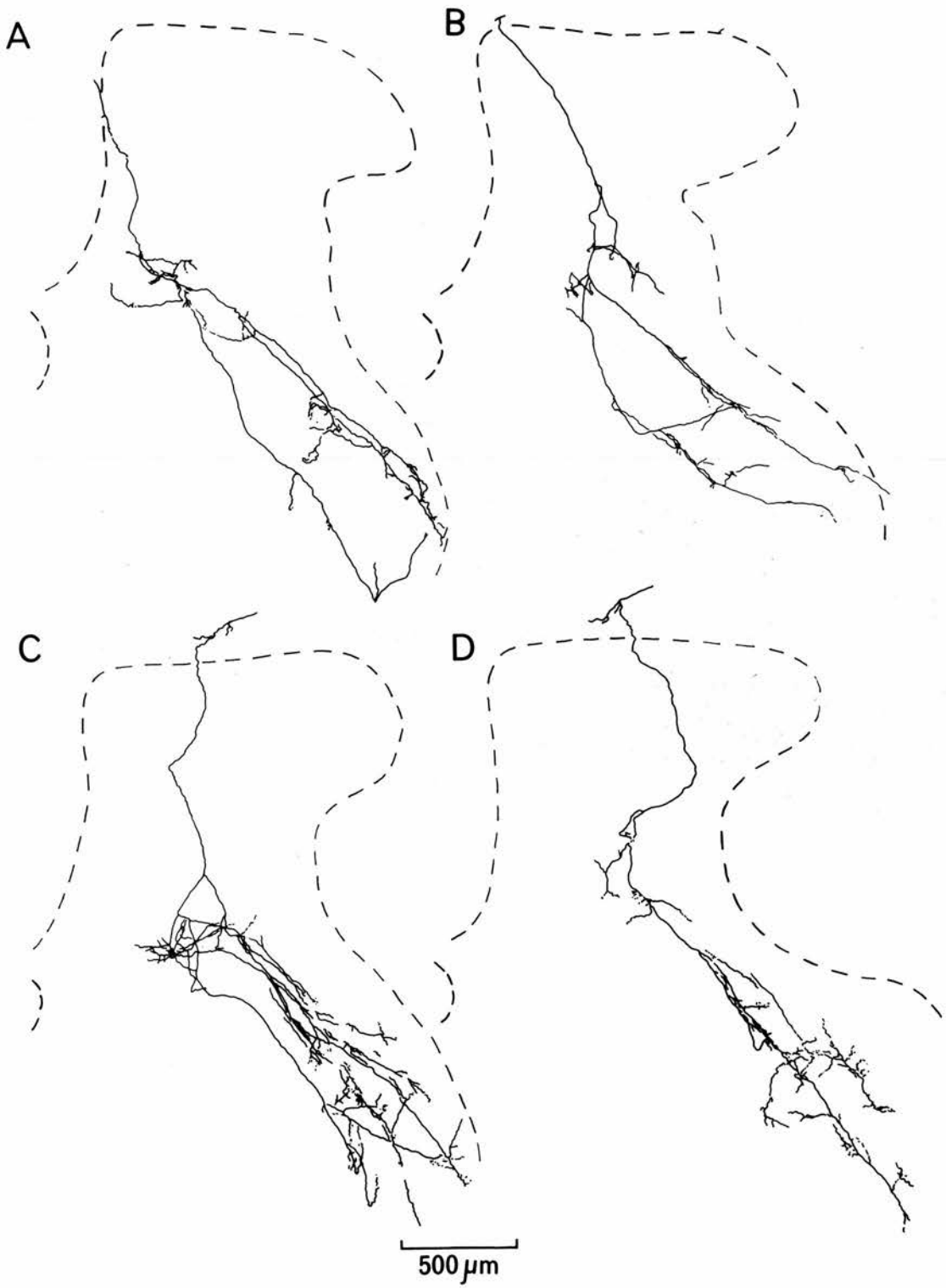
All Ia afferent fibres bifurcated after entering the spinal cord. Collaterals arose from the main ascending or descending branches and were spaced at about 1.0mm intervals.


caudal
rostral
Mg
Lg/s
Pt
position of dorsal root entry
position of collateral
injection site
5x1mm

PART 3: Figure 2

Ia afferent fibre collaterals.

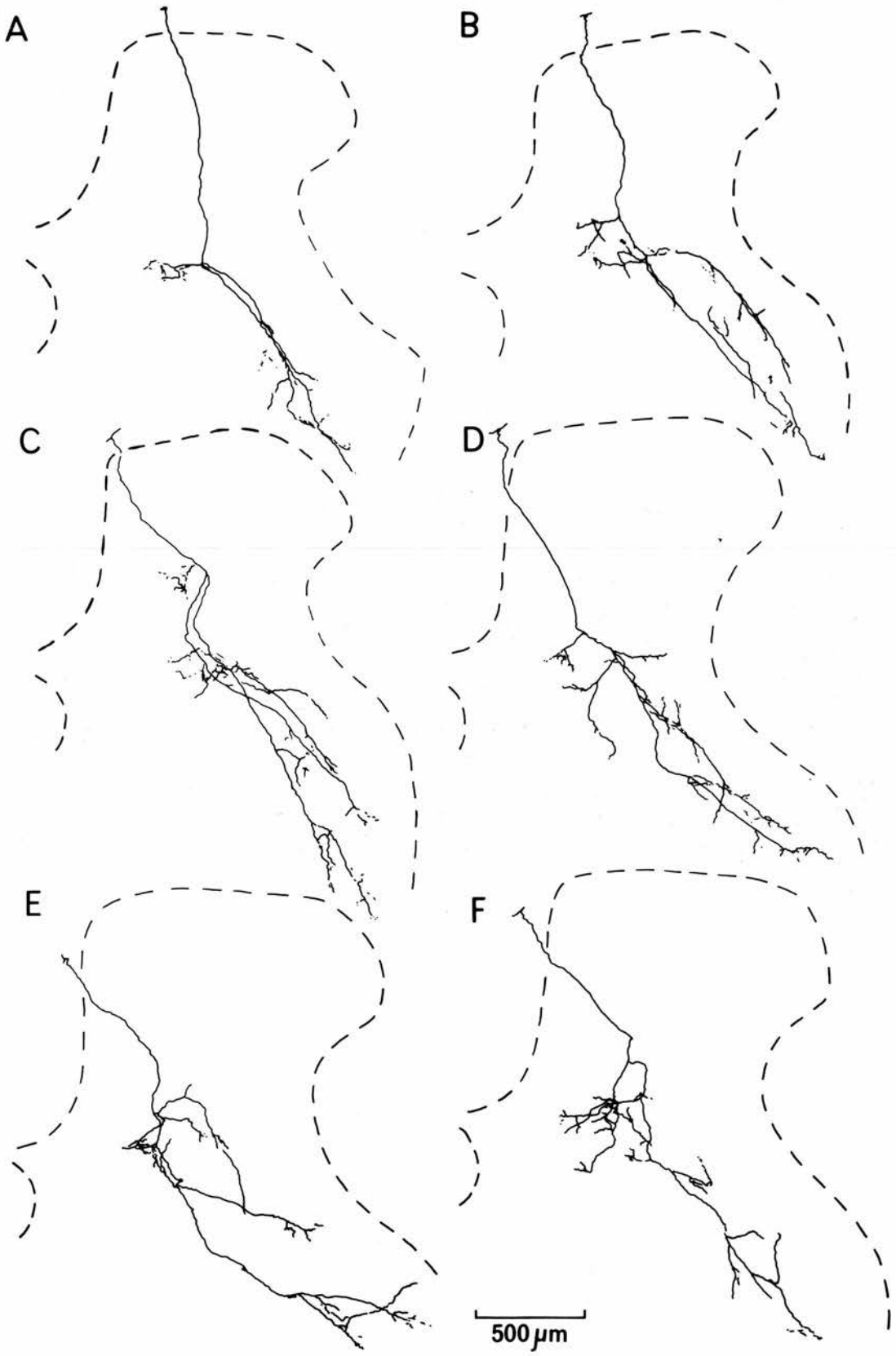
These camera lucida drawings are reconstructions in the transverse plane of 4 adjacent collaterals from a single Lg-S Ia afferent fibre. A - C arise from the descending and D from the ascending intraspinal branch of the afferent fibre. Dashed lines represent the outlines of the central canal and the dorsal and ventral horns of the cord. The origin of each collateral is shown. A is most caudal, and at this level the main axon is coursing deeply within the dorsal columns, close to the grey-white interface at the medial edge of the dorsal horn. Collaterals C and D arise close to the dorsal root entry and bifurcation of the main fibre. The collaterals enter the dorsal horn at its dorsal (B - D) or medial (A) border and run directly to lamina V or VI before beginning to subdivide. Terminal branches and boutons form arborizations in the intermediate region; one or more larger diameter branches however project ventrolaterally towards the motor nuclei in the ventral horn. These branches generate some terminations in lamina VII and then further fine branching develops extensive arborizations in lamina IX. The lamina IX terminals of these 4 collaterals form an almost continuous column of boutons extending more than 1.3mm rostro-caudally.


A
B
C
D
500 μm

PART 3: Figure 3

Ia afferent fibre collaterals.

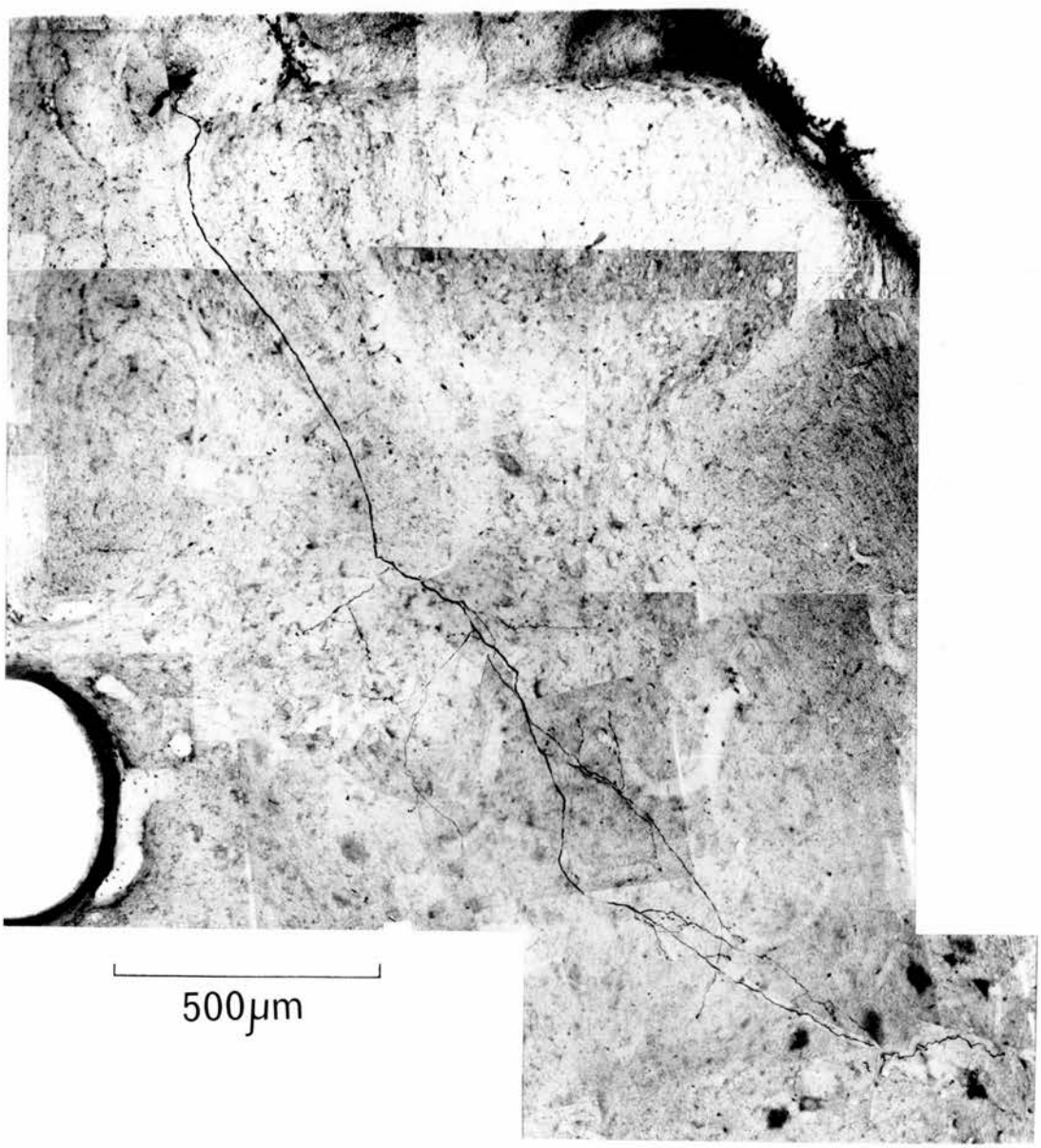
A - F are reconstructions of six adjacent collaterals from the ascending branch of a Mg Ia afferent. A is caudal (500 μm rostral to the entry zone of the fibre) whilst the other collaterals are in sequence to the most rostral one (F). The course of the main fibre can be determined - it ran medially from its entry into the cord and eventually ascended in the dorsal columns, fairly close to the edge of the dorsal horn. As the axon ascended, it gradually assumed a deeper position in the dorsal columns. The collaterals arose successively at intervals of 300, 500, 800, 1200 and 400 μm from the main axon. They all terminated in similar manner in three main locations, in the intermediate region, lamina VII and lamina IX.


A
B
C
D
E
F
500 μm

PART 3: Figure 4

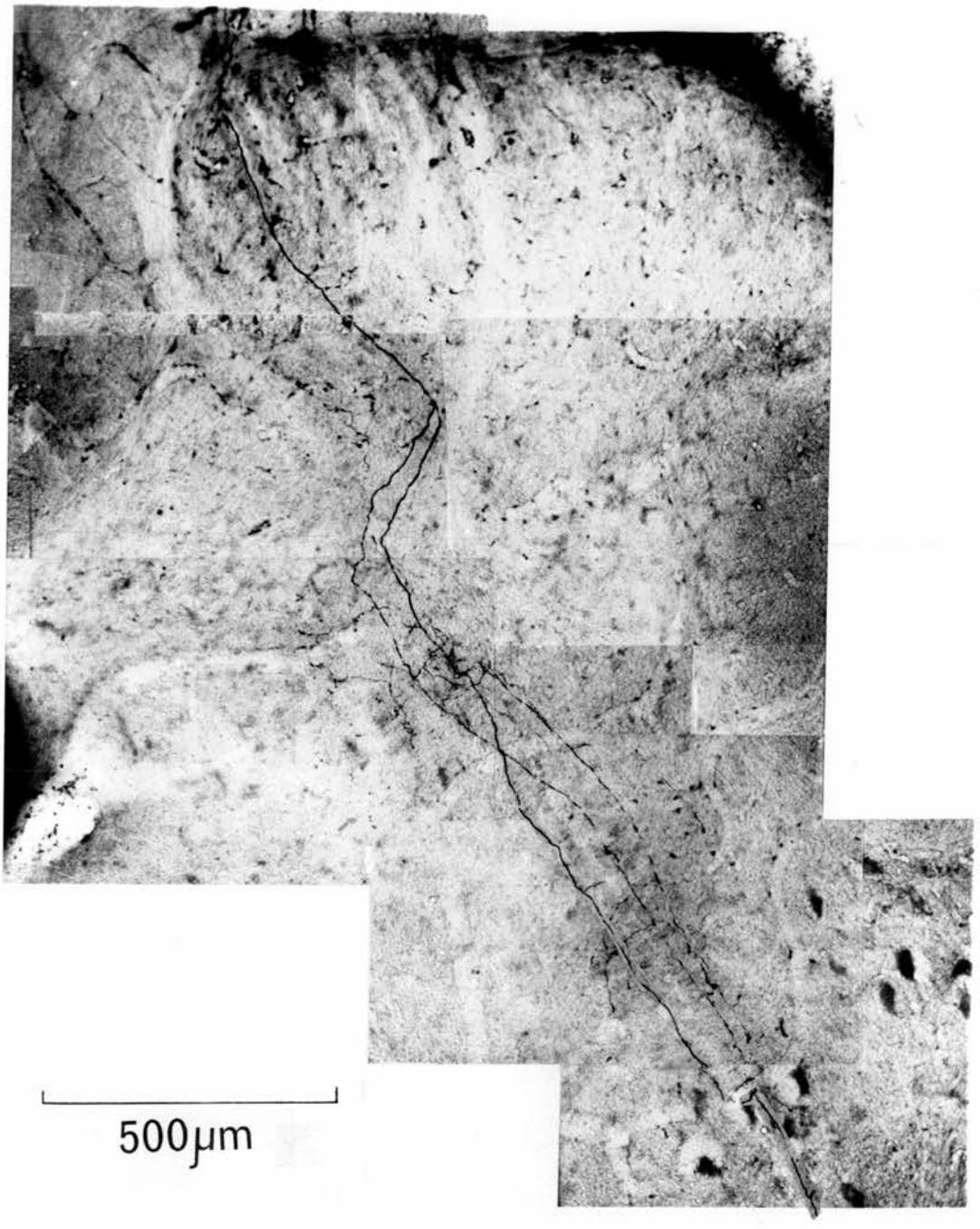
A Ia afferent fibre collateral.

This photographic reconstruction, from 4 serial 100 μm thick transverse sections of cord illustrates the trajectory and branching pattern of a single Mg Ia collateral. The central canal and outline of the dorsal horn can be seen. Some ventral horn neurones are evident in the bottom right hand corner of the figure; they are demonstrated by counterstaining. Fine branches and boutons are distributed to the intermediate region, lamina VII and to the motor nuclei. The main axon is in the dorsal columns, level with the dorso-medial corner of the dorsal horn. This collateral is reconstructed in Part 3, Figure 3D.


500μm

PART 3: Figure 5

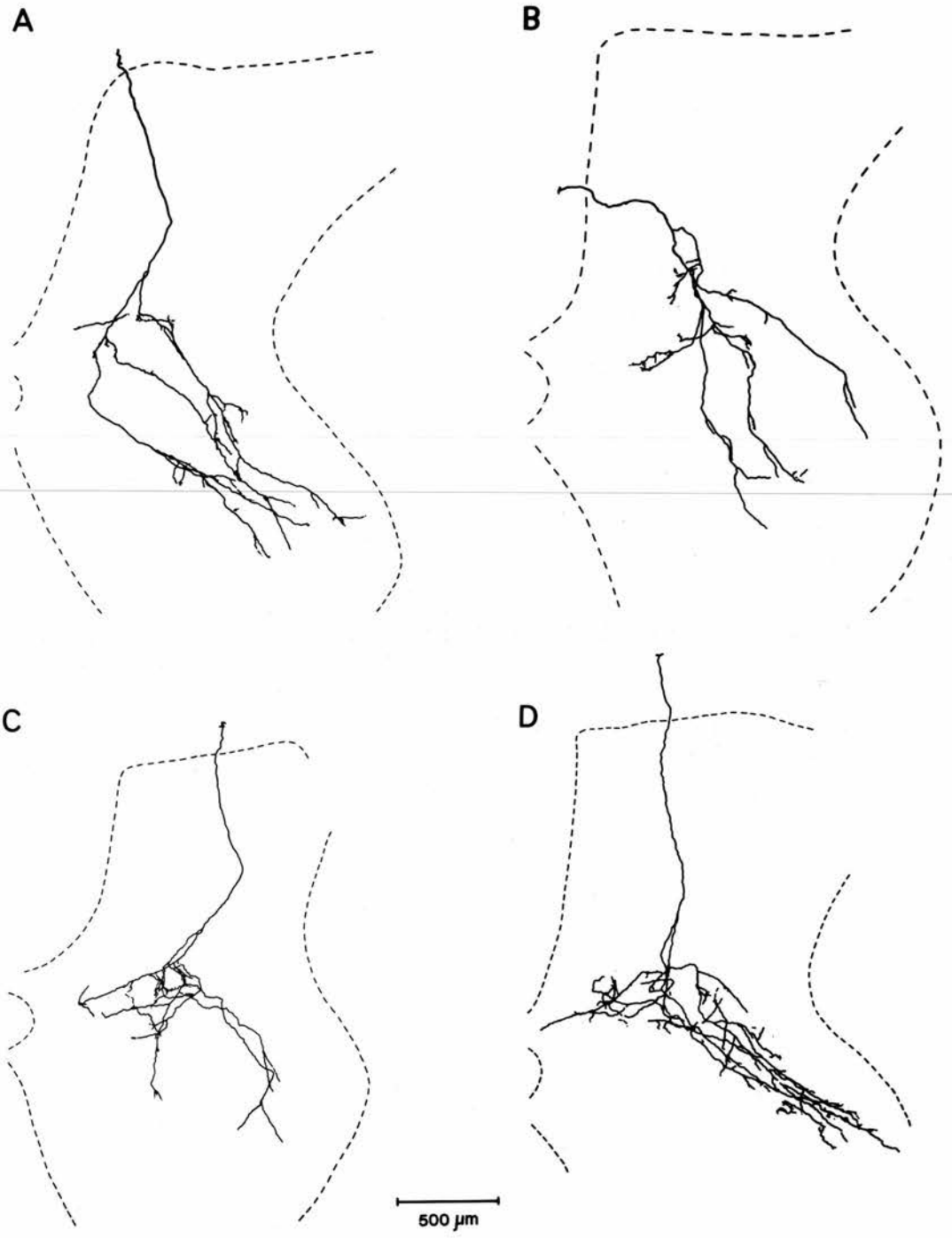
Like the previous figure, this montage illustrates a single Mg Ia afferent fibre collateral. Major branching commences in the dorsal horn. Terminal arborizations are generated in the intermediate region and then several branches course ventro-laterally into the lamina IX motor nuclei. This collateral is reconstructed in Part 3, Figure 3C.


500μm

PART 3: Figure 6

Ia afferent fibre collaterals.

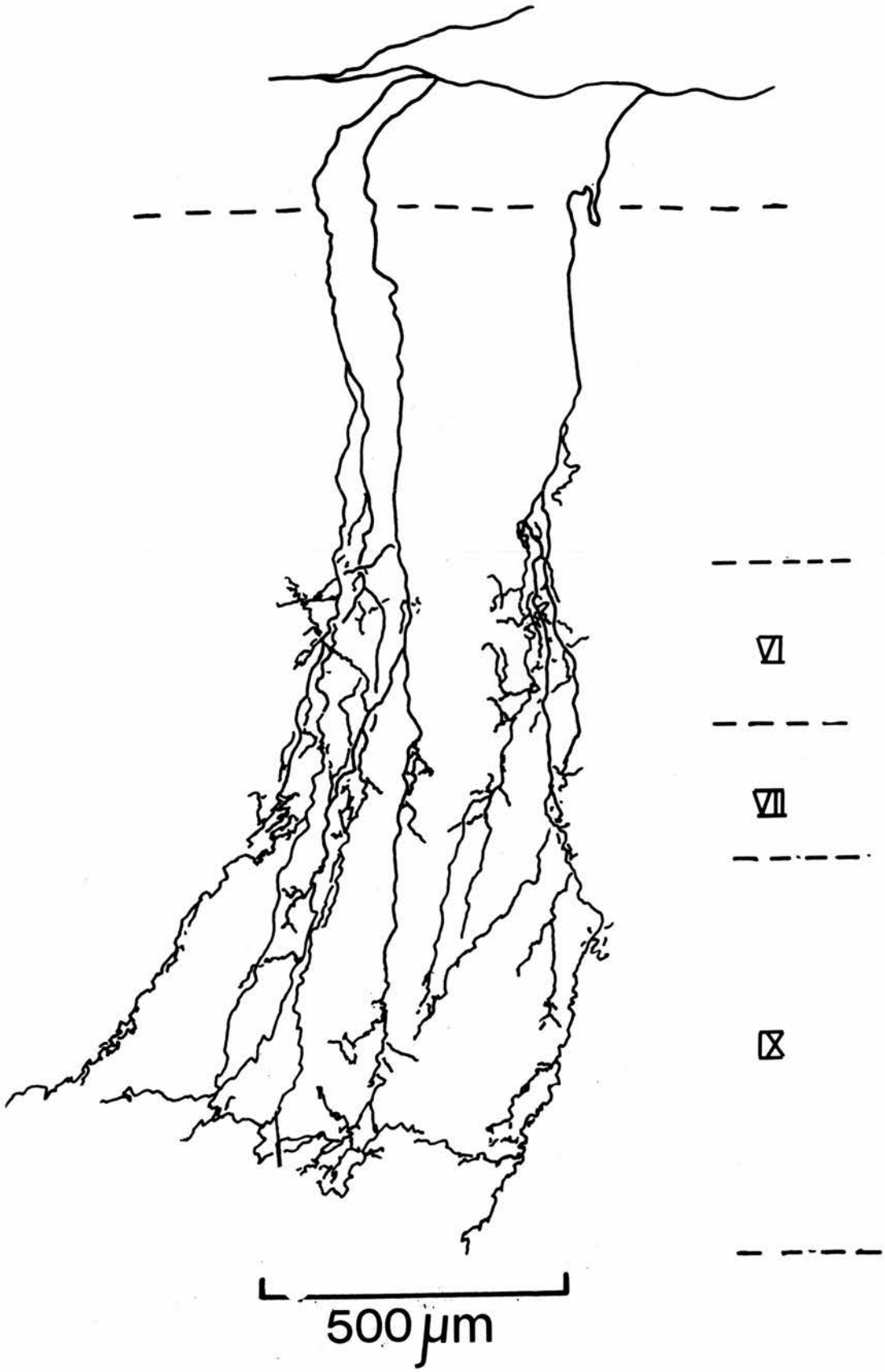
Reconstructions, in the transverse plane of Ia collaterals from different primary afferent fibres. A from Mg; B from Lg-S and C, D from axons in the PT nerve. Note that in C, D the intermediate region arborizations are more extensive than for the majority of triceps surae afferent collaterals. The collateral in B arises from an ascending axon deep in the dorsal columns. The overall morphology and projection pattern varies from collaterals closer to the position of dorsal root entry in that there is a less localized distribution of terminals in the triceps motor nuclei.


A
B
C
D
500 µm

PART 3: Figure 7

Ia collateral arborizations.

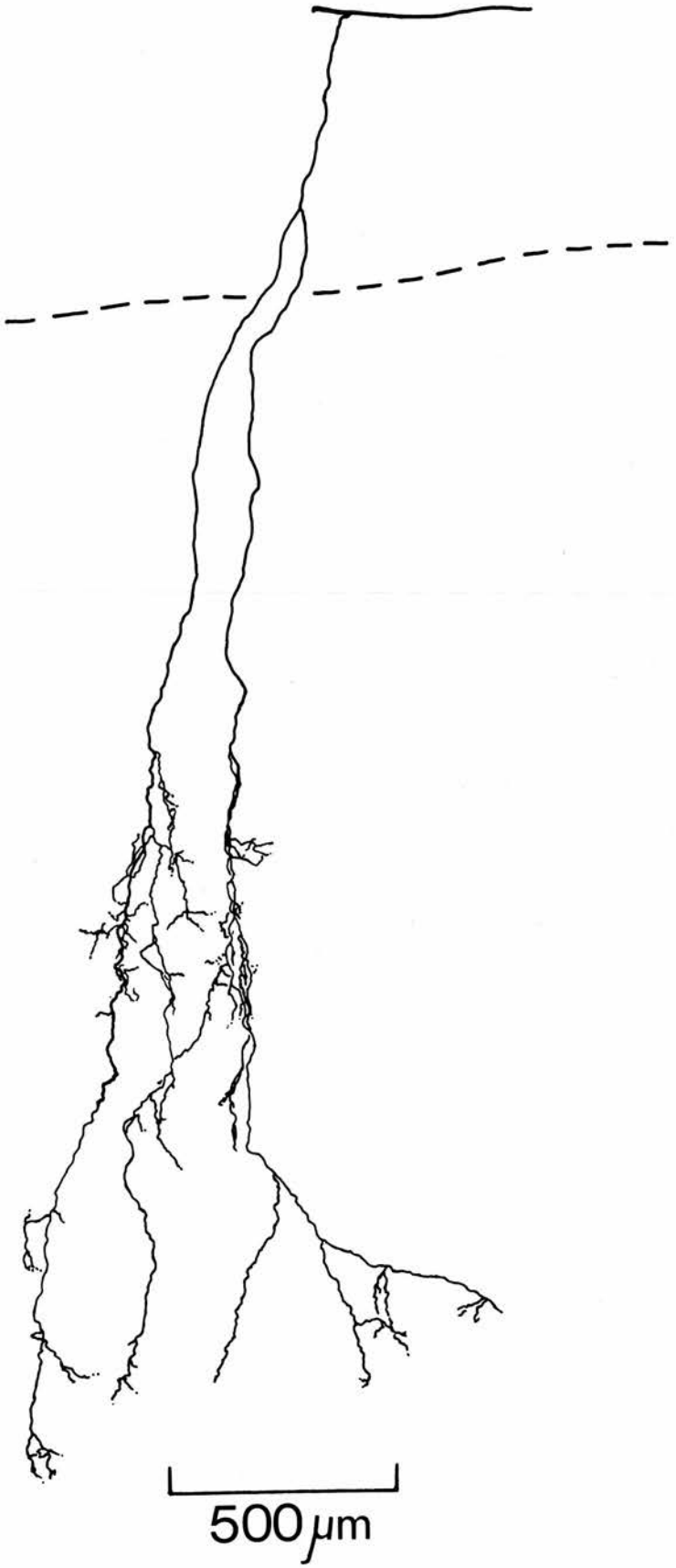
The reconstruction is of 3 adjacent Mg Ia collaterals viewed in the sagittal plane. Rostral is to the left. The bifurcation of the main axon is shown at the top - the collaterals arise from the descending branch. The two rostral collaterals had an apparently identical origin. It should be noted that collaterals course rostrally from their origin. Terminal arborizations are generated in lamina VI, VII and IX. There is overlap of terminal axons and boutons in lamina IX and to a lesser degree in the more dorsal regions.


VI
VII
IX
500 µm

PART 3: Figure 8

This reconstruction, in the sagittal plane, shows the morphology of a single Ia afferent fibre collateral. Branching begins in the dorsal columns, but all terminal branches are in laminae VI, VII and IX. In this example the lamina IX arborization extends over about 1mm rostro-caudally.

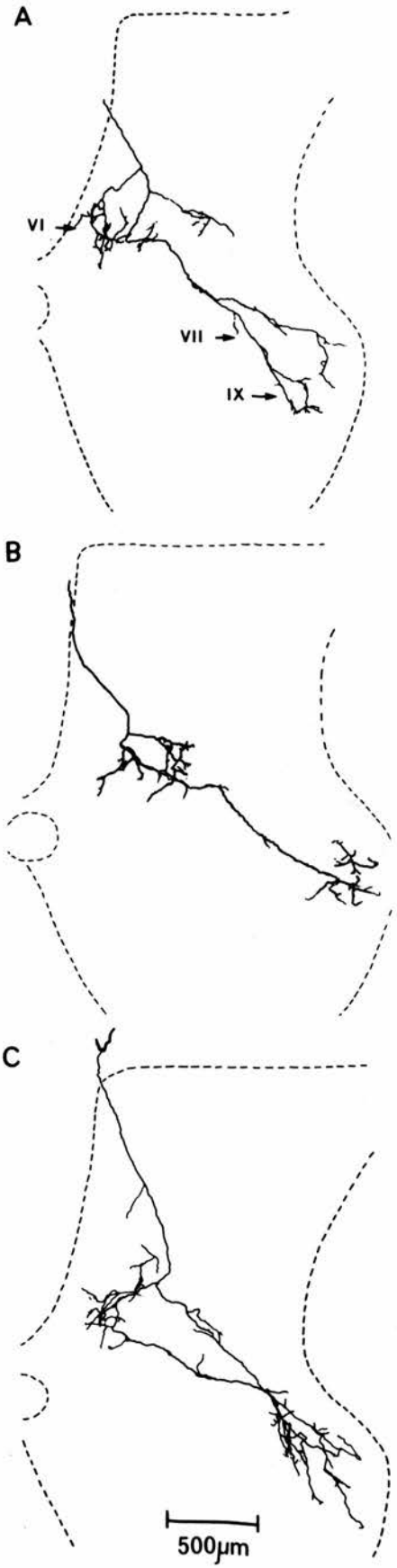
Rostral is to the left; dashed line represents the dorsal border of the dorsal horn.


500 μm

PART 3: Figure 9

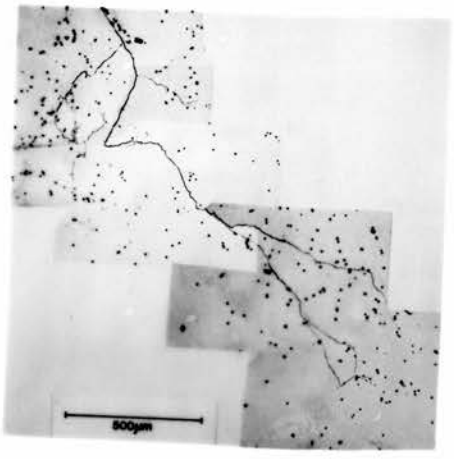
Ia afferent fibre collaterals.

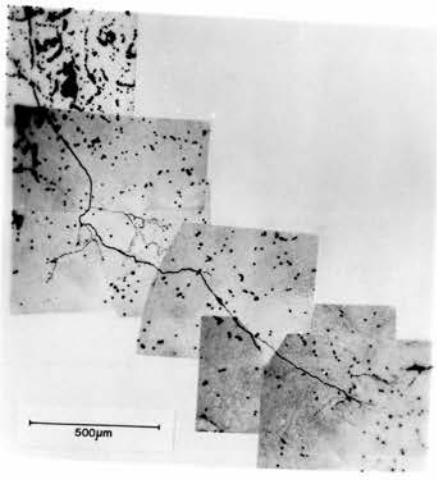
The reconstructions (in the transverse plane) are of three adjacent Mg Ia collaterals arising from the descending branch of the axon (A most caudal, C most rostral). The collaterals originate in the dorsal columns, enter the grey matter at the medial border of the dorsal horn and run ventrally to lamina VI, where they branch and terminate extensively, particularly in the medial half of that region. Some of the branches change direction and run ventro-laterally towards the motor nuclei in the dorso-lateral part of the ventral horn - the location of the triceps surae motor pools. On the way, a few fine branches terminate in lamina VII. The terminals generated in lamina IX are restricted within the appropriate motor cell column region.


A
VI
VII
IX
B
C
500μm

PART 3: Figure 10

These three photomontages illustrate the collateral branches reconstructed in the previous figure. DAB processing produces numerous stained blood corpuscles in the background. It can be seen that the collaterals narrow somewhat as they course ventro-laterally through the grey matter.

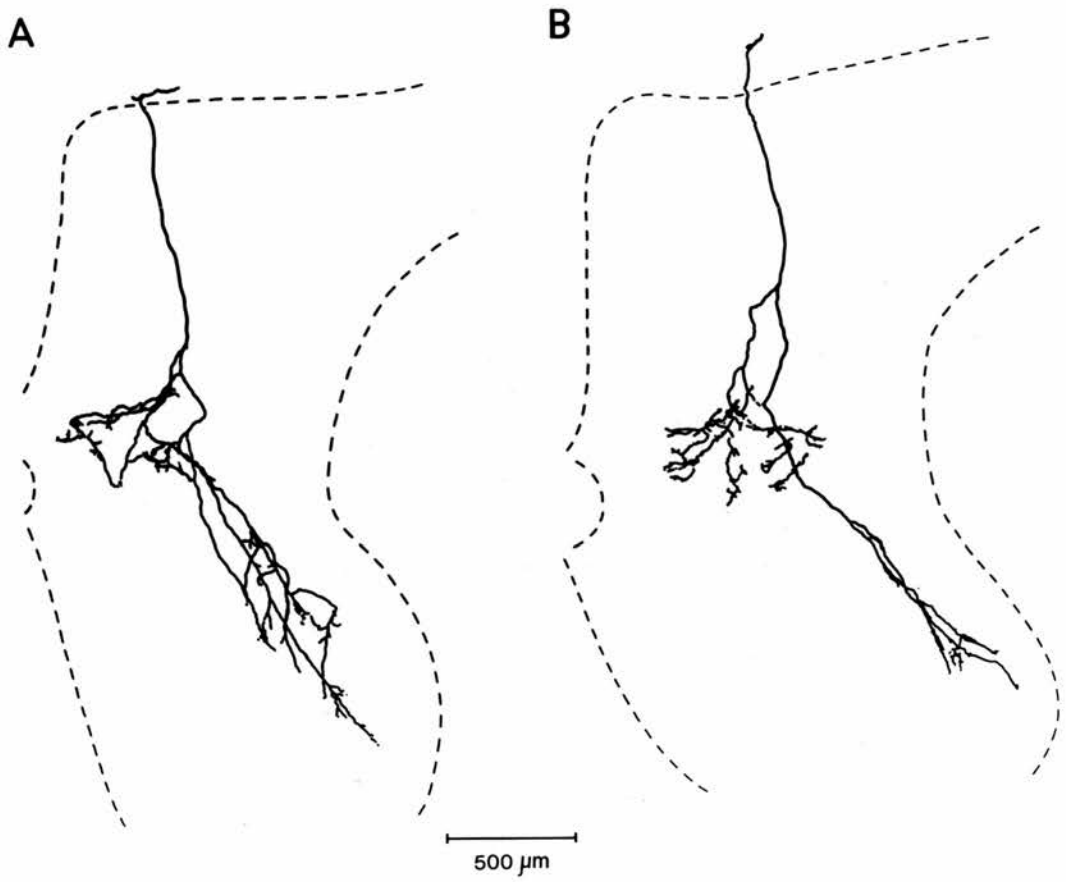

500µm


500µm


500µm

PART 3: Figure 11

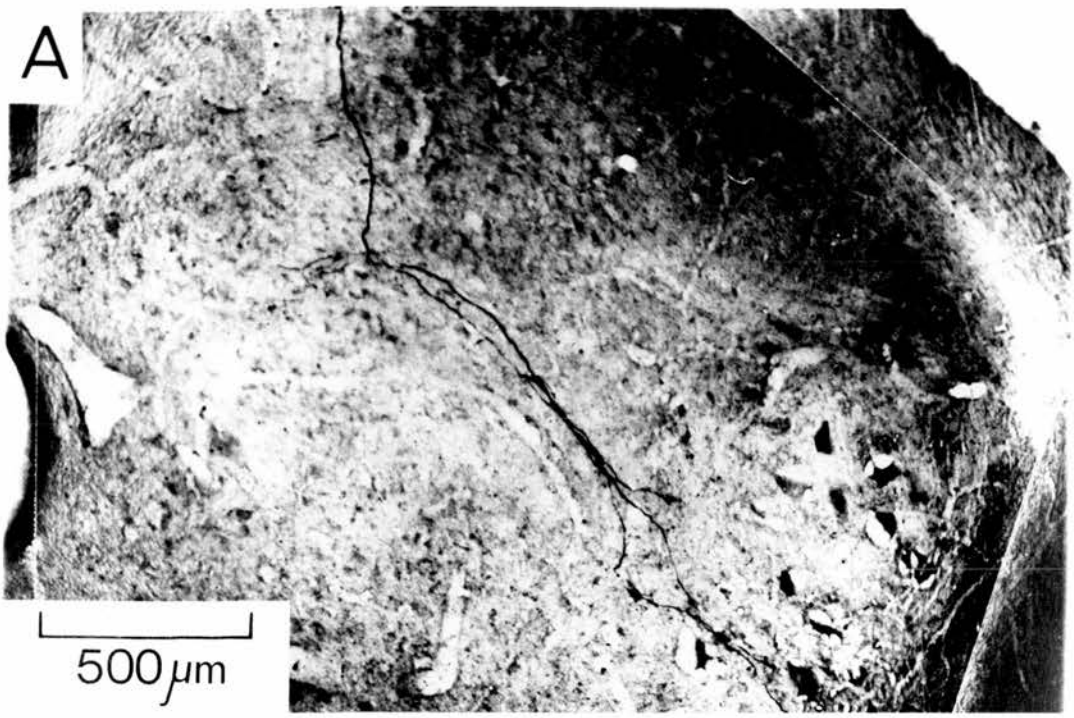
Two adjacent (A caudal) collaterals from a Lg-S Ia fibre are reconstructed in the transverse plane. They both arborize characteristically in the intermediate region (lamina VI) then course ventro-laterally across lamina VII (where further terminals are given off) towards lamina IX to terminate in the region of the triceps surae motor nuclei.

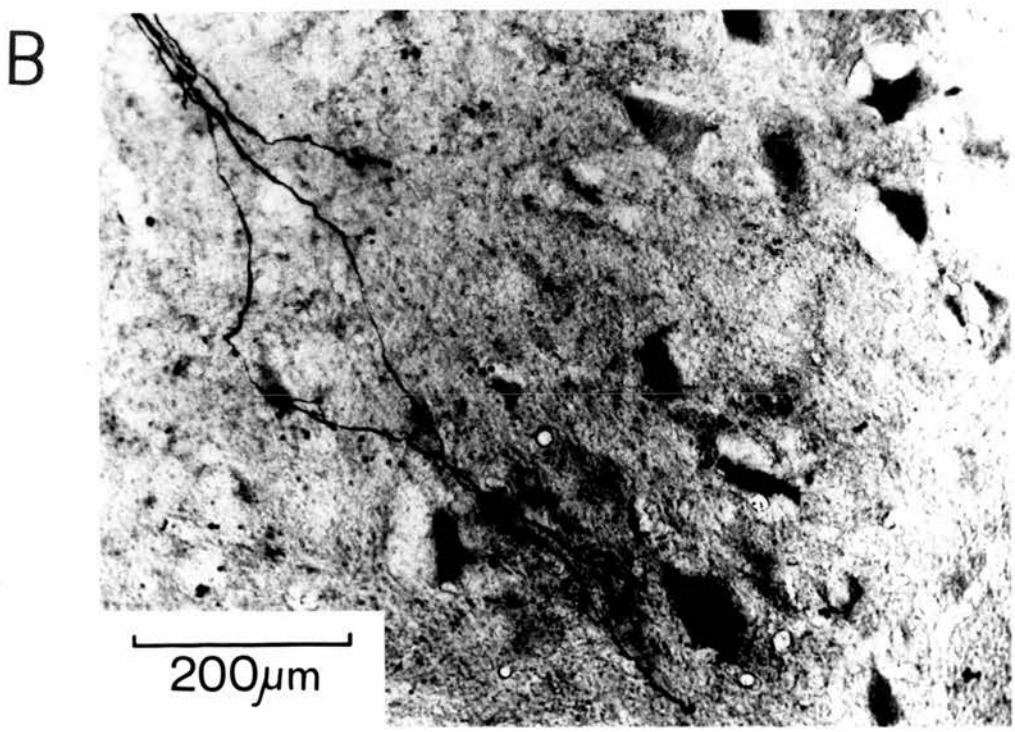

A
B
500 μm

PART 3: Figure 12

A. Part of a Mg Ia collateral photographed from a single 100 μm thick transverse section of cord counterstained with methylene green.

B. A higher magnification view of the ventral horn branches of the above collateral. Terminal axons and boutons lie between large neurones in the motor nucleus. A smaller cell dorsomedial to the main group of cells receives a single somatic contact.

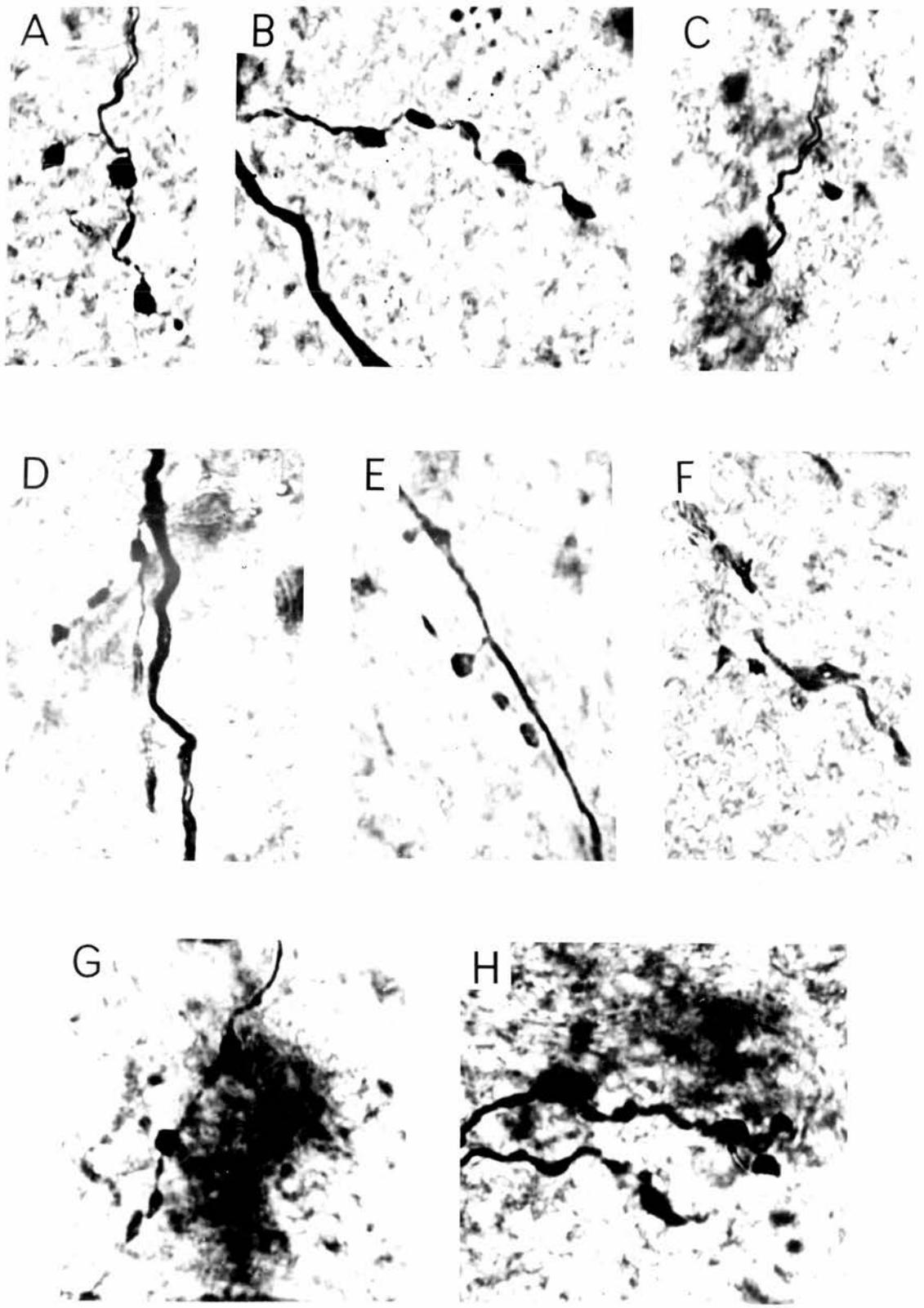

A
500μm


B
200μm

PART 3: Figure 13

Ia boutons.

This figure illustrates examples of Ia terminal branches and boutons. A - C were located in lamina VI; D - F in lamina VII and G, H in lamina IX. C, D, G, H are counterstained; cell bodies in C, D are outlined by dashed lines.

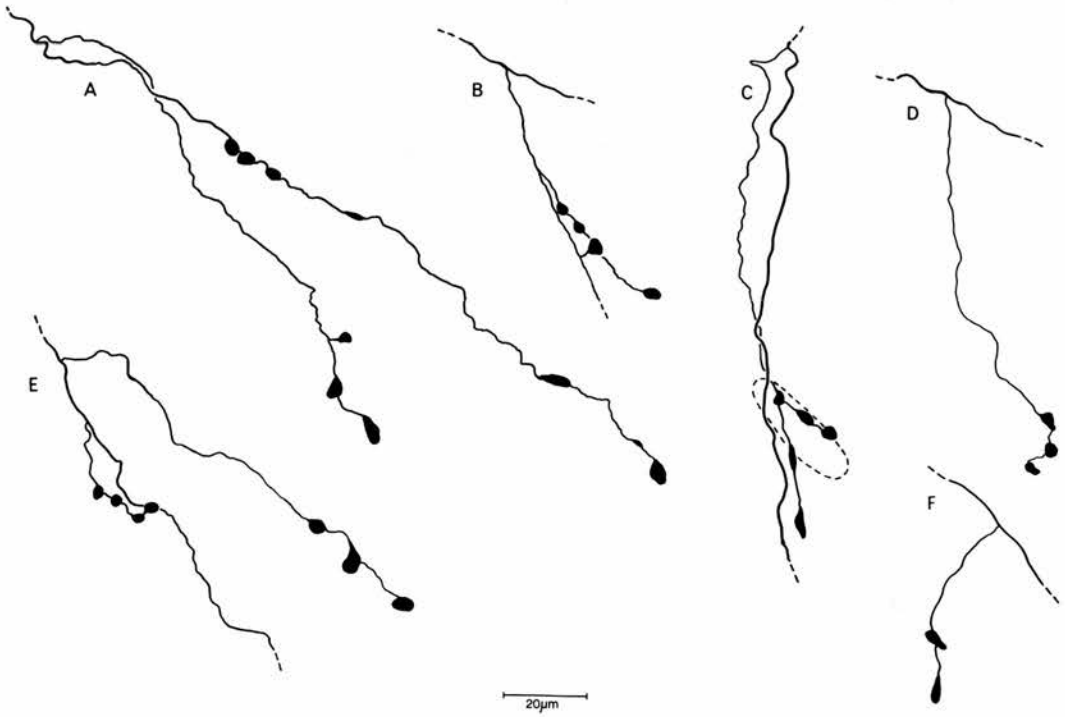

A
B
C
D
E
F
G
H
20μm

PART 3: Figure 14

Ia terminal axons and boutons.

The arborizations reconstructed in the upper part of this figure are some examples from lamina VI projections. Terminal branches often carry several 'en passant' boutons as well as a terminal bouton and, additionally, single boutons are sometimes offset from the major terminal axon. The lower group of terminals arose from Ia collaterals passing through lamina VII. Terminal branches carrying 2 - 3 'en passant' plus 1 terminal bouton lie close to the major branch running towards the motor nuclei. In all reconstructions medial is to the left, dorsal to the top. Spinal neurones which were visualized by counterstaining are indicated by dashed lines.

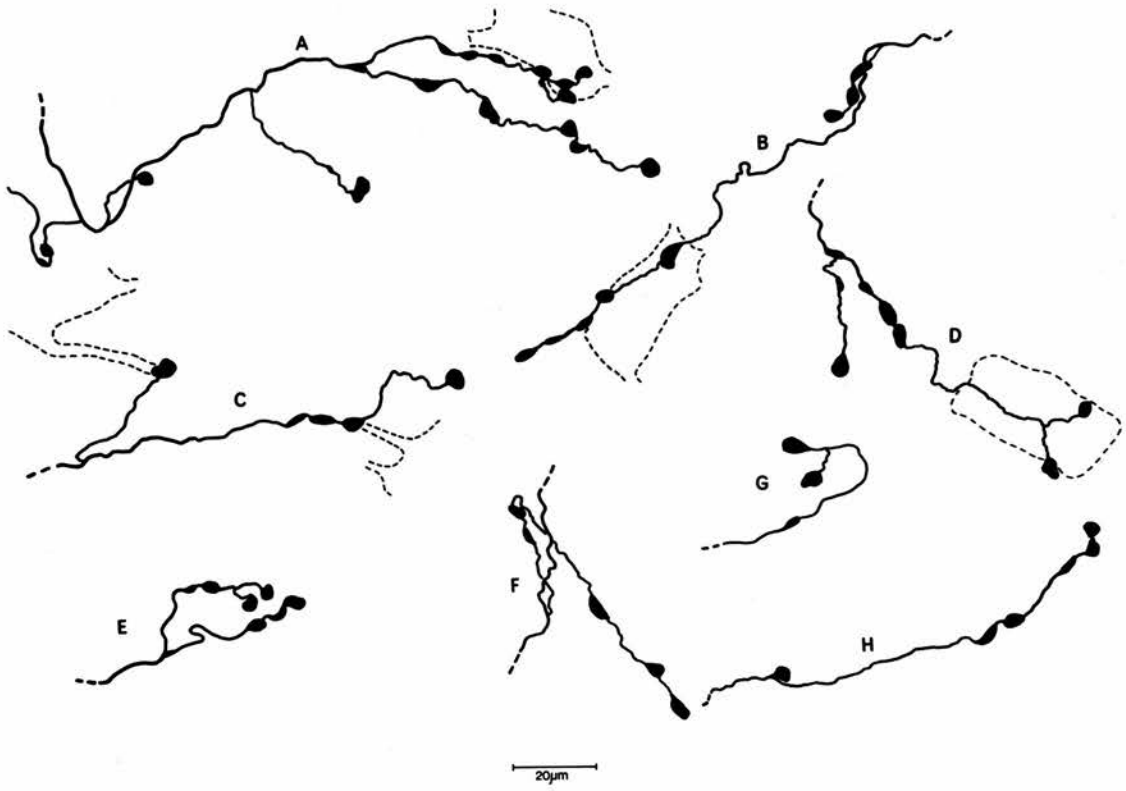

A
B
C
D
E
F
G
H
I
J
20µm


A
B
C
D
E
F
20µm

PART 3: Figure 15

Ia boutons in lamina IX.

Reconstructions of some terminal arrangements are shown (parts of A, B are seen in Part 3, Figure 13G, H). Most terminal branches carry boutons 'en passant' as well as terminal. Relationships with some ventral horn neurones (outlined by dashed lines) are illustrated in some cases. In fact most of the boutons did not appear to contact cell bodies in the motor nucleus (see also Part 7).

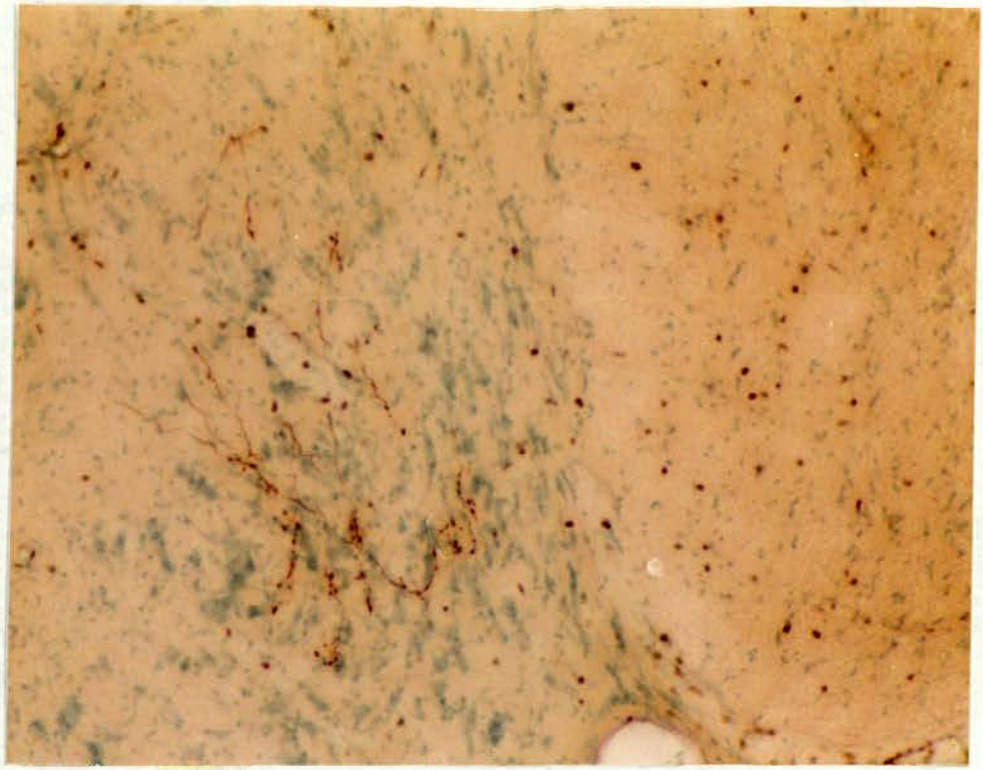

A
B
C
D
E
F
G
H
20µm

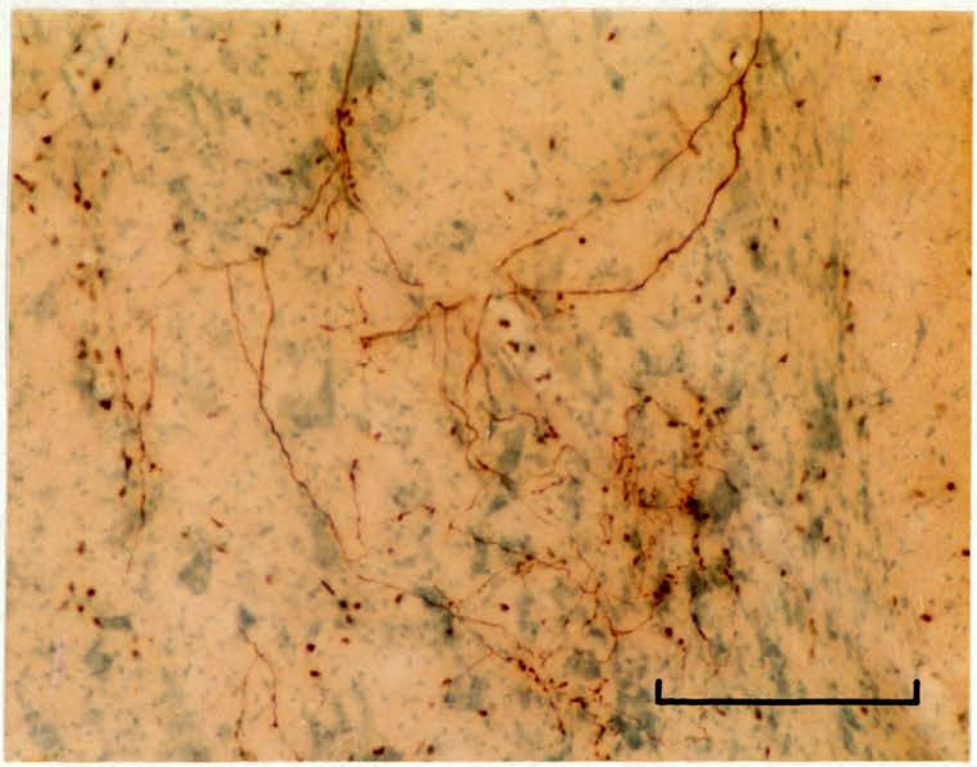
PART 3: Figure 16

Ia afferent fibre boutons.

The micrographs illustrate some of the terminal arrangements seen in the intermediate region. Collateral branching and numerous 'en passant' and terminal boutons create quite extensive arborizations. Boutons frequently appear to contact counterstained neuronal profiles. The boundary between the dorsal horn and dorsal columns is seen towards the right hand edge of these figures.

Scale Bar: 200 μm.

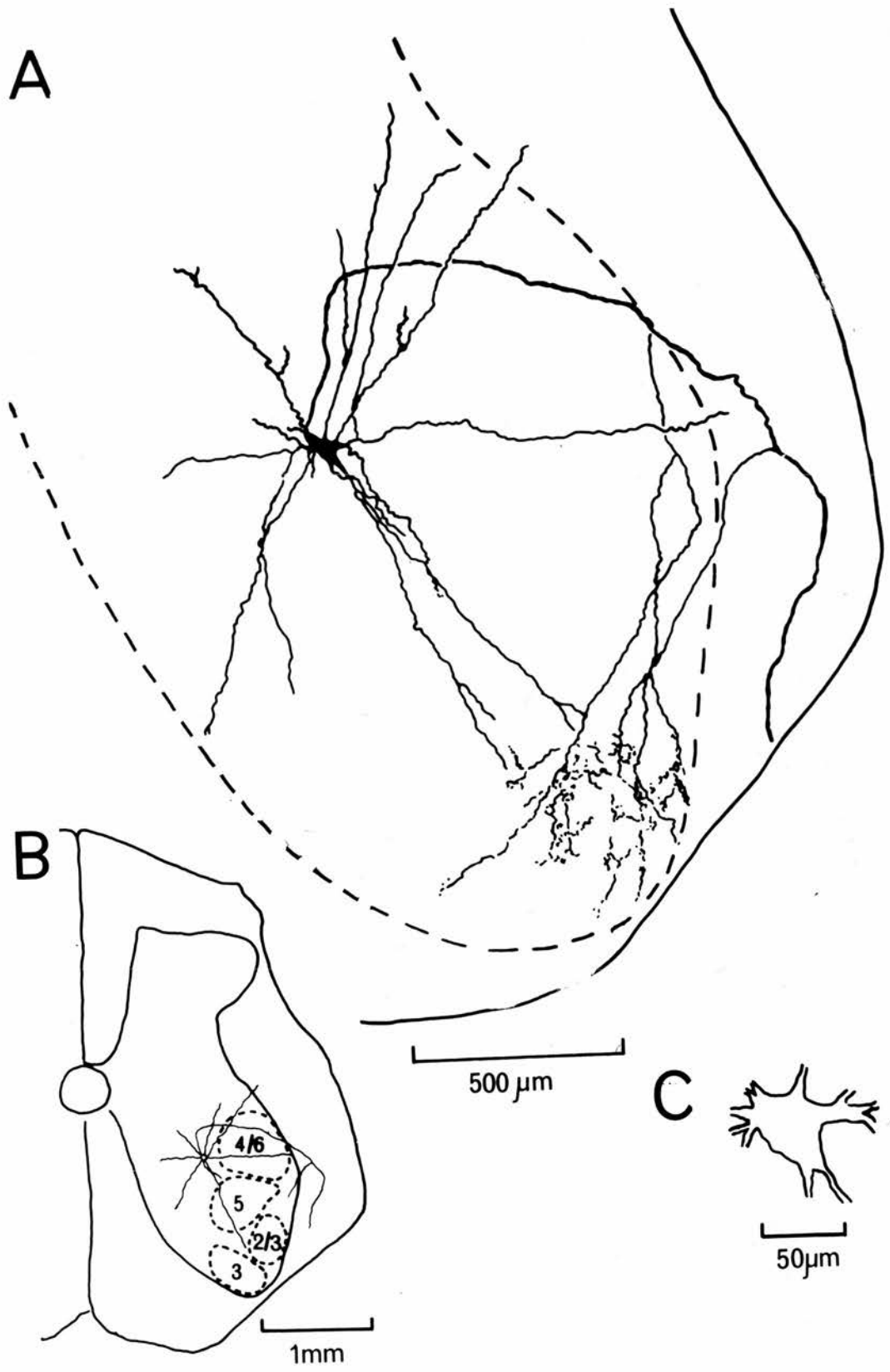




PART 3 : Figure 17

The anatomy of a presumed Ia inhibitory interneurone.

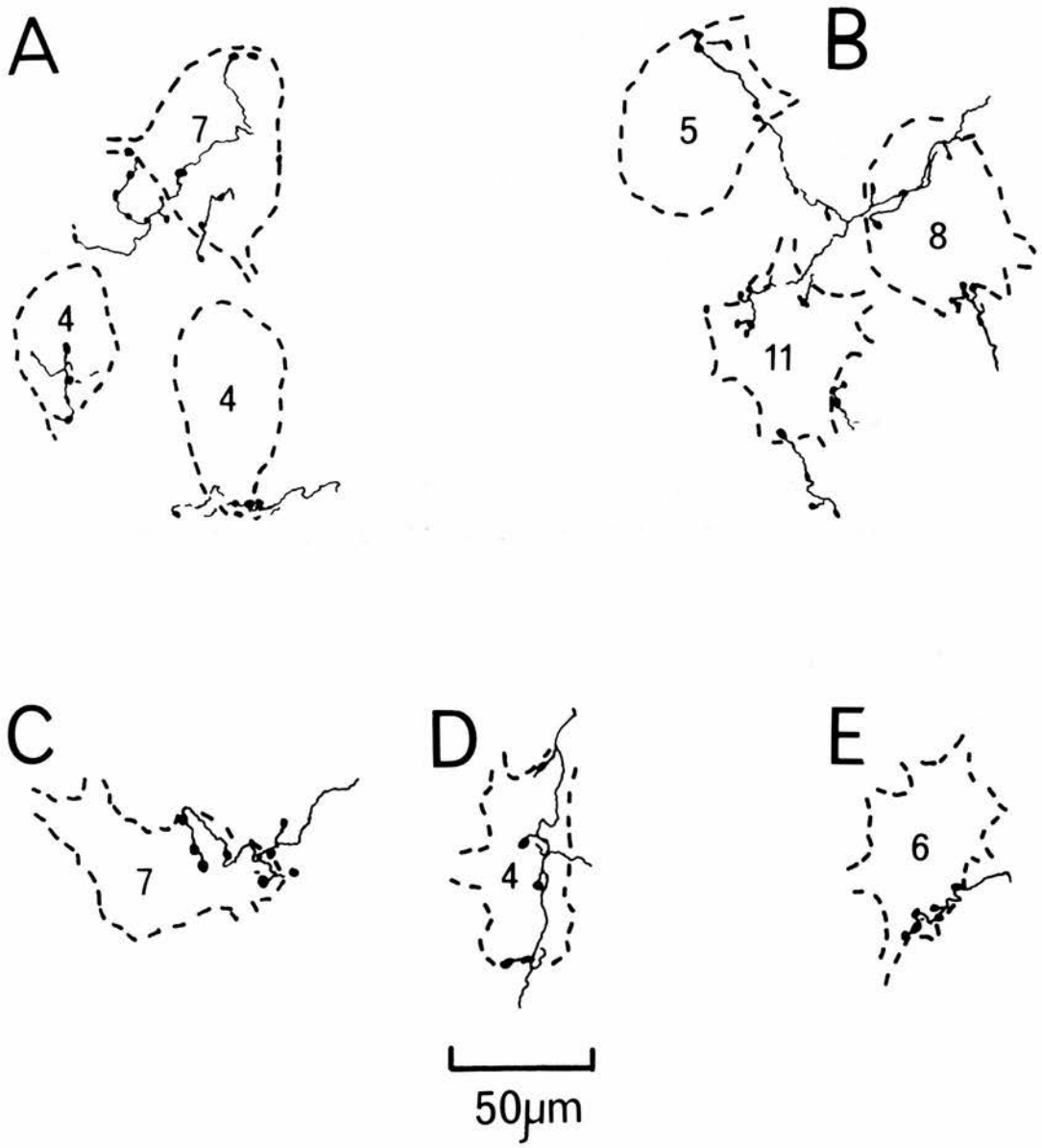
A. The cell (see text for details) is located in lamina VII. Dendrites are long (500 - 900 μm) and straight and radiate from the soma largely restricted to the transverse plane. One dendrite ran caudally for about 900 μm. The dendrites encompass a large area of the ventral horn but (see B), apart from one or two exceptions, do not penetrate the motor cell columns (Romanes, 1952). C illustrates the soma and dendritic origins. The axon (thick line in A) runs dorsally then laterally to enter the lateral white matter. In doing so it courses around the perimeter of the triceps motor pool. The axon ascended for at least 1.6mm (this was the extent of the processed tissue) and gave off two collaterals (200 and 700 μm rostral to the soma). Collaterals re-entered the grey matter and arborized extensively in motoneurone pools 2 and 3. Terminal branches and boutons of these collaterals overlapped longitudinally and together extended over 1.5mm of tissue.


A
500 µm
B
4/6
5
2/3
3
1mm
C
50µm

PART 3: Figure 18

Interneurone contacts upon motoneurones.

These reconstructions show some of the synaptic arrangements generated by the neurone shown in the previous figure. Cell outlines were drawn from counterstained sections (dashed lines) and the numbers refer to the number of contacts observed on each cell, including those on proximal dendrites. Most of the boutons arising from the labelled interneurone were involved in making juxtasomatic contacts on large ventral horn neurones (presumed to be antagonistic motoneurones) in the terminal regions. Each neurone received from 2 - 12 synapses (mean about 6.5).


A
7
4
4
B
5
8
11
C
7
D
4
E
6
50µm

PART 4 : THE MORPHOLOGY OF Ib AFFERENT FIBRES

Introduction

Impulses in group Ib axons from Golgi tendon organs produce di- or trisynaptic excitatory or inhibitory effects in α-motoneurones (Eccles et al., 1957a, b: Watt et al., 1976). Ib afferent fibres also excite, monosynaptically, some of the cells of origin of the DSCT and VSCT (Oscarsson, 1965, 1973; Lundberg, 1971) and cells in the intermediate nucleus (Lucas & Willis, 1974). But the central projections of Ib afferents have proved difficult to characterize anatomically. Rethelyi & Szentagothai (1973) tentatively identify Ib afferent collaterals in Golgi stained material as the relatively large diameter collaterals which arborize widely in the intermediate region (corresponding to the area in which FSP's > 20% of maximum were evoked by electrical stimulation of Ib fibres by Eccles et al., 1954) and do not reach the motor nuclei. The present work presents direct information on the central organization of Ib collaterals.

Results

These results were obtained from eleven adult cats, anaesthetized with chloralose ($70mg.Kg^{-1}$) and paralysed with gallamine triethiodide. Successful intrafibre staining was achieved with HRP injections of 150-200 nA. min. All the material was processed for HRP demonstration by the method of Hanker et al. (1977).

Thirteen Ib axons with their collaterals were stained, seven from Lg-S, three from Mg and three from muscles with axons in the posterior tibial nerve. The axons had peripheral conduction velocities of 80 - 98 $m.sec^{-1}$ (mean 87) and had no ongoing activity when first penetrated. Under the same experimental conditions as for the recording of Ia afferents, the Ib afferents required noticeable stretch (manual extension of flexion of joints) to excite them.

Collaterals of Ib afferent fibres. The distribution of Ib collaterals from the stained axons is shown in Fig. 1. The axons were stained for total lengths of 5.1 - 9.9 mm (mean 7.68 mm); ascending branches ranged from 5.0 - 9.6 mm and the descending branches from 0 - 3.8 mm. Most of the axons (except one axon from Lg-S) were traced into the dorsal roots. Usually they bifurcated soon after entering the cord to send main branches rostrally and caudally. A single PT Ib axon did not bifurcate but simply ascended from the level of its entrance zone. None of the main branches terminated as a collateral and can be presumed to extend for greater lengths and give off more collaterals. Generally the rostral branch of the stem axon was thicker than the caudal branch.

The distance between the most caudal and rostral collaterals from an axon ranged from 3.4 - 8.0 mm. Again, on the well founded assumption that HRP labels all the collaterals in between the extremes, useful data on

collateral distribution is obtained. A total of ninety nine collaterals were observed with a range of five to eleven from each axon (7.6 ± 2.1; mean ± S.D.). Of these, fifty seven were from ascending axons, thirty five from descending branches and seven from the axon which was not traced into the dorsal roots. The latter axon was probably part of an ascending branch. It was injected in the dorsal columns near the rostral end of L7 segment and as it was traced rostrally its course assumed a deeper position in the dorsal columns.

Collaterals were given off at intervals of 100 - 2600 μm (890 ± 506 μm; mean ± S.D.) which is not significantly different from the spacing of the Ia collaterals. However intervals on the descending branches of Ib axons were 200 - 1500 μm (690 ± 331 μm; mean ± S.D.), a much closer spacing than on the ascending branches (100 - 2600 μm; mean 1080 ± 524 (S.D.)). This difference is highly significant ($P < 0.001$ Student's 't' test). Again, as for Ia fibres, there was no significant difference in collateral spacing with distance from the dorsal root entry (except for ascending versus descending branch intervals) - which was in most cases in L7 segment.

Ib collateral morphology. The characteristic branching patterns of Ib collaterals are strikingly similar throughout the sample (see Figs. 2 - 11). Like the Ia collaterals, Ib collaterals shift rostrally from their origin (see the sagittal view in Fig. 6). They enter the dorsal horn at its dorsal or dorsomedial border

and run ventrally in a direct course through the grey matter to lamina V. Branching usually commenced in lamina V or ventral lamina IV and gave rise to an extensive fan-shaped arborization located mainly in the medial and central parts of lamina VI and in the dorsal part of lamina VII. Terminal branches and boutons were spread over a 400 - 800 μm wide area in the transverse plane (see Figs. 3, 4 and 11). But in contrast the rostro-caudal extent of the terminal arborization was restricted to 200 - 400 μm. This is illustrated best in the sagittal reconstructions (see Figs. 6, 7) which also illustrate how Ib collaterals run almost in parallel with each other and with a rostral tilt to their path. The limited longitudinal extent of individual arborizations meant that there were nearly always clear gaps of 300 - 400 μm between adjacent terminal fields even when the collaterals arose close together.

There were some variations in the general morphological pattern and distribution of terminals. Some collaterals (e.g. Fig. 8) had minor branches and boutons more dorsally than usual, in the central part of lamina IV. In other cases there were marked projections into more lateral portions of laminae V and VI (see Figs. 2, 7, 10). The latter observation applied particularly to collaterals arising from ascending axons in rostral L7 and caudal L6 segments. In all cases however, there was maintained a major terminal arborization in central and medial parts of lamina VI. When several collaterals

from a single ascending Ib axon were well stained there was usually an abrupt transition in collateral morphology - whereas a collateral might have terminals only in the medial part of the intermediate region, its rostral neighbour and subsequent more rostral collaterals projected additionally to these lateral regions.

Terminal arborizations and synaptic boutons of Ib collaterals. Fine terminal branches (see Figs. 3, 4, 11, 12, 13) were located mainly in lamina VI and in dorsal lamina VII. They were generally oriented within the transverse plane (vertically, horizontally or obliquely). This terminal organization is expressed in the overall morphological pattern of a wide transverse arborization with limited longitudinal spread.

Boutons 'en passant' were predominant in Ib terminal arborizations and, like the fine branches, lay in the transverse plane. There were however frequent examples of single terminal boutons at the end of short fine stalks. Single Ib collaterals gave rise to 56 - 384 boutons (179 $\pm$ 114 mean $\pm$ S.D.). This reflects the profuse terminal branching and occurrence of 'en passant' boutons and is a much greater number of boutons than is produced in the intermediate region by single Ia collaterals.

In counterstained material contacts were observed on cell bodies and proximal dendrites in laminae V - VII (and IV in the few examples of terminations in this area). Up to 6 boutons, on 2 - 3 terminal branches formed

synaptic arrangements with small - medium sized lamina VI neurones (Fig. 13). Boutons ranged in size from 1.0 x 1.0 to 5.0 x 3.0 μm (3.1 ± 0.97 x 1.7 ± 0.53 μm: mean ± S.D.).

Discussion

The results confirm the suggestions of Rethelyi & Szentagothai (1973) on the identity of Ib collaterals. The collaterals do indeed generate characteristic terminal arborizations in the intermediate region. The zone of termination corresponds very closely to the localization of FSP's described by Eccles et al. (1954). They (Eccles et al., 1954) used electrical stimulation to separate the effects of Ia and Ib fibres (see Bradley & Eccles, 1953). That technique is not applicable to most hindlimb muscles so that single fibre techniques as used in this work are much more relevant to current physiological and anatomical problems. Adequate stimulation (see Lucas & Willis, 1974; Lundberg & Winsbury, 1960; Stuart et al., 1970; Brown et al., 1967) can selectively activate Ia fibres, but directly to activate a pure group Ib population of fibres is a difficult problem. In this study, application of criteria laid down in many previous investigations (see Matthews, 1972) were used to classify single Ib afferents. In the event, all of the Ib axon collaterals had comparable anatomic features and were distinctly different from any other kind of primary afferent collateral. The question may be posed that the

present observations might only be examples of incompletely filled Ia collaterals which also arborize in the intermediate region. In this sample, no branches or boutons were found ventral to the central canal, even when collaterals were very densely stained. All collateral branches in the intermediate region subdivided and ended in terminals. Finally the actual terminal zone of the Ib fibres in the intermediate region was more profuse in extent and numbers of boutons than the best stained Ia arborizations.

In fact some collaterals did project to lateral regions and terminated slightly dorsal and medial to the triceps surae motor nucleus. These collaterals arose from axons which had other well stained collaterals terminating only in medial regions. Hongo et al. (1978) in a preliminary study published while this work was under way concluded from a very small sample that there were two types of Ib afferent fibre; one which projected to both the intermediate region and the ventral horn and one which terminated only in the intermediate region. The present results refute this suggestion and indicate that the situation arises because individual collaterals from a single axon can have differential projections. Collaterals arising several millimeters rostral to the dorsal root entry of the Ib axon often project ventro-laterally to lateral laminae V, VI and dorso-lateral lamina VII. They do not actually enter the motor nucleus. The functional significance of this projection is discussed later.

There was also seen, on occasion, a Ib projection to more dorsal laminae of the dorsal horn. There are so far no descriptions of Ib activated neurones in these regions. It is not unreasonable to suggest, in the absence of a more exciting possibility, that these rare arborizations are involved in contacts on the dendrites of deeper neurones. However, some of these terminal regions contain many boutons and one axon (Fig. 8) had at least three collaterals which all had this dorsal projection. It is therefore suggested that Ib axons distribute terminals over at least one segment of the cord, above and below the dorsal root entry. The collaterals caudal to the entry zone tend to be closer together than those on the ascending branch. The significance of these shorter inter-collateral distances is obscure as yet. The wide fan-shaped arborization had restricted rostro-caudal extent and there was never any overlap between adjacent collaterals, in contrast to the Ia collaterals. The collaterals thus occupied discrete zones of the spinal cord, with all terminal branches and boutons oriented in the transverse plane (see the summary diagrams in Fig. 14). This tallies with the known dendritic tree patterns of cells in this area (Fyffe, 1980) and it is appealing to suggest that the frequently observed chains of 'en passant' boutons along Ib terminal branches make climbing type synaptic contacts along such dendrites. As far as juxtasomatic contacts on intermediate region neurones are concerned, terminals are

often provided by two or more branches of the collateral and were more frequently observed than upon investigation of Ia collaterals. Indeed several workers (Eccles, 1965; Lucas & Willis, 1974) have noted that the majority of interneurones in the intermediate region which are monosynaptically activated by Group I fibres receive their excitation preferentially from Ib afferents rather than from Ia fibres. This correlates with the more extensive Ib projections.

Clearly then, intermediate region neurones are involved in the Ib pathways. Such effects as autogenetic inhibition and reflex facilitation of antagonistic motoneurones (Laporte & Lloyd, 1952) could be associated with the lamina VI neurones which project to the motor nuclei (Czarkowska et al., 1976). The Ib produced presynaptic inhibition of Ia, Ib and cutaneous afferent fibres (Eccles, Schmidt & Willis, 1963; Eccles, Holmquist & Voorhoeve, 1964) might also involve participation of the lamina VI neurones. Involvement of intermediate region interneurones in other possibly Ib generated effects, e.g. the inhibition of fusimotor neurones (Eldred, Granit & Merton, 1953) which is perhaps a component of the clasp knife effect (Sherrington, 1906; Matthews, 1972) or in crossed spinal reflexes (Holmquist, 1961; Perl, 1958, 1959) must await determination of the synaptic actions of the cells to be verified.

There is detailed electrophysiological information that Ib afferents distribute their effects

monosynaptically to pathways ascending from the spinal cord to the cerebellum (see review by Oscarsson, 1973). The cells of Clarke's column (see Rexed, 1954) extend from the fourth lumbar segment to thoracic segments. The DSCT arises from these neurones. But primary afferents entering the cord at lower lumbar levels have to ascend for at least two segments before giving collateral inputs to these cells. None of the present sample of 13 axons was stained for that distance but if the collateral patterns of L7 and L6 remain characteristic in upper lumbar segments then it would provide a sound anatomical basis for monosynaptic Ib excitation of DSCT cells. Recently Aoyama, Hongo & Kudo (1973) demonstrated that in segments L5 and L6 of cat there are intermediate region neurones (not anatomically belonging to Clarke's nucleus) which project ipsilaterally to the cerebellum. These cells receive convergent monosynaptic Ia and Ib excitation. Their locations correspond to the terminal areas of the Ib afferent fibre collaterals. Thus not all of the Ib effects mediated by cells in the segment of entry should be considered as being actions restricted to the segmental or spinal level.

Additionally Ib afferents monosynaptically excite some VSCT neurones (Eccles et al., 1961; Hubbard & Oscarsson, 1962; Burke et al., 1971; Lundberg & Weight, 1971; Lindström & Schomburg, 1974). Cells of origin of the VSCT are distributed in various regions of the cord. Hubbard & Oscarsson (1962) described the localization of

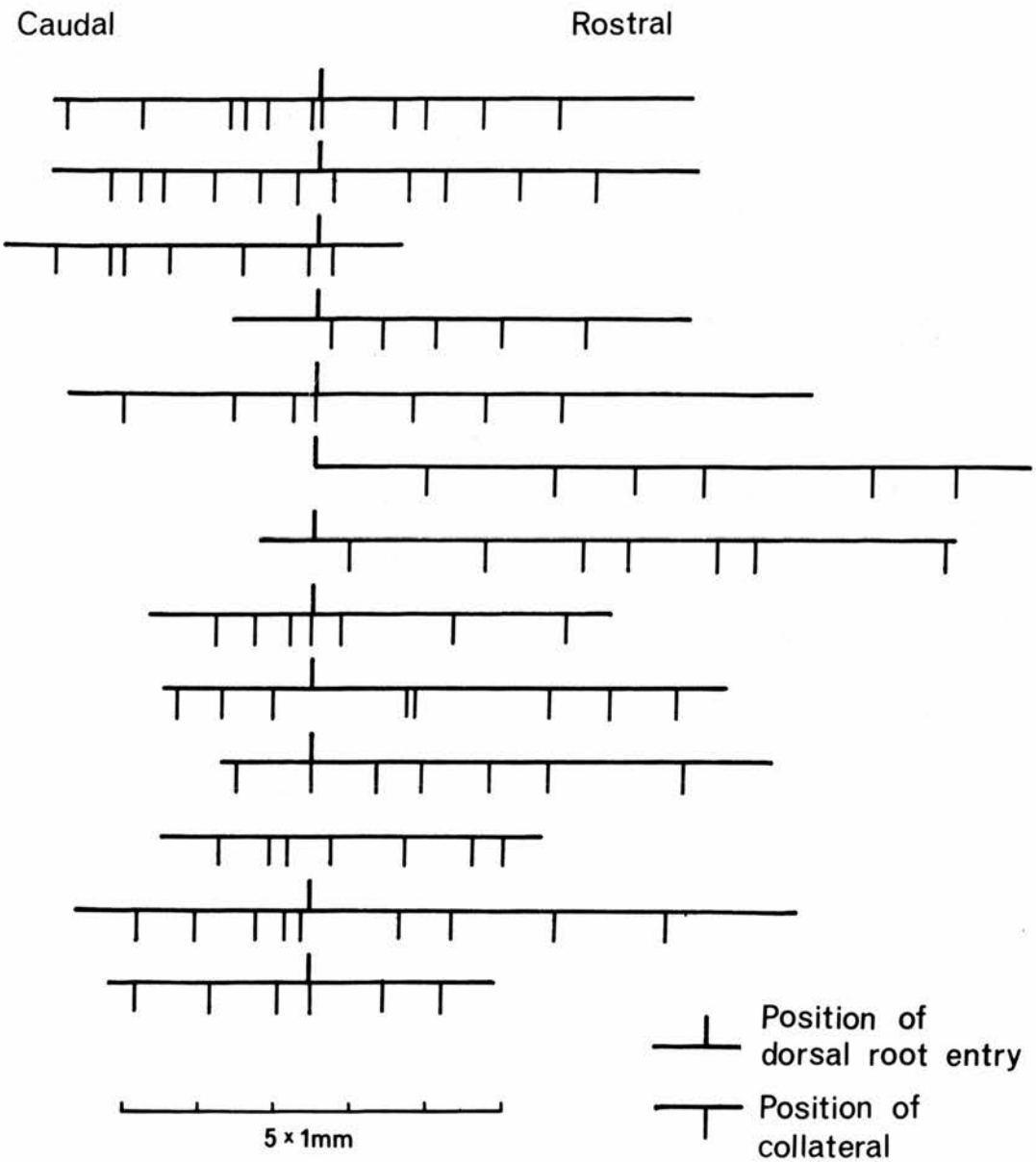
VSCT cell bodies as a widespread region across the base of the dorsal horn and dorso-medial parts of the ventral horn. Later Burke et al. (1971) described the spinal border cells of Cooper and Sherrington (1940) as being the major source of the VSCT and noted that their main monosynaptic primary afferent input was from Ia afferent fibres although some also received monosynaptic Ib input. Since the cells of Eccles et al. (1961) received primarily Ib inputs the two sources of the VSCT may complement each other. The present description of Ib collateral organization suits both schemes. The more dorsal neurones might be activated by the main Ib arborization in the intermediate region; spinal border cells by laterally projecting branches. It is interesting to note that while spinal border cell VSCT neurones have been found as far caudally as L6, Ib collaterals at that level (the most rostral collaterals successfully stained by our injections at L7) project laterally in lamina VI and into the dorso-lateral part of lamina VII. VSCT cells have been intracellularly stained in these regions (Jankowska & Lindström, 1970). VSCT cell dendrites could also be contacted by Ib boutons carried on more medial collateral branches since they also overlap with the dendritic tree areas of the VSCT neurones.

PART 4 : Figure 1

Branching patterns of Ib afferent fibres.

This diagram represents data from the thirteen stained Ib afferent fibres. The total stained length of each axon is shown, together with (where possible) the position of its entry into the spinal cord through the dorsal root and the origin of stained collaterals. The dorsal root entry positions have been aligned in this figure for simplicity. Twelve of the axons were traced into the dorsal root, all but one of these could be seen to bifurcate upon entering the cord. None of the main ascending or descending axons terminated as a collateral or projection into the grey matter; rather, towards the rostral and caudal extremes staining intensity became progressively fainter.

Ib Afferent fibre branching patterns.

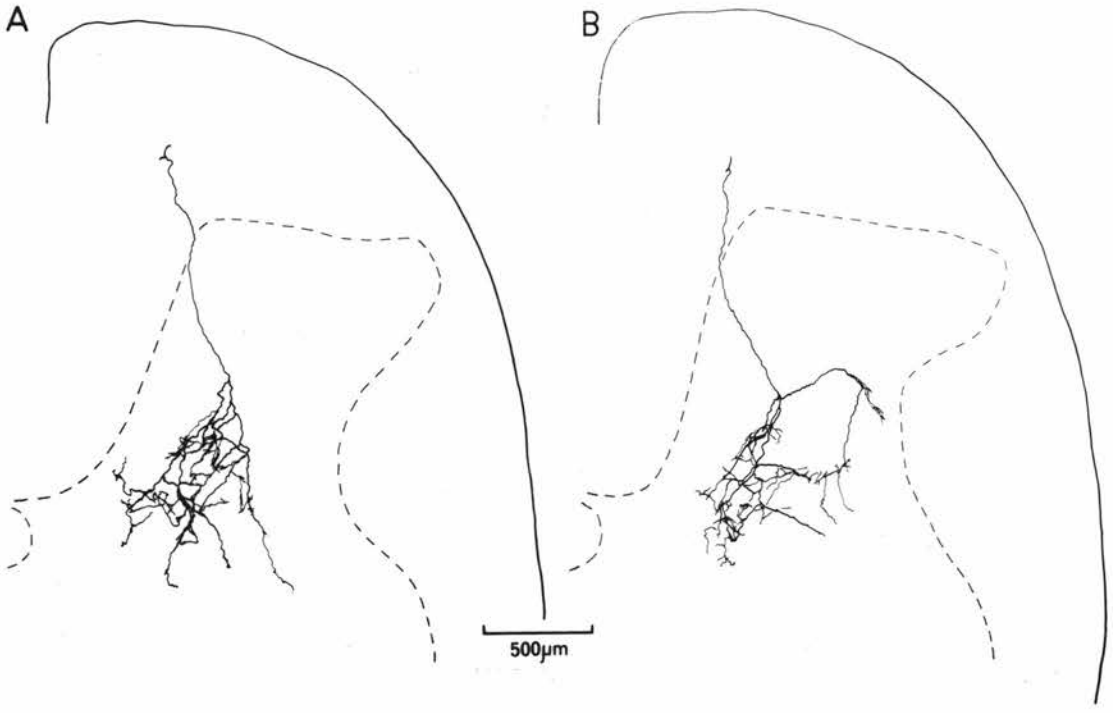


PART 4 : Figure 2

Ib afferent fibre collaterals.

These reconstructions, in the transverse plane, are of two adjacent (A more caudal) collaterals from the ascending branch of a Lg-S Ib afferent. The collaterals run ventrally through the dorsal horn and generate extensive arborizations in the medial half of the intermediate region, with boutons distributed in laminae V, VI and the most dorsal levels of VII. There are no boutons dorsal to lamina V or ventral to the central canal. The rostral collateral (B) especially makes additional projections to lateral parts of lamina V and VI.

The collaterals are organized essentially in two dimensions, in the transverse plane, as viewed, and there is thus little rostro-caudal distribution of boutons. The terminal arborizations do not overlap with each other.

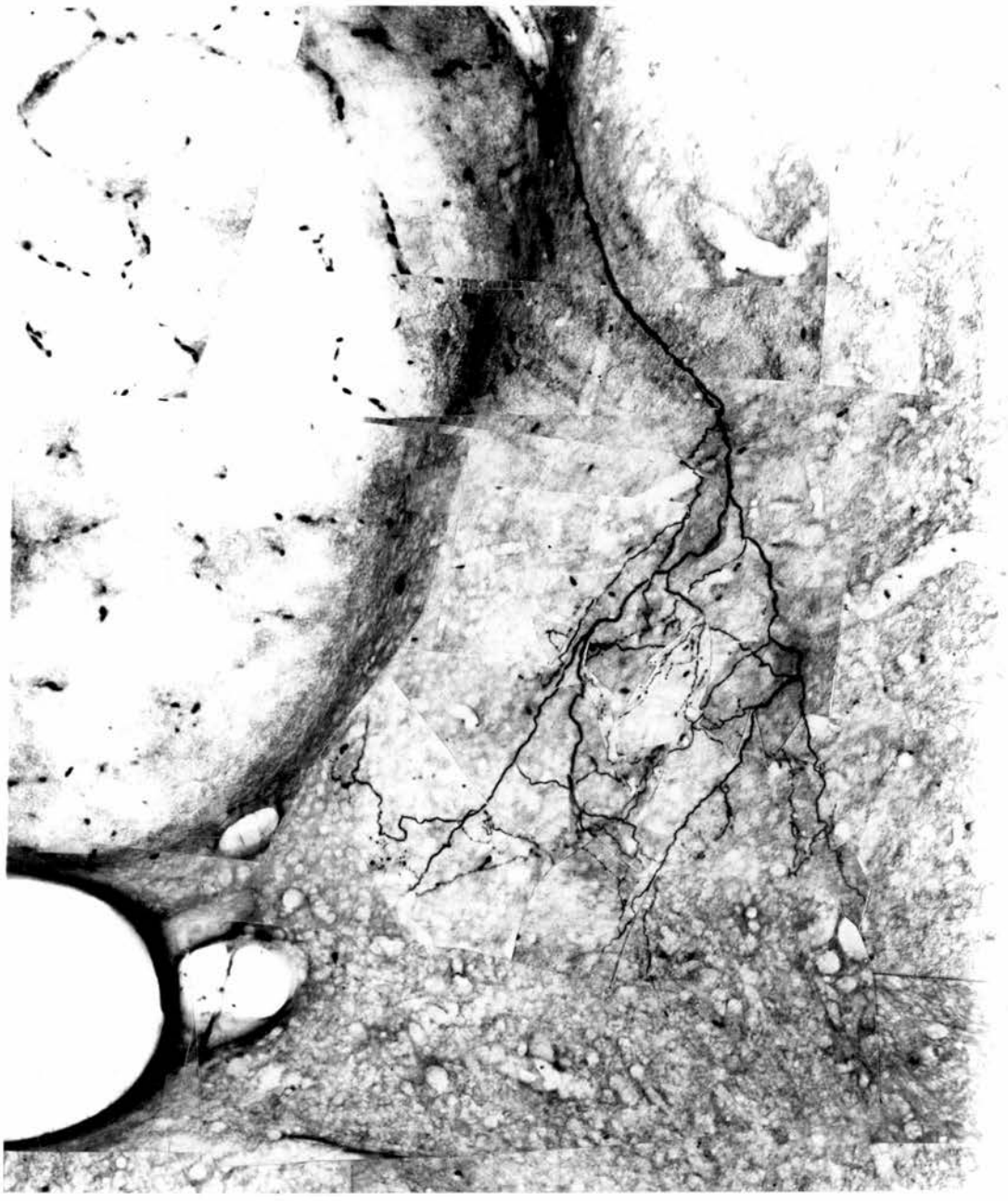

A
B
500μm

PART 4 : Figure 3

Collateral arborization of a Ib afferent fibre.

This collateral, from the ascending branch of a Lg-S Ib afferent fibre is also reconstructed in Fig. 2A. The main collateral branch enters the grey matter from the dorsal columns. It runs ventrally to lamina V before repeatedly dividing to form extensive terminal arborizations in the medial part of laminae V and VI, also projecting close to the central canal. Even in this fairly low power photomontage presentation boutons can be seen on some of the fine terminal branches.

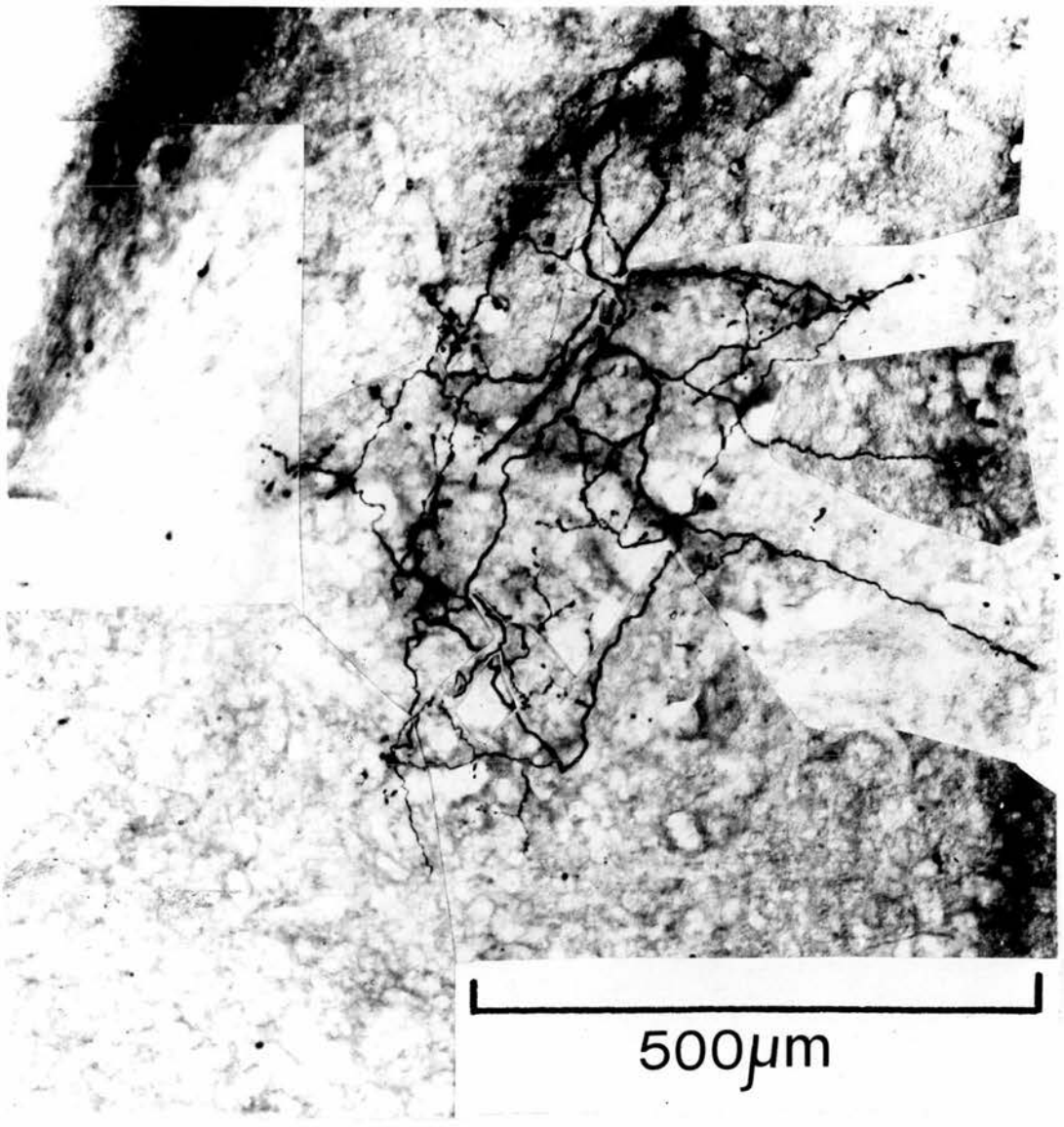
From 100 μm thick transverse sections of spinal cord.


500 μm

PART 4 : Figure 4

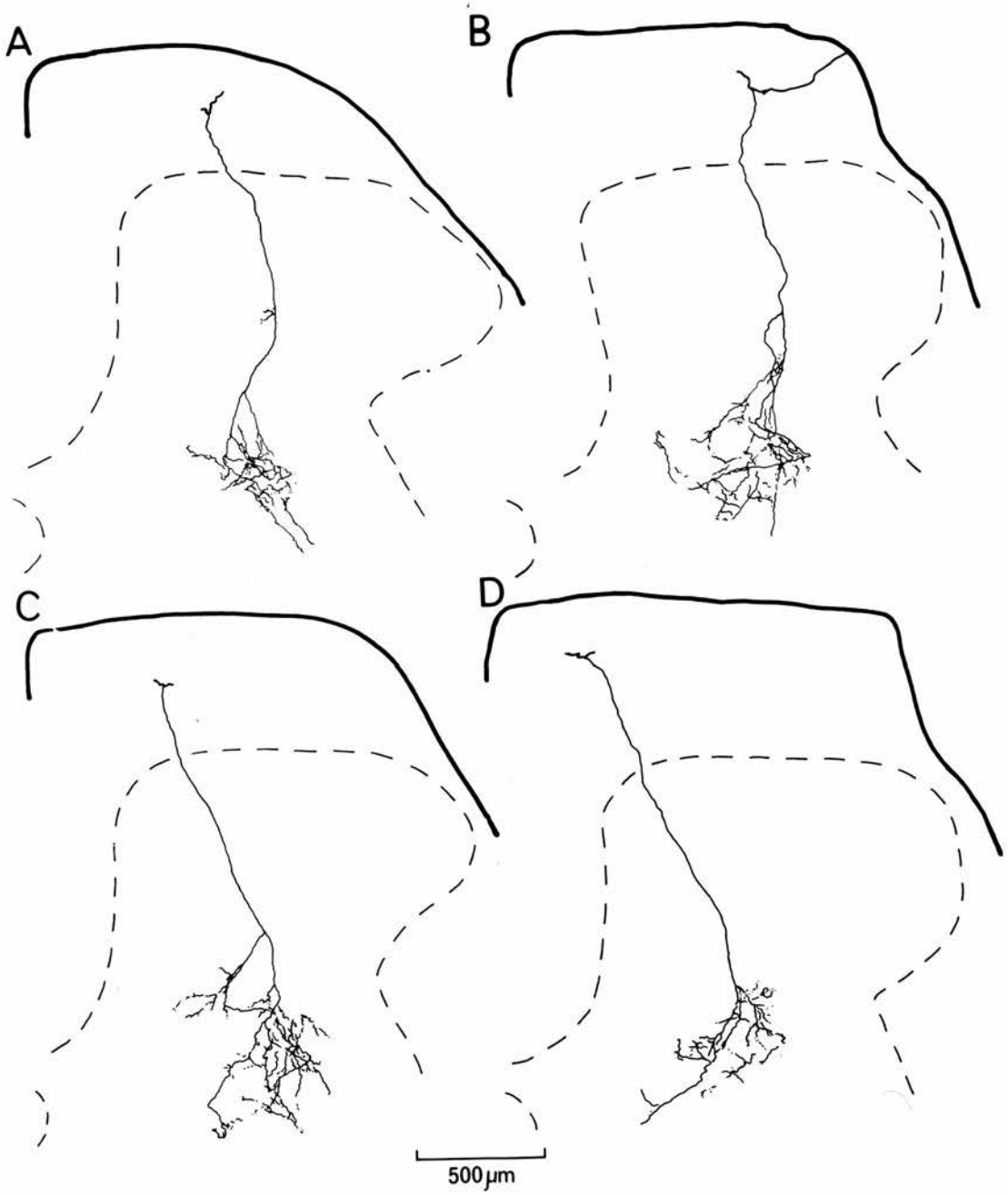
The micrograph illustrates some details of the fine branching and terminal arrangements in the terminal arborization of the collateral reconstructed in figure 2B. Medial is to the left - part of the medial border of the dorsal horn can be seen. Some of the collateral branches extend out of the main arborization region and are directed to more lateral areas.

Ib Terminal Arborization.



PART 4 : Figure 5

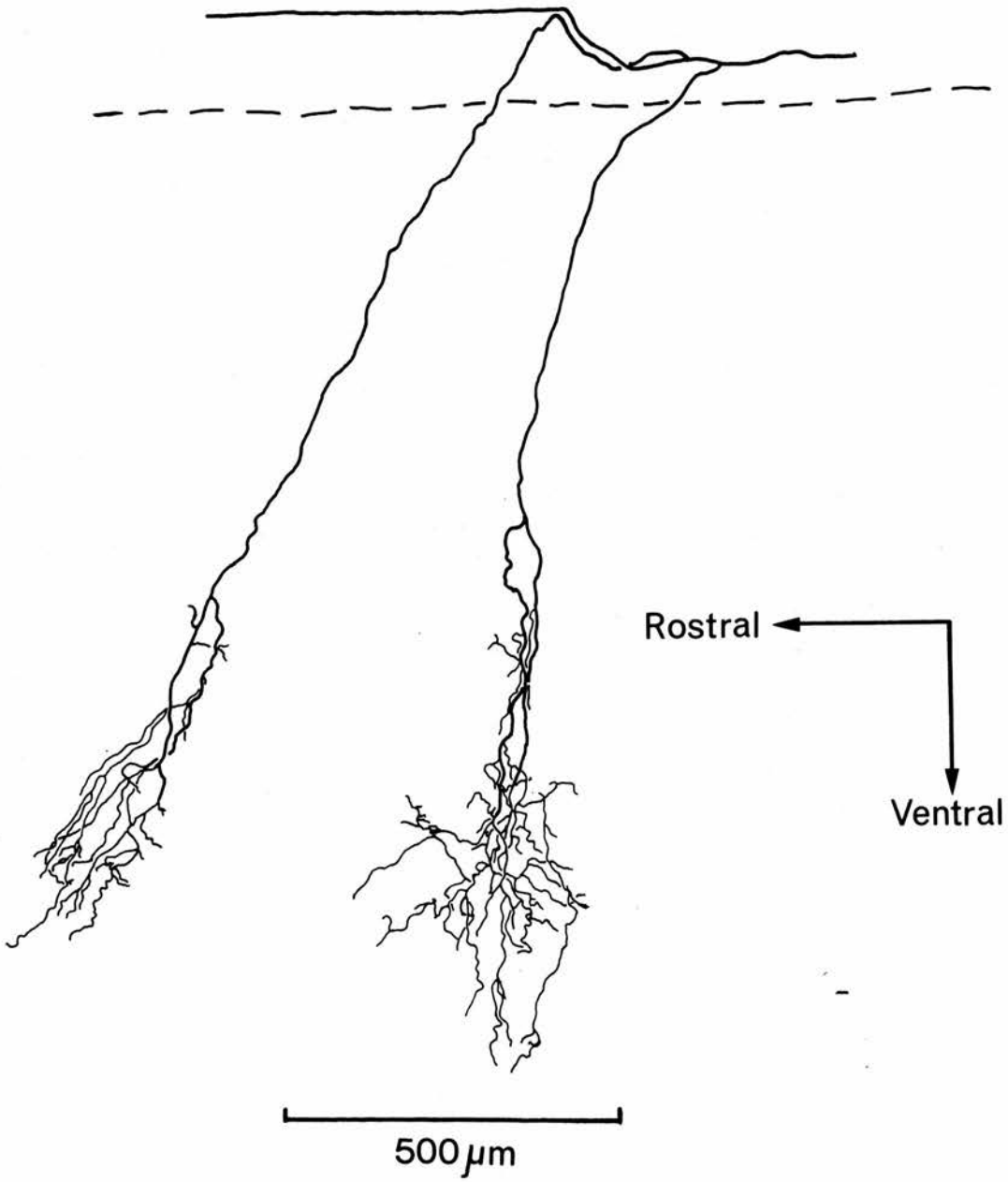
This figure shows reconstructions, from transverse sections, of four adjacent collaterals from a Lg-S Ib afferent fibre. Collateral A is most caudal, D most rostral. A and B arise from the descending branch of the axon near to the dorsal root entry, C and D from the ascending branch, up to 2.0mm rostral to the entry zone.


A
B
C
D
500 µm

PART 4 : Figure 6

Ib afferent fibre collaterals.

This reconstruction, from 100 μm thick sagittal sections of spinal cord, shows two adjacent collaterals from the ascending branch of a Lg-S Ib afferent. The main Ib axon ascending the dorsal columns is shown as a solid line. The dashed line indicates the dorsal border of the dorsal horn. Both collaterals have a cranial trajectory as they pass ventrally through the grey matter, so that the terminal arborizations are rostral to the level of collateral origin. Despite the close proximity (within 50 μm) of the origins of these collaterals on the parent axon, there is no overlap between the two arborizations. Boutons were located at depths which would correspond to laminae V - VII. It can also be seen in this plane of view that the rostro-caudal extent of each arborization is restricted to less than 400 μm.

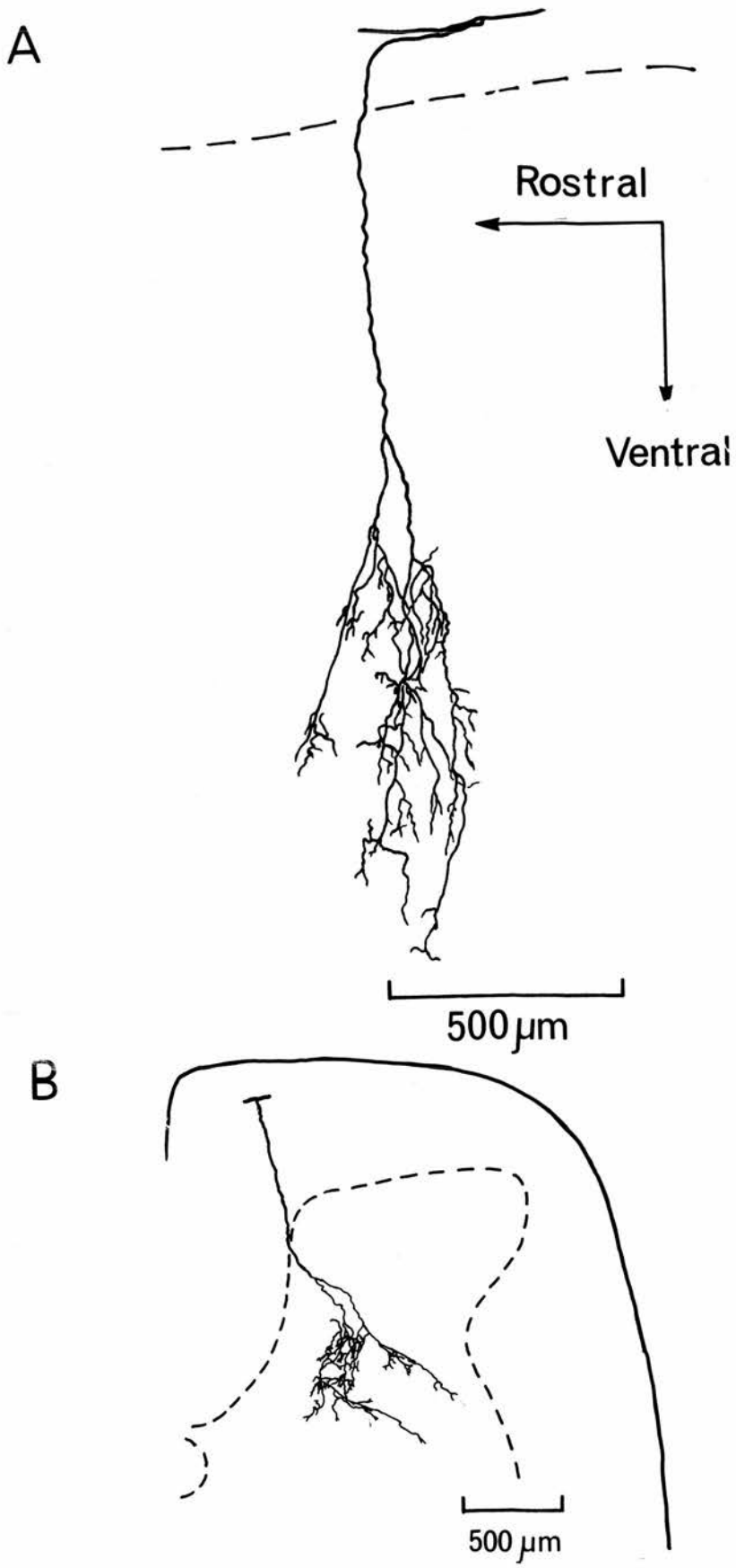

Rostral
Ventral
500 μm

PART 4 : Figure 7

Ib collaterals.

A is a sagittal view of a Mg Ib afferent collateral. Note again the restricted rostro-caudal development.

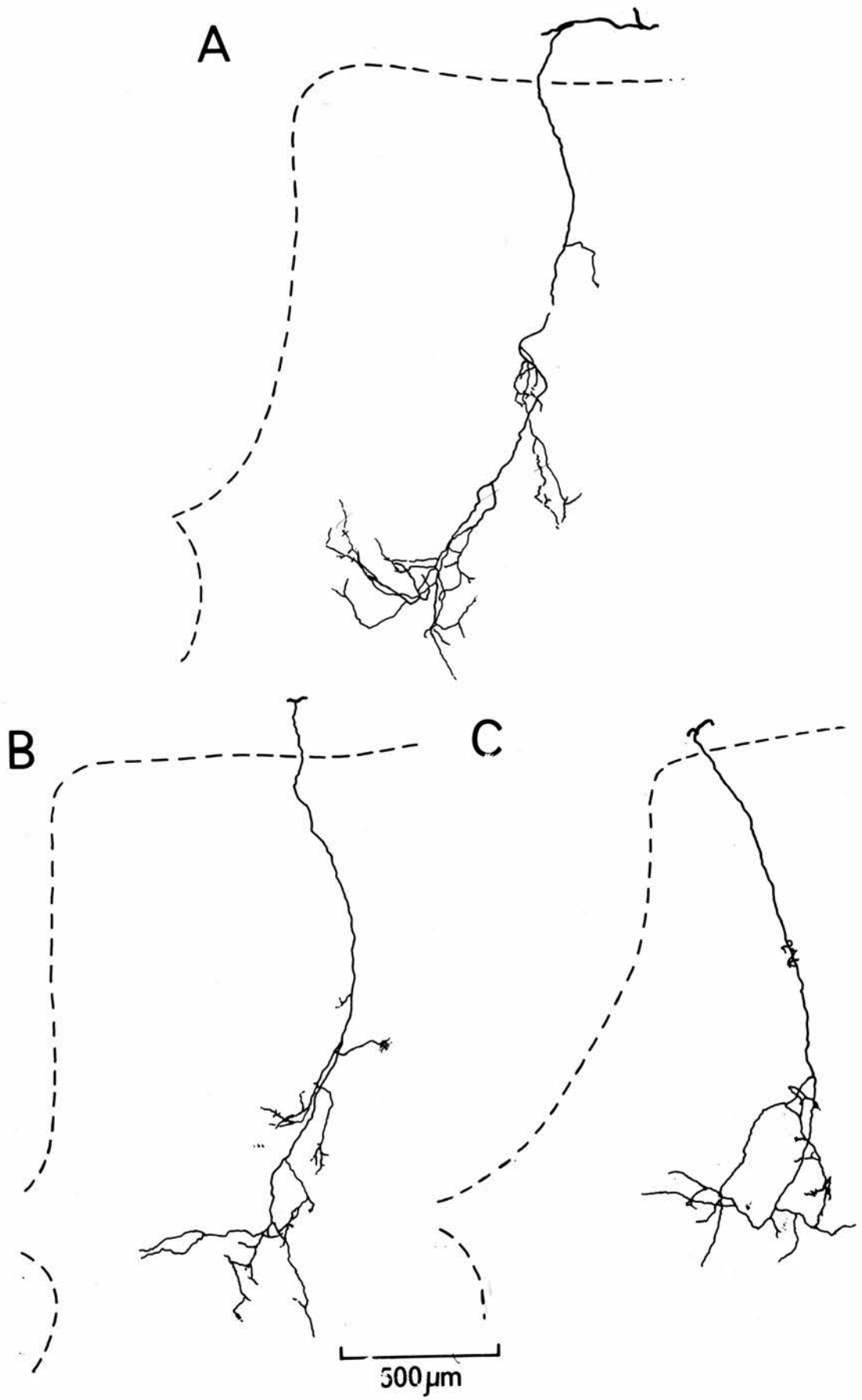
B is a reconstruction of a Lg-S Ib collateral in L_6 spinal segment - the parent axon is located quite superficially in the dorsal columns, some 4.5mm rostral to its point of entry to the cord via an L_7 dorsal root. The collateral is similar to those previously illustrated in having terminals in medial laminae V and VI. It differs however in that branches also extend laterally and terminate in lateral lamina VI and dorso-lateral lamina VII. There are some boutons also in the central part of lamina VI.


A
Rostral
Ventral
500 µm
B
500 µm

PART 4 : Figure 8

Ib collaterals.

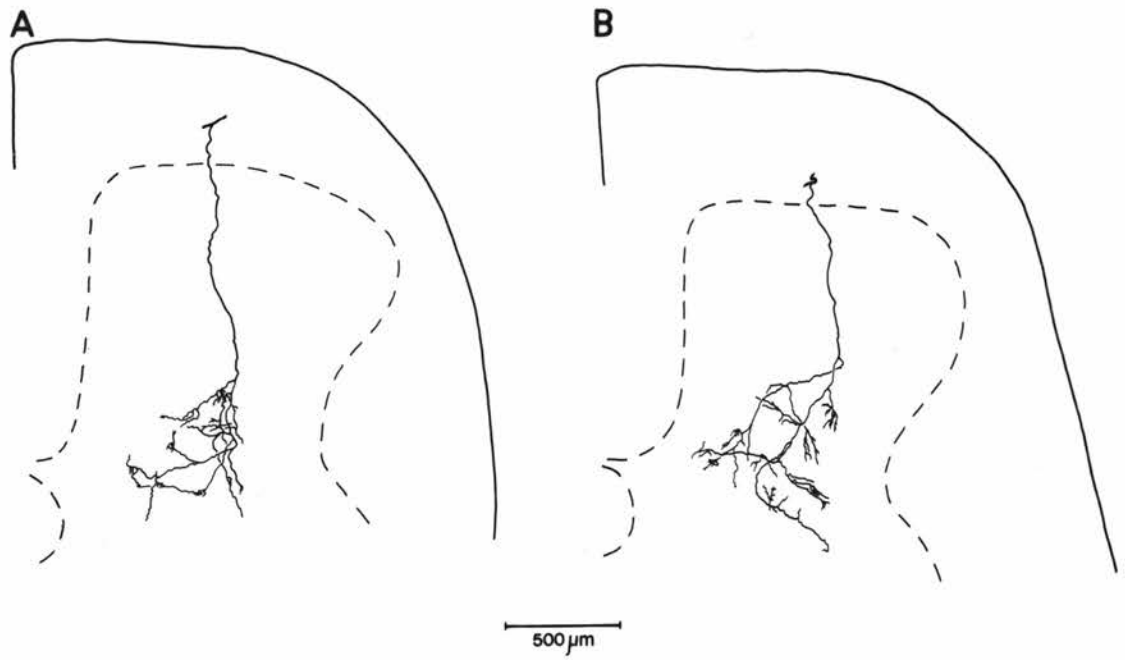
These three adjacent collaterals, from the descending branch of a Lg-S afferent fibre are interesting because they demonstrate some variability compared to most of the other Ib collaterals investigated. A is rostral and C is most caudal. Each collateral generates terminal arborizations in the medial half of the intermediate region but also sends boutons to discrete areas more dorsally in the centre of the dorsal horn.


A
B
C
500 µm

PART 4 : Figure 9

Ib collaterals.

This figure shows reconstructions of two collaterals from different Mg Ib afferent fibres. Both were from ascending axons, and both have essentially similar terminal regions, with extensive arborizations in the intermediate region.

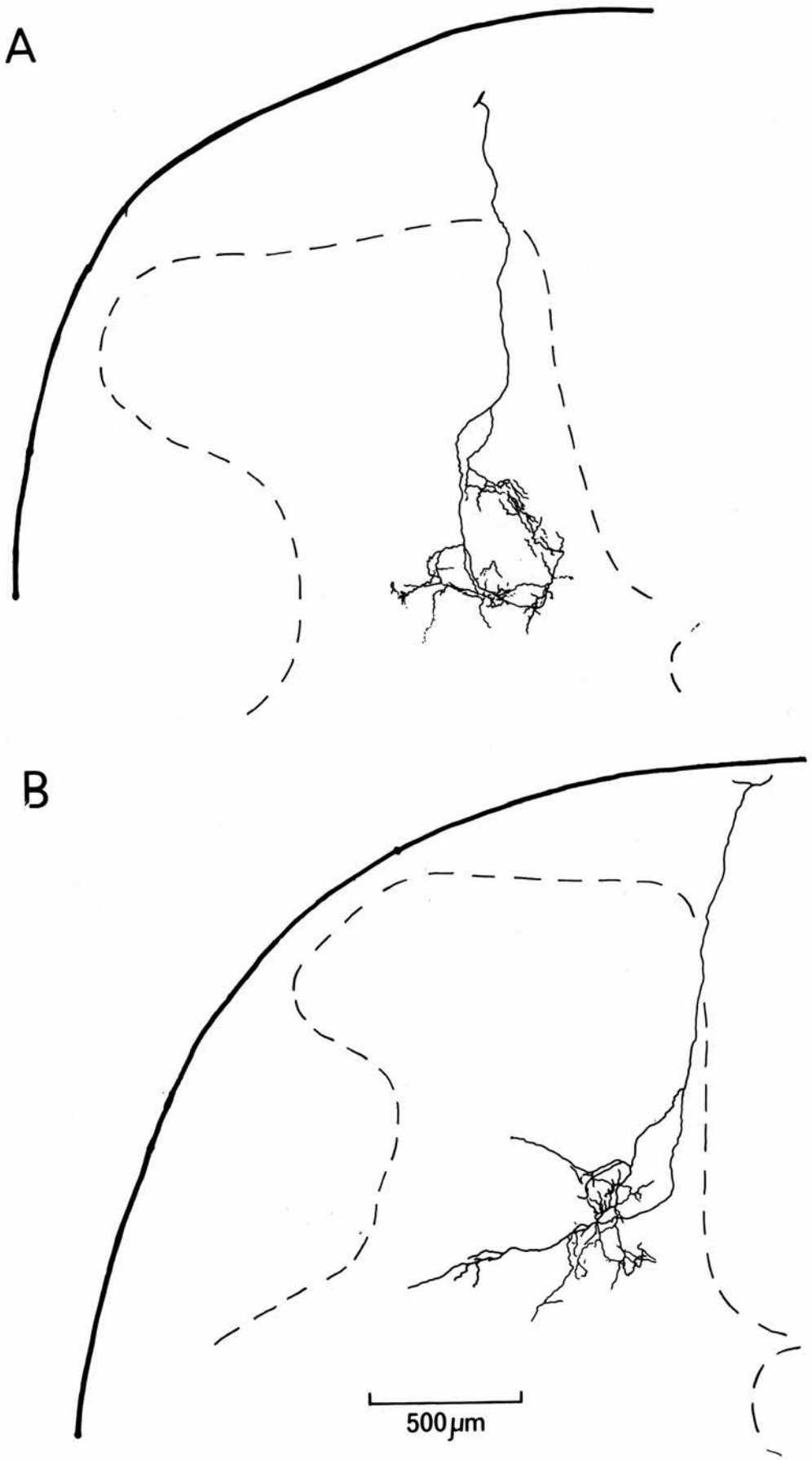

A
B
500 μm

PART 4 : Figure 10

Ib collaterals.

Two adjacent collaterals from a single Ib axon (ascending branch: B more rostral). Collateral B has a prominent projection to the lateral part of lamina VI as well as the typical fairly extensive arborization in the medial half of the intermediate region.

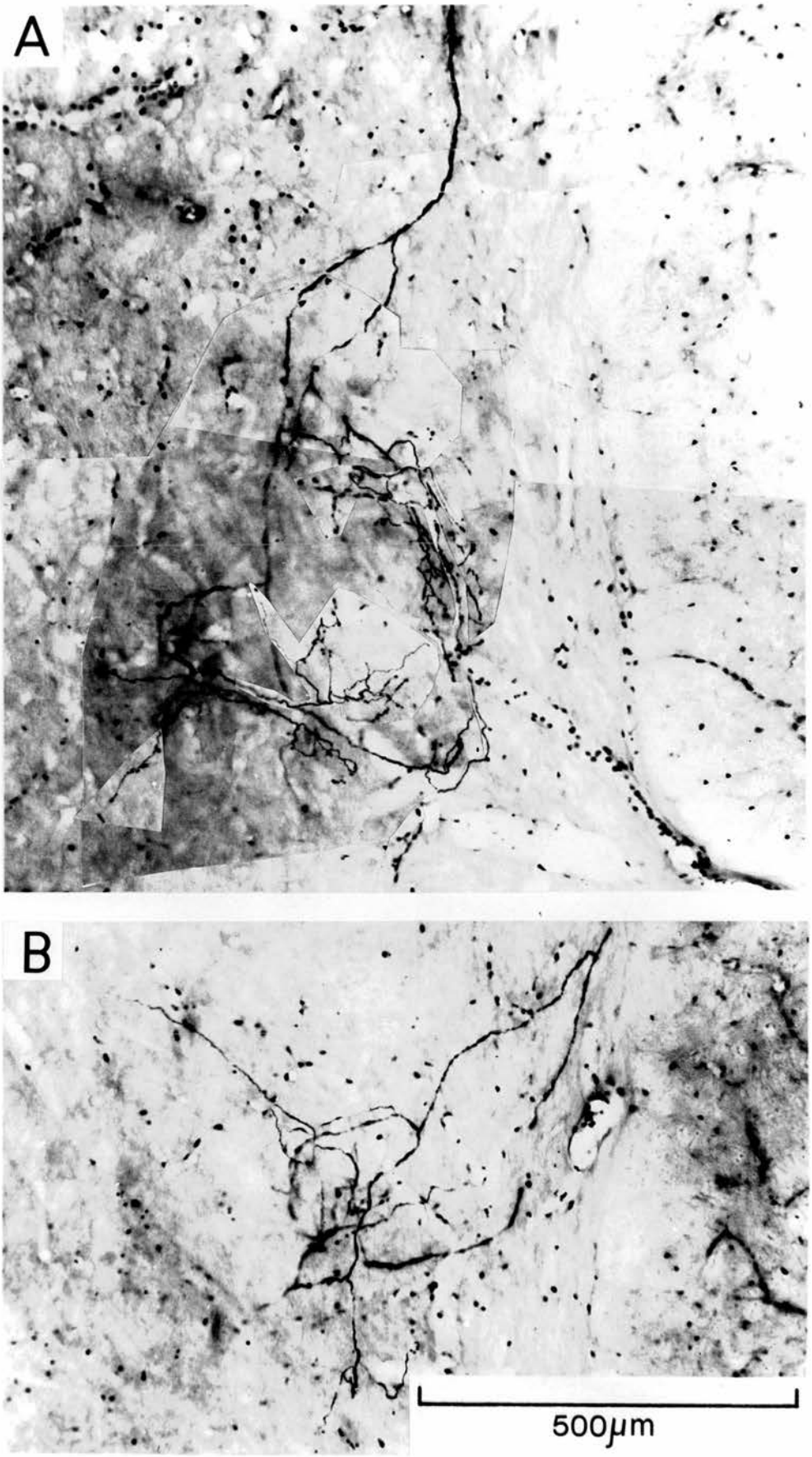
Photomicrographs of parts of these collaterals are shown in the next figure.


A
B
500 µm

PART 4: Figure 11

Ib collateral terminal arborizations.

These micrographs, from single 100μm thick transverse sections of cord, illustrate part of the terminal regions of the collaterals reconstructed in the previous figure. The collateral branching is seen, and even some bouton carrying terminal axons are resolved. Terminal axons and boutons are organized in the plane of the section.


A
B
500μm

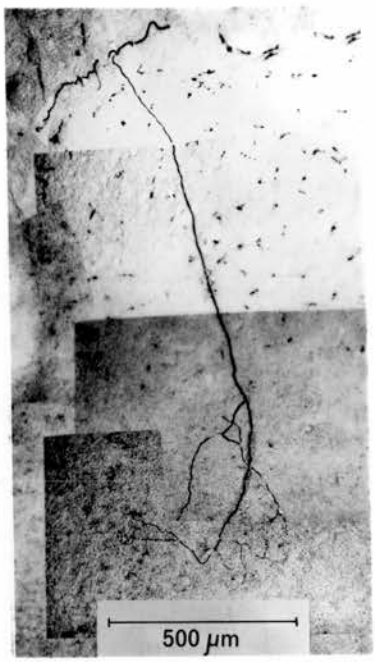
PART 4 : Figure 12

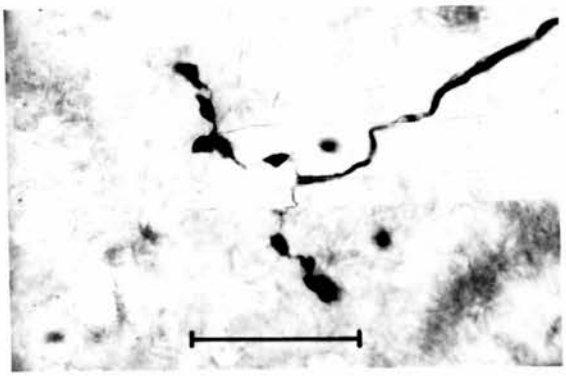
Ib collateral arborizations.

The upper photomontages show Ib collaterals from different axons. Both enter the grey matter at the dorsomedial edge of the dorsal horn and course ventrally to terminate in the medial parts of laminae V and VI. However the collateral on the left (see also fig. 8C) also has a minor arborization situated in a more dorsal position.

The lower micrograph shows details of the terminal part of a Ib axon collateral and its synaptic boutons. The scale bar is 20 μm.


500 μm


500 μm



PART 4 : Figure 13

Terminal arborizations and synaptic boutons of Ib afferent fibre collaterals.

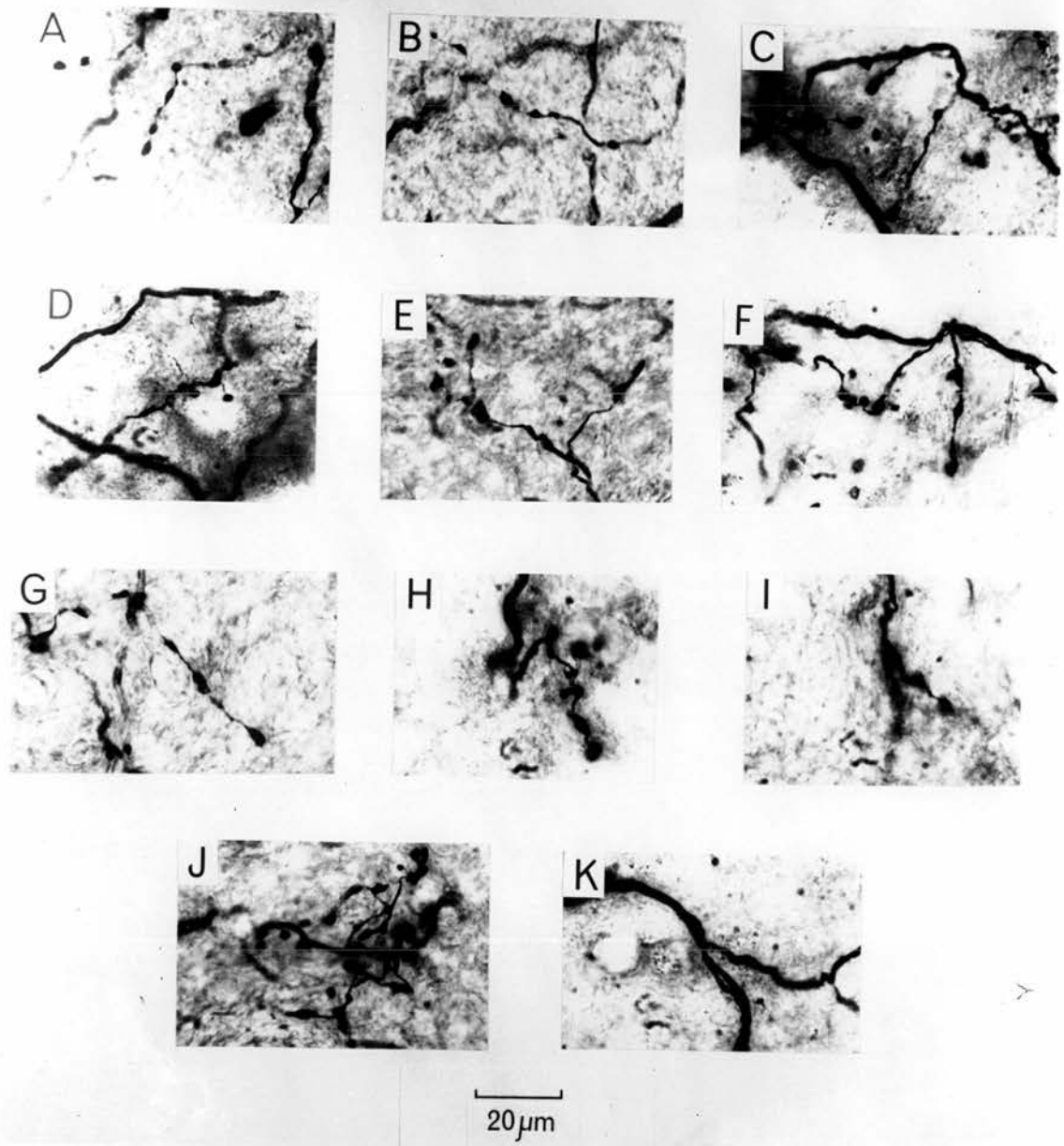
A - F are terminals in the medial half of the intermediate region.

G - I are examples of terminals of branches which project to more lateral zones.

J shows some of the boutons which comprised one of the unusual terminal zones located dorsally in lamina IV.

K shows branching of a Ib collateral. The division occurs at a presumed node of Ranvier (note constriction) and generates <u>three</u> daughter branches.

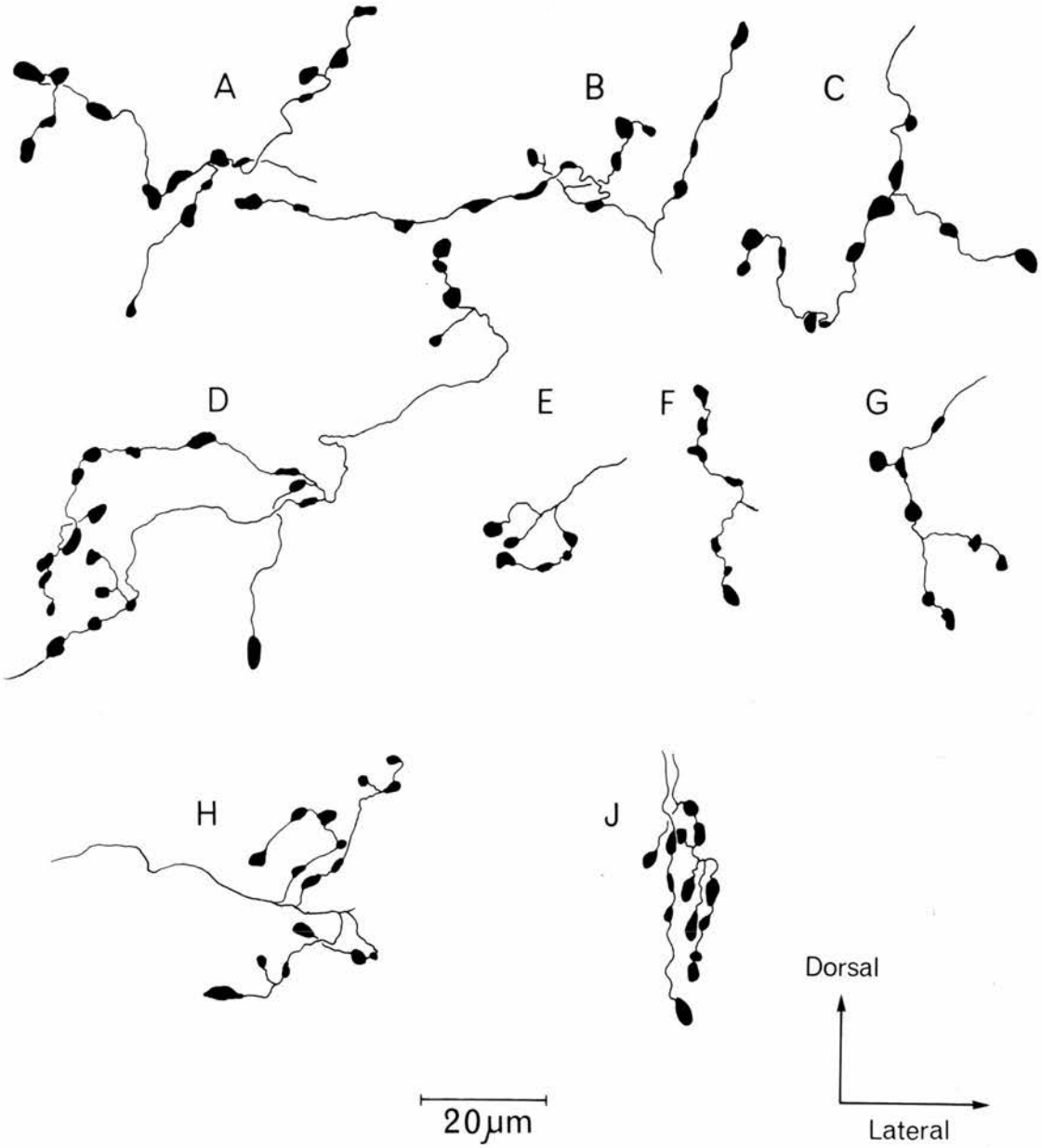
All micrographs are from single 100 μm thick sections of cord.


A
B
C
D
E
F
G
H
I
J
K
20μm

PART 4 : Figure 14

Ib terminal arborizations and boutons.

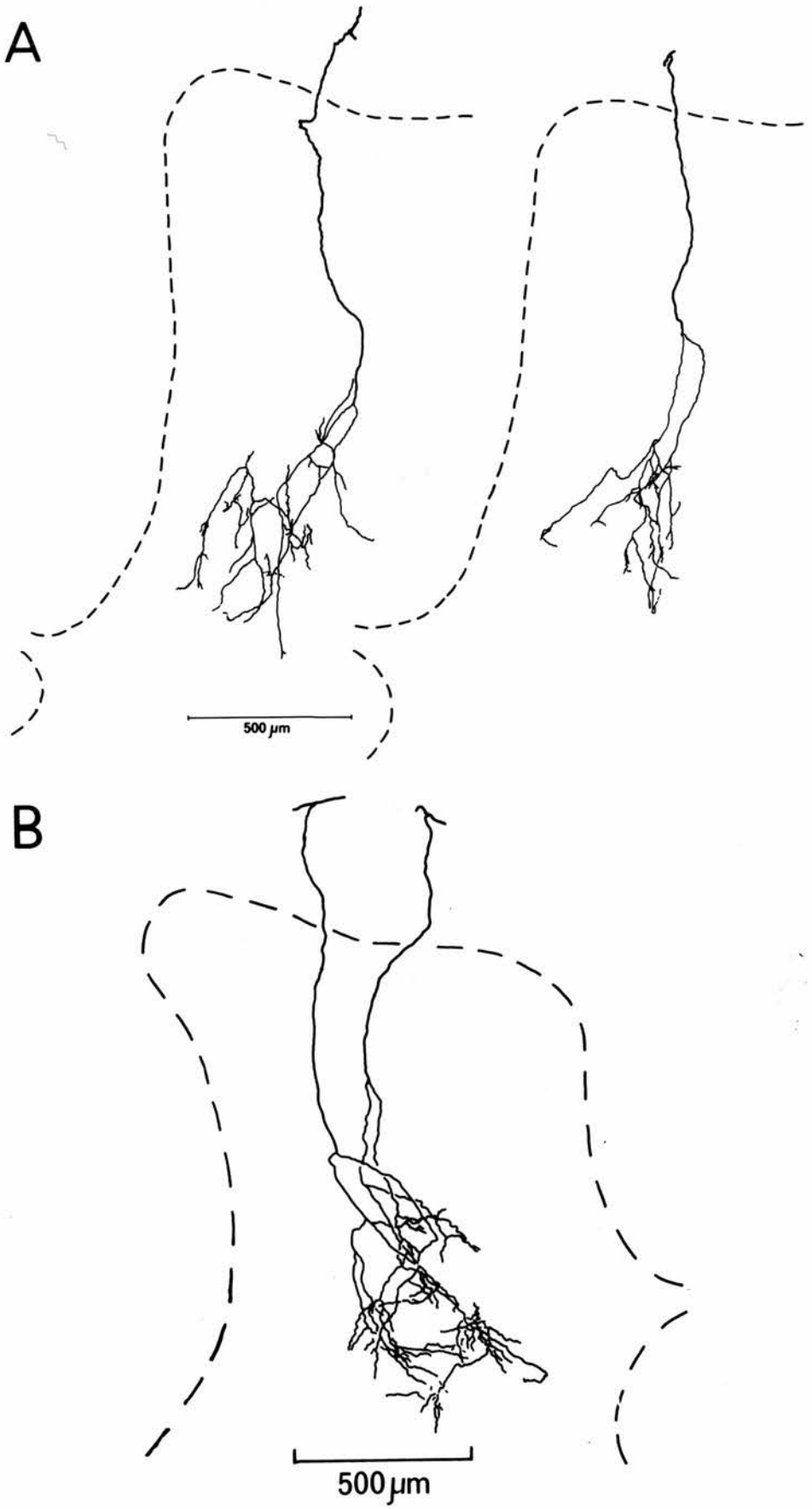
Camera lucida reconstructions of some of the terminal arrangements. Note the predominance of 'en passant' boutons and the occurrence of clusters of terminals. Drawing H shows part of the complex arrangement shown in micrograph J of the previous figure.


A
B
C
D
E
F
G
H
J
20μm
Dorsal
Lateral

PART 4: Figure 15

This figure shows further examples of Ib afferent fibre collaterals which terminate in the intermediate region. The collaterals shown in A are from a Lg-S Ib fibre. The collateral reconstructed on the left (most caudal of the pair) is also shown in Figure 12.

B. Is a reconstruction of two adjacent collaterals from the ascending branch of a Mg Ib fibre. This is one of the very few examples where Ib collateral arborizations overlapped slightly with their neighbour.


A
500 µm
B
500 µm

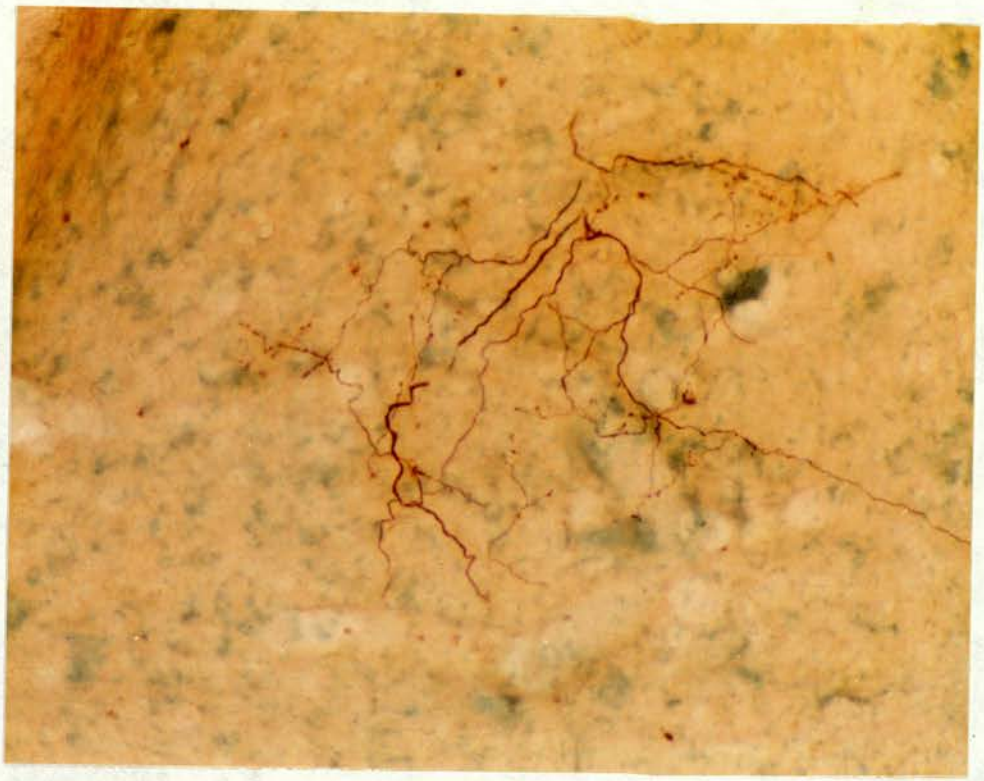
PART 4: Figure 16

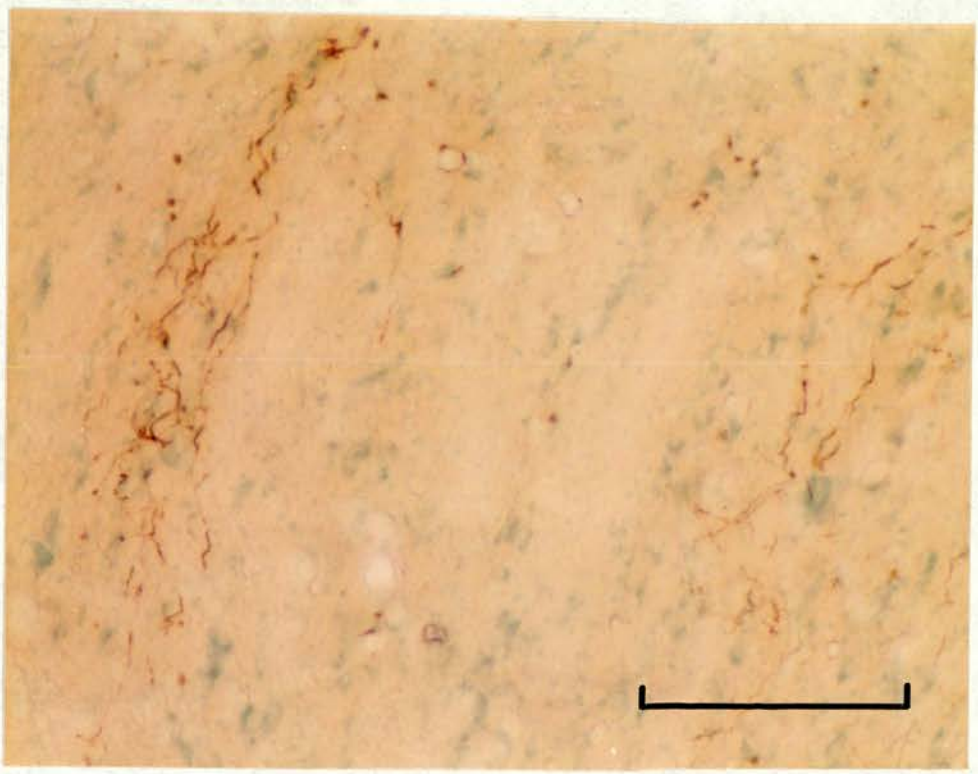
Ib collateral arborizations.

Upper figure: shows, in the transverse plane, the branching and terminations of part of the collateral reconstructed in Figure 2B.

Lower figure: this low power micrograph, from a single 100 μm thick sagittal section, shows the relationship between two adjacent collaterals from a Ib axon. The arborizations lie in parallel, but with a clear gap in between. Each collateral generates a terminal field which has restricted rostro-caudal spread, although the arborizations are tilted away from the vertical.

Scale Bar: 200 μm.





PART 5 : THE MORPHOLOGY OF GROUP II MUSCLE AFFERENT FIBRES

Introduction

The role of muscle spindle secondary endings and their associated afferents is unclear and has been recently interpreted in rather controversial fashion by some workers (Matthews, 1972). Only very recently has physiological evidence been presented to show that the Group II afferent fibres from spindle secondary endings make monosynaptic connexions with α - motoneurones (Kirkwood & Sears, 1974, 1975; Stauffer et al., 1976). These findings make interpretation of classical anatomical studies of dorsal root fibre projections even more complicated (see Iles, 1976). Direct anatomical evidence is required to supplement the current scanty electrophysiological data and perhaps aid the development of concepts regarding the effects generated by impulses in muscle spindle Group II afferent fibres.

Results

Technical difficulties. The techniques used were identical to those used for injecting Ia and Ib afferent fibres, excepting of course the identification parameters. Although there are roughly the same numbers of spindle primary and secondary afferent fibres from triceps surae muscles, microelectrode sampling bias was clearly in favour of the larger diameter Group I

axons. As a consequence these fibres were more likely to be injected at a relatively early stage of the experiment. Subsequent electrode tracks searching for muscle afferent fibres were then carried out at a distance (usually at least 5mm) from the first injection site. This lessened the probability of penetrating triceps surae Group II fibres (or other Group I fibres) near their entry into the cord. Searching in the dorsal columns for specific axons which have entered the cord at adjacent segmental levels was unproductive in most cases. Even in experiments committed to recording Group II axons, the percentage of useful penetrations was small. Staining of the collaterals from Group II axons was further complicated because of the long course taken by these collaterals through the grey matter. Injections of 150 - 200nA.min were necessary adequately to label the collaterals and this required stable intra-axonal penetration and current passing for 15 - 30 minutes.

The intraspinal trajectories of muscle spindle Group II axons. Three Group II muscle afferents, from different cats, have been successfully stained. The axons had peripheral conduction velocities of 54, 59 and 61m.sec^{-1} respectively and all innervated Lg - S muscles. Each unit had a regular ongoing discharge when recorded and could be excited by fairly light stretch of the muscle. Threshold to electrical stimulation was about 1.5 - 1.8 times threshold for the most rapidly conducting axons in the Lg - S nerve (see also Brock et al.1951.) The

stained lengths of the axons are shown in Fig. 1; they were labelled for up to 7.1mm which must represent only a small fraction of their total length in the spinal cord. The axons gave rise to 3 - 8 collaterals at intervals of 100 -1400 μm (mean 796 μm; n = 14). The sample size is obviously very small and therefore it is unwise at this stage to infer too much from the observation that Group II intercollateral spacing is shorter than the Group I spacing. There was no tendency for collaterals to be more closely spaced on the thinner descending branch of the main axon, as was found in the Ib axon sample. Both axons which were traced into a dorsal root did in fact bifurcate on entering the spinal cord; the rostral and caudal branches took courses in the dorsal columns. It was not possible to decide whether the third, short, stained axon was part of an ascending or descending branch because the position of its trajectory in the superficial part of the dorsal columns did not alter markedly from section to section.

Morphology of muscle spindle Group II axon collaterals. Two adjacent collaterals from each of the labelled axons are illustrated in the reconstructions of Figs. 2 and 3. The collaterals differ markedly from the Group I collaterals described previously. They enter the dorsal horn at its dorsal or dorso-medial border, and like other primary afferent collaterals are directed rostrally from their point of origin. The detailed

anatomical patterns of termination are complex, with boutons distributed to the dorsal horn, intermediate region and to the motor nuclei region (lamina IX) of the ventral horn. The most dorsal region of terminal arborization was usually the central and/or lateral parts of lamina V although boutons were observed in central lamina IV and even occasionally in lamina III (see Fig.2). In each case one or sometimes two main branches of the collateral continued on a ventral trajectory towards the base of the dorsal horn. These branches subdivided in lamina VI and VII to generate terminal arborizations in the central and lateral parts of the intermediate region (lamina VI and dorsal lamina VII). In the case of one apparently well-stained collateral (Fig. 3B), there were no branches ventral to the level of the central canal. But in all the other examples each collateral sent a single major branch (one collateral in Fig. 2 had two branches) ventro-laterally to terminate in the lamina IX motor nucleus. These branches were usually quite thin (< 1 μm diameter) and faintly stained, but were seen to divide and terminate in boutons.

Terminal arborizations of Group II axon collaterals. Some terminal branches and boutons are shown in Figs. 4 and 5. The most complex terminal patterns were seen in the centre of the dorsal horn, in laminae IV, V and VI. Here the main collateral gives off several branches as it passes ventral-ward. The location of these

arborizations was more dorsal and lateral than the zone of termination generated by Ia or Ib afferents in the intermediate region. These in turn subdivide profusely and give rise to fine terminal axons carrying up to 10 boutons 'en passant'. The bouton carrying terminal branches are oriented within the transverse plane, but the rostro-caudal extent of a single collateral arborization may range up to 600 - 700 μm. In two examples (from axons B and C of Fig. 1) there was very little gap between the dorsal group of terminals of one collateral and its neighbour.

In the intermediate region the pattern was similar although the rostro-caudal extent of terminals was less than in the more dorsal region. Again fine branches carried boutons 'en passant' along their final 20 - 50μm, and lay predominantly in the transverse plane. In this region there were many examples also of single 'terminal' boutons arising from fine axon branches.

Boutons were faintly stained and few in number within the lamina IX region. The faintness was probably due to the long distance, often up to 3mm, over which the HRP had to be transported from the main axon running in the dorsal columns to these ventral horn terminal regions. Coupled with this, the collateral branches reaching the ventral horn were extremely fine. From five to twenty one boutons per collateral were observed in lamina IX, with up to 3 boutons per terminal branch.

Bouton sizes ranged from 1.0 x 1.0 μm to 4.3 x 3.3μm.

In counterstained sections some boutons contacted a variety of dorsal horn cells, but no contacts were seen on the somas or proximal dendrites of ventral horn neurones. In one case a triceps surae α - motoneurone had been subsequently injected with HRP. Although the fine ventral branch of the Group II afferent collateral penetrated the dendritic tree of the α - motoneurone, no contacts were seen. This single negative result has little significance on its own but may be related to the projection frequency of spindle Group II afferents to the synergistic motoneurone pool. Stauffer et al. (1976) give a value of about 50% for Group II connectivity compared to the almost complete projection of Ia fibres (Mendell & Henneman, 1971; Watt et al., 1976; Lüscher et al., 1979).

Discussion

The small sample of stained Group II axons has provided some extremely interesting information regarding the possible central actions of these fibres. In the first instance, the observed collateral anatomy correlates very well with the focal synaptic potential patterns described by Fu et al. (1974). Group II collaterals terminate in three distinct regions of the spinal grey matter, viz: in the dorsal horn (laminae III - V) in an area dorsal and lateral to the main intermediate region terminal zone of Group I muscle

afferent collaterals; secondly, in the centre and lateral parts of laminae VI and dorsal VII and, finally, in a ventral region in the lamina IX motor nuclei. Of course these three anatomic terminal zones do not equate with the three types of Group II FSP described by Fu et al. (1974) because their 'third' type referred to a long latency, presumably polysynaptic response. The location of Group II terminals in the dorsal horn and intermediate region corresponds with their "dorsal Group II FSP" whilst their "ventral Group II FSP" can be related to the ventral terminations of the Group II collaterals. Collaterals arise from the ascending and descending branches of the main axon and could thus transmit spindle secondary ending information to segments rostral and caudal to the level of entry into the cord. Collaterals are more closely spaced than those on Group I muscle afferents: it is tempting, but perhaps premature in view of the small Group II sample, to postulate that 'thinner' axons have as a rule shorter intercollateral distances. Support for this premise is that Ib axons have collaterals closer together on their thin descending axon branch. This however was not observed for Ia axons, and the intercollateral spacing on hair follicle afferent axons (Brown et al., 1977) depends less on conduction velocity (hence fibre diameter) than on the region of collateral termination - collaterals which arborize in the lateral part of the dorsal horn are more widely spaced than those directed to medially sited termination areas.

If each collateral generates the same average number of boutons (in a particular region) regardless of axonal conduction velocity (as yet the samples of stained axon and bouton counts are too small to determine this) then closer spacing of collaterals on smaller axons would present a greater terminal density and distribution than would be the case for larger afferents. This would in effect contradict the size principle analyses of Henneman and his colleagues (Lüscher et al.,1979, 1980). Clearly it would be interesting to extend the sample of stained Group II axons and compare their intercollateral spacings and numbers of lamina IX terminals with their conduction velocity and also with similar parameters of Ia afferents.

In this sample, as with the Ia and Ib afferents, there was no correlation of intercollateral spacing against distance from the dorsal root entry. Lüscher et al. (1980) suggest from their investigation of postsynaptic population potentials that Group II terminals are distributed quite evenly over the whole extent of the motoneurone pools they supply; the present results support this suggestion, although in one case a Group II collateral (Fig. 4B) did not actually penetrate into the ventral horn. Unfortunately the next collateral rostrally was too faintly stained to determine whether it did or did not enter the motor nucleus. These collaterals were in L7 segment and so would be expected to be at the level of the triceps surae motoneurone pool.

Before discussing further the relevance of the

spinal projections of Group II axons to their possible effects it must be noted that, even in the present small sample of axons, there was considerable variability in morphology between collaterals from different axons although collaterals from the same axon tended to have similar terminal fields. On the whole, Ia and Ib fibres innervating the same muscle have remarkably similar features although (see Ishizuka et al., 1979) Ia collaterals from varied muscles of origin had different trajectories and terminal zones. Furthermore, the extensive investigations of cutaneous afferent projections (see Brown, 1981) clearly demonstrated the uniformity of organization of similar types of axons, even from different cats. In the present work, the Group II axons all innervated Lg - S muscles but still exhibited differences, particularly in the complexity and location of terminal areas in the dorsal horn. These observations may of course have some relation to the identity of the Group II axons. The axons clearly had conduction velocities within the Group II range (Hunt, 1954). The functional homogeneity of muscle afferent fibre populations conducting (or having diameters) in the Group II range has been previously studied. It is accepted that spindle afferents conducting in the Group II range innervate the secondary endings (Matthews, 1963). Hunt (1954) believed that almost all Group II muscle afferent fibres innervated muscle spindles. However, further evidence indicated that a sizeable percentage of

Group II muscle afferent fibres do not innervate muscle spindles (Boyd & Davey, 1968; Barker, 1962, 1967; Hunt & McIntyre, 1962) but exhibit for example the characteristics of Pacinian corpuscle afferents or innervate the (slowly adapting) Ruffini spray endings. These latter afferent types conduct at around 20m.sec^{-1} and are therefore unlikely to be included in the present sample. Skoglund (1956) showed that some joint afferents run in muscle nerves also.

When the triceps surae muscles are considered (Matthews, 1972) the situation does seem to be rather simpler, with the concensus of opinion that most Group II axons do innervate spindle secondary endings. Given the characteristic response of the three axons to muscle stretch, it seems reasonable to assume that they did innervate spindle secondary endings.

Since topographical parameters and receptor of origin are probably equivalent for the three stained Group II fibres, the function of the variable anatomical features needs explaining from some other point of view. Eccles & Lundberg (1959) and Holmqvist & Lundberg (1961) used electrical stimulation to investigate the reflex actions of Group II fibres. These studies generated the concept of the 'flexor reflex afferents' or FRA, a term of convenience which included Group II and III muscle afferents along with cutaneous and some joint afferents. Stimulation of any of these components elicited a non-specific flexor reflex and activated various ascending

spinal pathways. The drawbacks of electrical stimulation techniques have of course been emphasised frequently (see Matthews, 1972). Interestingly, these and other workers (Eccles & Lundberg, 1959b; Kuno & Perl, 1969; Sherrington & Sowton, 1915; Wilson & Kato, 1965) also found contradictory effects; for example, recording IPSP's in flexor motoneurones, EPSP's in extensor motoneurones and facilitation of extensor reflexes. In particular, the state of the preparation influenced the observed effects. In the decerebrate animal (Eccles & Lundberg, 1959b) supraspinal control mechanisms suppress flexor actions, due to tonic inhibition of interneurones involved in the FRA pathway. This inhibition is released by lesions at various brain stem levels, although even in spinal cats (Wilson & Kato, 1965) extensor motoneurones were excited by Group II muscle afferent fibres. Holmqvist & Lundberg (1959b) proposed alternative central pathways to flexor motoneurones, one excitatory and one inhibitory, and that supraspinal centres can select either path for function. They suggested that excitatory and inhibitory actions from the FRA to flexor motoneurones are evoked by impulses in the same afferent fibres.

There is little direct information on the interneurones responding to muscle Group II fibres. Fukushima & Kato (1975) located Group II activated interneurones in dorsal horn laminae IV - VI and also in the ventral horn. The positions correlate with the

focal synaptic potential location described by Fu et al. (1974a) and with zones in which the present labelled axons terminate. Clearly, convergence patterns on to Group II activated dorsal horn neurones require definitive analysis. As well as pathways (excitatory or inhibitory) to flexor motoneurones there will be excitatory and inhibitory pathways to extensor motoneurones (see later). Supraspinal control might act directly upon the Group II pathway interneurones or alternatively, since many of the descending tracts end in similar areas to the Group II collaterals, might be mediated by non-specific, or interneuronal, mechanisms upon the afferent fibre terminals. These mechanisms would be largely appropriate for differential effects to be evoked by the same fibre under different conditions. But, in entirely speculative fashion, the effects could be mediated by the two types of afferent fibre observed anatomically. Two of the three axons had collateral arborizations in the dorsal horn, the third had terminals only in the lateral part of the intermediate region. There is no electrophysiological evidence to support this premise. The responses of interneurones and motoneurones to activity in single Group II afferent fibres, whose own responsiveness to stretch, vibration and γ - activation has been thoroughly checked, should be carefully analysed.

But of special interest is the observation that muscle Group II afferent collaterals project directly

into the ventral horn motor nuclei. This represents an arrangement suitable for monosynaptic linkage with α - motoneurones (of course α - motoneurone dendrites also project into the more dorsal Group II terminal areas, but (see General Discussion) connexions in these regions are unlikely). Such, unexpected, connexions have been recently demonstrated by Kirkwood & Sears (1974, 1975), whose important spike triggered averaging work was then confirmed by Stauffer et al. (1976). The new anatomical and physiological findings lend firm support to Matthews' (1969) suggestions on the function of spindle Group II afferents. Prior to 1969 the observed excitation of extensor motoneurones after stimulation of muscle nerves at Group II fibre thresholds was regarded as a paradoxical phenomenon, somewhat related to the general state and condition of the animal. The FRA concept was in fairly widespread use (e.g. Oscarsson, 1967) although many (e.g. Wilson & Kato, 1965) thought that continued use should be discarded. Matthews (1969) re-examined Group II spindle afferent effects by comparing the reflex tension due to stretch and stretch plus vibration of the tendon. Assuming that vibration only excites the primary endings (Granit & Henatsch, 1956; Bianconi & van der Meulen, 1963; Brown, Engberg & Matthews, 1967) he calculated that more tension was developed during the tonic stretch reflex than could be attributed to Ia action alone. He postulated that the spindle secondary afferents were the

most likely candidates as the source of the extra reflex tension developed. This hypothesis generated quite considerable controversy, as well as stimulating some newer experimental work. Matthews (1972) and McGrath & Matthews (1973) have considered some of the contradictory arguments (e.g. Grillner, 1970, 1973; Barnes & Pompeiano, 1970a, b) in some detail. Several groups worked towards methods of selectively activating Group II fibres in order to approach the problem directly. Cangiano & Lutzemberger (1972) used DC anodal block to inactivate Group I fibres. They only noted hyperpolarizing responses in extensor motoneurones following Group II stretch evoked activity. Cook & Duncan (1971) found that compression block of Group I afferent fibres abolished the tonic stretch reflex. Jack & Roberts (1974) used an electrical shock stimulation technique to activate selectively Group II fibres. In summary, none of these results disproved Matthews Group II hypothesis but rather pointed towards possible indirect modulatory roles of Group II afferents in the general mechanisms in the idea of autogenetic excitatory effects from Group II afferent fibres. Westbury (1972) provided evidence to support the work of Matthews (1969). By looking at subthreshold effects in motoneurones, thus obviating any muscle contraction induced errors, Westbury (1972) confirmed the lack of occlusion when the muscle was vibrated (to drive the Ia afferent fibres) and stretched.

Whilst Matthews (1970, 1972) and Kanda & Rymer (1977) considered schemes such as disinhibition and direct polysynaptic activation to explain the Group II excitatory action on extensor motoneurones, Kirkwood & Sears (1974, 1975) directly demonstrated the monosynaptic excitatory projection from secondary spindle afferents. The earlier ideas were still investigated - Lundberg, Malmgren & Schomburg (1975) thought that the excitatory link was disynaptic because it had the same latency as the commonly observed Group II excitatory link to flexor motoneurones. Kanda & Rymer (1977), like Cook & Duncan (1971), suggested a need for central facilitation from Ia afferents (or other afferents) before the Group II effects were manifest. Fromm, Haase & Wolf (1977) demonstrated a depression of recurrent inhibition of extensor motoneurones by the action of Group II afferents; i.e. a disinhibitory action. Following Kirkwood & Sears, Lundberg et al. (1977) reinvestigated their earlier 1975 results and found a previously undetected monosynaptic excitation of both flexor (cf. Holmqvist & Lundberg, 1961) and extensor motoneurones. Detailed studies of the actions of single identified spindle secondary afferents on motoneurones of different types - tonic and phasic - and twitch characteristics would be useful to define further the part they play in stretch reflexes.

These results establish that it is not reasonable to include the muscle Group II afferents amongst the FRA

group. The anatomy supports the now recognized Group II actions and differentiates the axons from the many cutaneous afferents (see Brown, 1981) which may contribute to the FRA. Indeed since all the primary afferents examined by intra-axonal HRP injection have their own characteristic projection patterns, the heterogeneity within the whole group appears so great as to render the concept, and use of the term FRA, virtually useless at the present.

The recent findings, especially the spike triggered averaging results of Kirkwood & Sears (1974, 1975) and Stauffer et al. (1976), emphasise the dangers and complications of using selective electrical stimulation or block for selectively activating afferent fibres (except perhaps some low threshold Group I afferents in some circumstances) with a view to determining their central connectivity.

The excitatory monosynaptic projection to motoneurones is not as extensive as the one from Ia afferents. Stauffer et al. (1976) reported 49% of synergistic motoneurones receiving EPSP's. The excitatory effects (monosynaptic or polysynaptic) require central facilitation (Kanda & Rymer, 1977). The impression from the anatomical material is that whilst collaterals project uniformly to the ventral horn over much of the extent of the motor nucleus, their lamina IX terminals are rather sparse. Faint staining made bouton counting difficult and there is no direct information on

the number of synapses a single afferent makes on any target motoneurone. Such quantitative data are required to enable estimates to be made of the contribution of secondary endings to the general excitatory state of synergistic motoneurones.

None of the Group II axons were stained at the level of Clarke's column (rostral to L_4). It is likely (Jansen & Rudjord, 1965; Jansen, Nicolaysen & Rudjord, 1967) that secondary spindle afferents make monosynaptic connexions with the cells of origin of the DSCT, either exclusively or convergently with Ia or Ib afferents. The Group II axons, unlike the Ia or Ib axons did not project to the area near the medial edge of the base of the dorsal horn which at more rostral levels becomes the location of Clarke's column. If Group II axons do indeed excite DSCT cells monosynaptically then a variation in collateral morphology would be expected at the level of Clarke's column. This postulated projection in more rostral segments can be likened to the observation that Ib collaterals in rostral L_6 (Part 4) begin to project to more lateral regions of the grey matter than was typical in L_7 or caudal L_6.

It seems that primary afferent fibres have the capability during development to project selectively to organized groups of target neurones. Where target neurone pools are 'staggered' along the rostro-caudal axis of the cord, only those collaterals at the appropriate level will terminate in the region of grey

matter containing these cells. Burke et al. (1979) state that Ia collaterals at the extremes of their labelled axons do not penetrate into the motor nuclei. This may be because they were unable to trace these branches because of faint staining, but alternatively it may indicate that the collaterals only enter the motor nuclei at segmental levels where monosynaptic connexions will be made among homonymous and heteronymous motoneurones. Outwith this part of the cord connexions would continue to be made with interneurones or tract cells in other regions of the grey matter.

Certainly, for the three types of muscle afferent fibres described in this thesis, the limited spread of HRP (over about 1 segment length of cord) has restricted the investigation of <u>all</u> their spinal terminations. While important observations have been made about their projections near their segment of entry the muscle afferents have such diverse central effects that descriptions of their terminal patterns at other cord levels would be valuable.

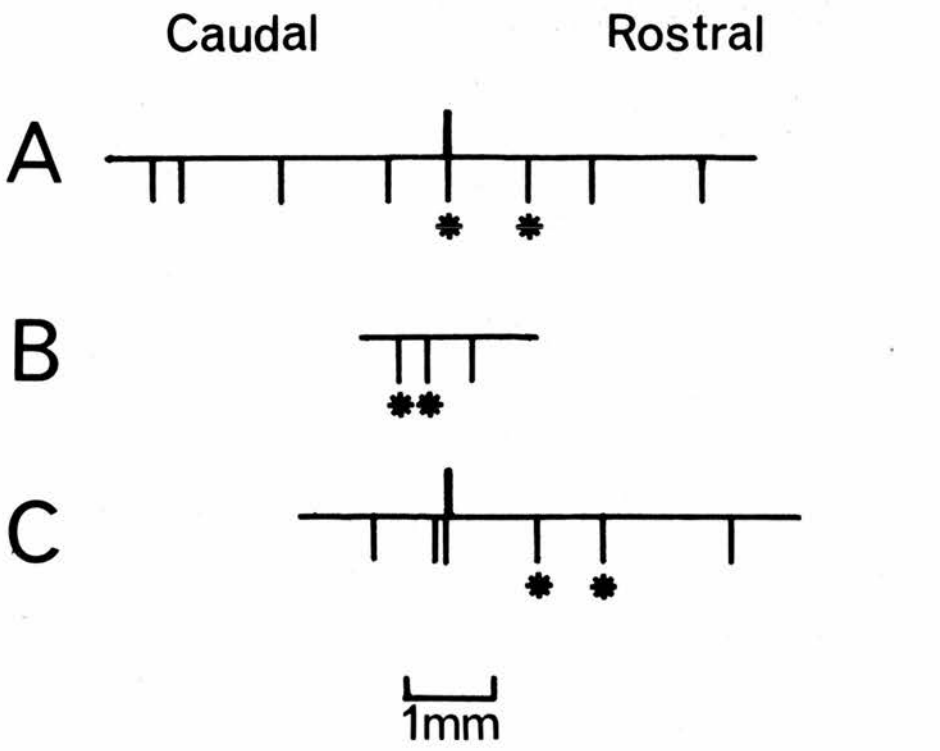
One final conclusion that the present anatomical results indicate is that significant confusion might arise in the interpretation of degeneration, Golgi or dorsal root labelling experiments. It is no longer valid to assume that primary afferent or dorsal root fibres entering the ventral horn come exclusively from spindle primary afferents.

PART 5 : Figure 1

Intraspinal branching of Group II afferents.

The stained parts of three Group II axons are represented showing length of axon traced, number and spacing of collaterals and in two cases the position of entry through the dorsal root. Axon B was injected in the dorsal columns and the fibre could not be traced back to the afferent entry zone. For each fibre two collaterals (asterisks) are reconstructed in subsequent figures.

Muscle spindle group II afferent fibres

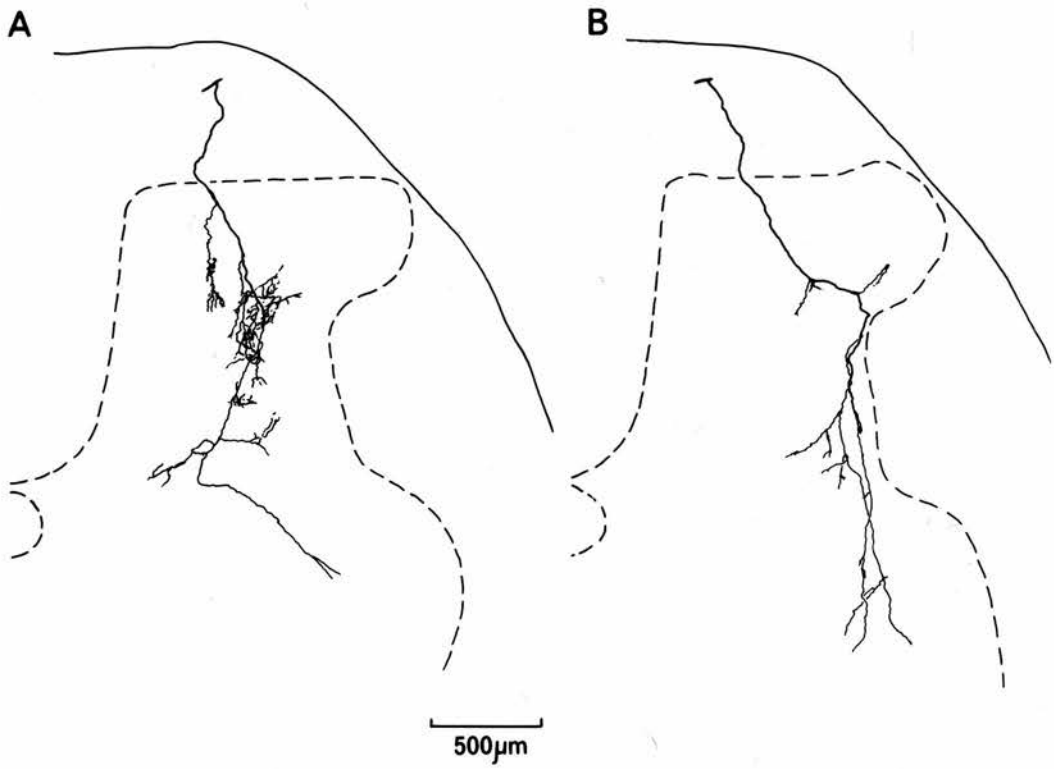


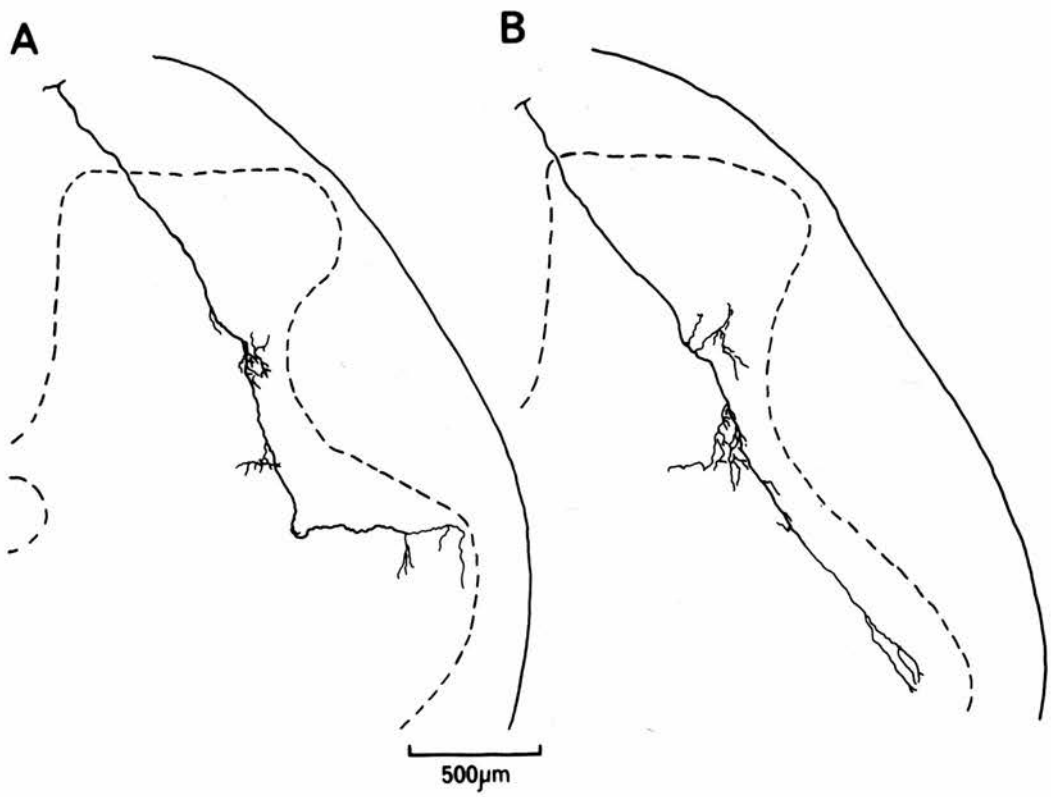
PART 5: Figure 2

The upper pair of collaterals are from axon A of Fig. 1. Collateral A arises very close to the dorsal root entry, whilst B is about 1mm rostral. A branches in the superficial dorsal horn and generates extensive arborizations in the centre of the dorsal horn. A major branch continues ventrally to terminate in the intermediate region and ventral horn. Collateral B has fewer terminals in the dorsal horn but also projects to the intermediate region and into the motor nuclei region.

The collaterals in the lower pair of reconstructions (from axon B in Fig. 1) have similar trajectories and terminal zones to collateral B above.

Muscle Group II collaterals

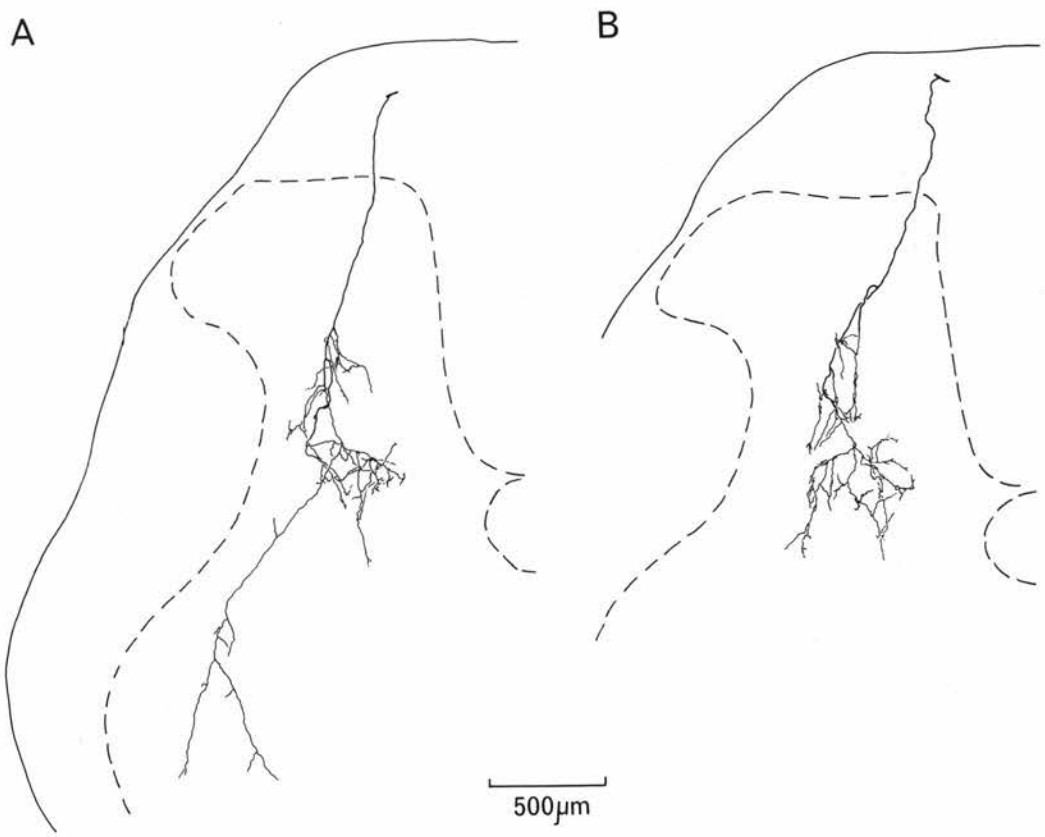




PART 5: Figure 3

These two collaterals are fom the ascending branch of axon C (Fig. 1). Both collaterals arborize in the centre of laminae V, VI and dorsal VII, but only the caudal collateral (A) projects to the ventral horn.

Muscle Group II collaterals

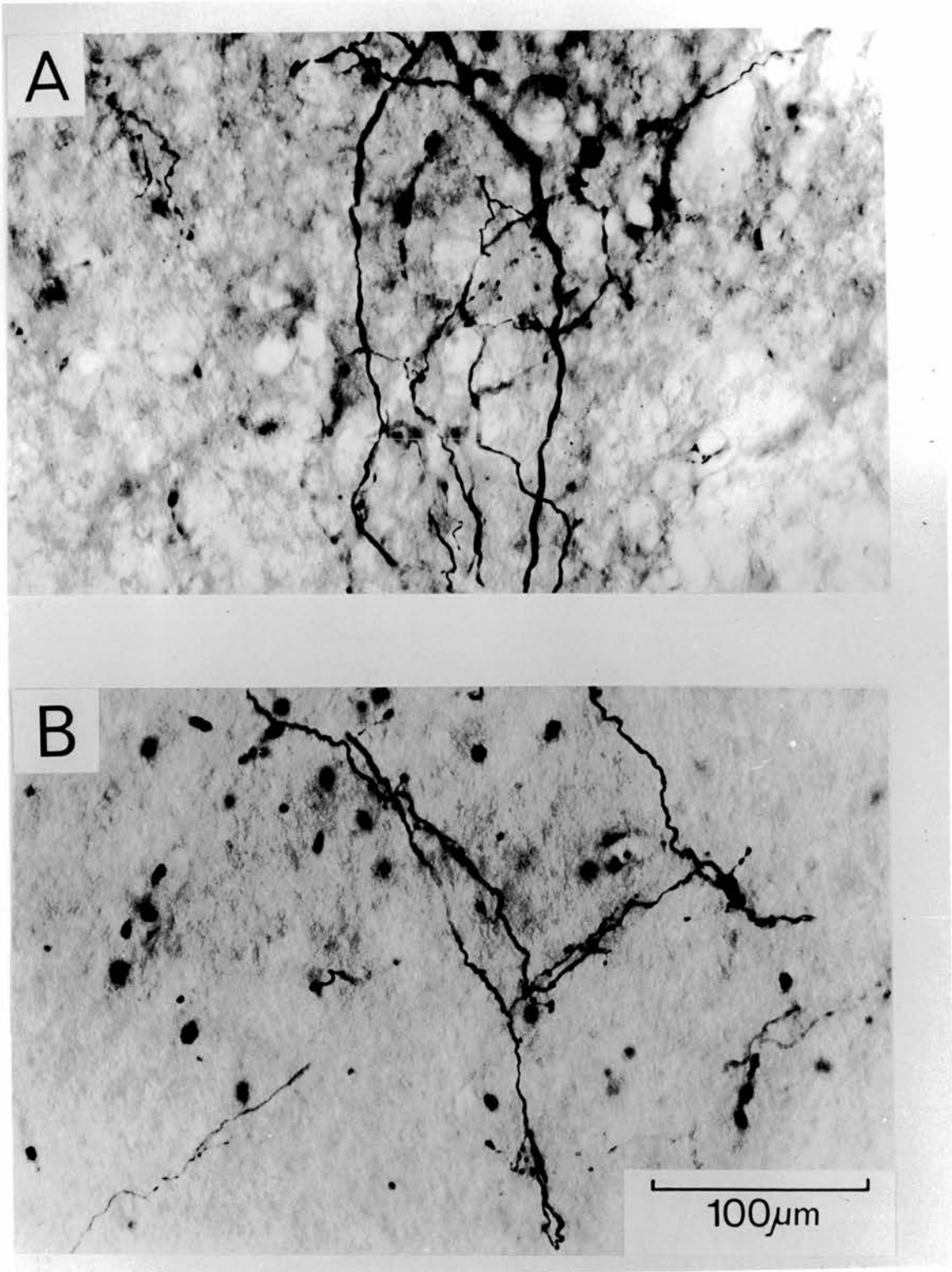


PART 5: Figure 4

Group II terminations

A. Part of the dorsal arborization of the collateral reconstructed in Figure 3A, upper.

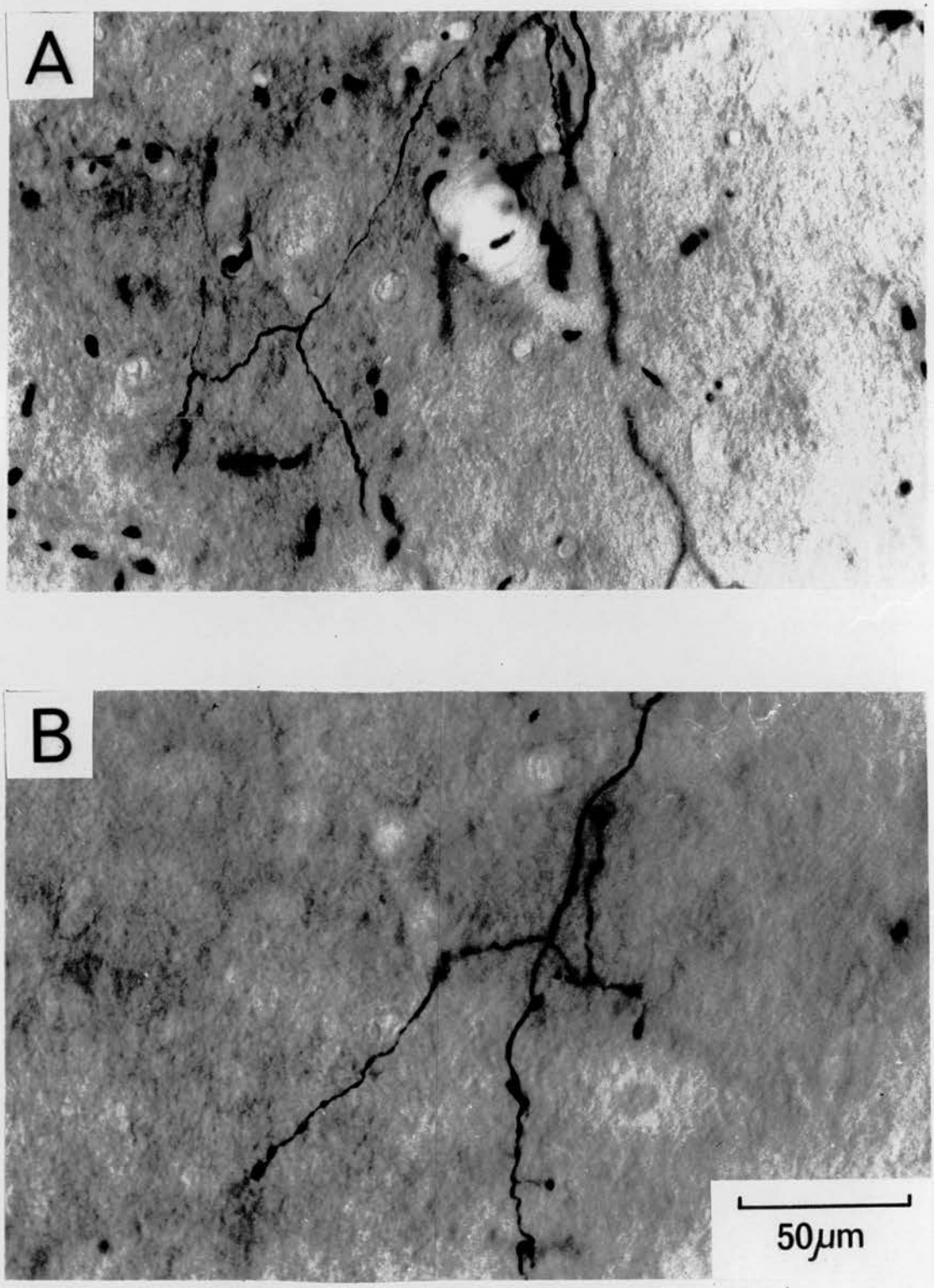
B. Some intermediate region terminations of the collateral from Figure 2A. Many of the boutons are 'terminal' types, offset from the collateral branches. At the bottom left is the small diameter branch which projects ventro-laterally into the lamina IX motor nuclei.


A
B
100μm

PART 5: Figure 5

Group II terminations.

These arborizations lie in the central part of the dorsal horn. 'En passant' boutons are commonly observed.


A
B
50μm

PART 6 : THE MORPHOLOGY OF TRICEPS SURAE α-MOTONEURONES

Introduction

The spinal motoneurone has had a predominant place in our thinking on neuronal function and integration. Since the pioneering intracellular studies of Eccles and his collaborators (see Eccles, 1957, 1964) many of its features have been exploited to provide intensive information which, coupled with dye injection, mathematical modelling and theoretical approaches, has to many workers represented the basic features of central neurones. However it is clear that extrapolation of results from motoneurones to other neural systems should be treated with caution. The α - motoneurone is, of course, a highly specialized projecting neurone with specific inputs and control systems.

Despite all the intensive electrophysiological and anatomical investigations, some confusion has recently crept into the literature with respect to the precise morphological features of these cells. Even the numbers and lengths of motoneurone dendrites as revealed by various staining methods is not clear (Aitken & Bridger, 1961; Barrett & Crill, 1974a; Lux et al., 1970). Although the original aim of the present work was to determine the distribution of Ia synapses on motoneurone dendrites, the intracellular injection of HRP into single identified α - motoneurones revealed some anatomical aspects which merit description. During the course of

this work, other groups have published some new observations on motoneurone anatomy following intracellular injections of HRP (Cullheim & Kellerth, 1976, 1978a, b, c; Cullheim, Kellerth & Conradi, 1977; Burke et al., 1979; Rose & Richmond, 1978). These confirm and complement the present results.

Results

The data described in this Part was taken from 34 α - motoneurones which were stained in conjunction with the appropriate Ia fibres (see Part 7). In these cases the afferent fibre was inadequately stained and so only the motoneurone was fully reconstructed. The neurones upon which contacts were established by Ia fibres are not included in this sample but are described briefly in Part 7.

All the neurones were antidromically activated from Mg or Lg - S muscle nerves and had peripheral conduction velocities of 70 - 102m.sec^{-1} (91 $\pm$ 17.4; mean $\pm$ S.D.).

To minimise staining of motor axon recurrent collaterals, which are described in detail elsewhere (Cullheim & Kellerth, 1978a, b, c) and which might complicate the reconstruction of Ia axon terminal arborizations, HRP injections were restricted to 60 - 120nAmm of current. This also helped to minimise possible cell damage (see Part 2). Nevertheless, some recurrent axon collaterals were well stained and examples are shown in Fig. 1. The main area of termination of

these branches was ventro-medial to the motoneurone pool, in the 'Renshaw cell area' (Renshaw, 1946; Eccles et al., 1954) whilst occasional terminals were located within the motor nucleus. The motor axons took variable courses through the ventral horn, and could be traced into the ventral roots. In fact the cautious approach to labelling motoneurones may have been unnecessary since the terminal areas of Ia afferents and recurrent collaterals in practice showed little overlap. Motoneurone cell bodies were located in the dorso-lateral lamina IX motor nuclei (see also Romanes, 1954) of spinal segments L_7 and L_6. When the triceps surae motor cell columns were located by initial microelectrode tracking, several identified motoneurones could successively be stained. The micrographs in Fig. 2 show triceps surae α - motoneurones stained virtually in line rostro-caudally after individual microelectrode tracks separated by some 500 μm or so. Even on these single 100 μm thick para-sagittal sections longitudinal overlap of dendritic trees is obvious.

Dendritic trees of α - motoneurones. Although the anatomic descriptions of even the early light microscopists (e.g. Ramon y Cajal, 1909) have conditioned more recent workers, physiologists and anatomists alike, to expect great dendritic extents, the lengths of some dendrites in the present results were surprising. Furthermore, the overall extensiveness and complexity of the dendritic arrangements was equally striking. Figs.3

and 4 illustrate some of the present findings.

Each motoneurone had between 7 and 18 (mean 11.6) primary dendritic trunks originating from the soma (see e.g. Table 1). Most branch points occurred as straightforward bifurcations, although about 5% of all branching occurred trichotomously (see e.g. Part 7, Fig. 4). Distances between successive branch points generally increased at greater lengths along the dendrite; each dendrite produced branches up to the 4th - 6th order.

One of the necessary assumptions for reduction of a dendritic tree to a uniform equivalent cylinder model is that Rall's (1959) power rule is applicable. For about 15 branch points (bifurcations) from each of 12 α - motoneurones the ratio

$$\frac{d_1^{\,3/2} + d_2^{\,3/2}}{D^{\,3/2}}$$

was measured, where d_1 and d_2 are the diameters of the daughter branches and D is the diameter of the parent dendrite. Since there were usually slight swellings at branch points, measurements were made 5 - 20 μm away from the branch point. The present findings indicated a range of values of the above ratio from 0.65 - 1.70 with a mean of 1.06 $\pm$ 0.15 (S.D.). This indicates, in agreement with the results of Lux et al. (1970), that Rall's simplifying assumption holds, at least for individual branch points.

There would of course be difficulty in relating this

finding to the equivalent cylinder model if there was significant tapering of dendrites between branch points (e.g. Barrett & Crill, 1974a, b), or if dendrites terminated at different distances, in which cases the compartmental model (Rall, 1964; 1967) would be rather more appropriate. Although as yet the combined dendritic tree parameters (d_0 3/2 at any particular electrotonic distance) and dendritic tapers have not been fully quantified, preliminary observations indicate that there was little tapering between branch points, at least up to 800 μm from the soma. It was sometimes difficult to interpret the degree of taper of dendritic trunks because of the contour of the soma at their origin. In definable cases there was no tapering. Distal dendrites often tapered along their final course.

Diameter measurements were further complicated by the presence of dendritic varicosities or beading. These usually occurred along the more distal dendrites (see Part 7, Fig. 11). They appeared to be fairly uniformly spaced and had smooth outlines. They were excluded from the diameter measurements. Varicosities appear in Golgi (Ramon y Cajal, 1909; Ellias & Stevens, 1980) and HRP stained material (Burke et al., 1979; A.G. Brown & R.E.W. Fyffe, unpublished results) on ventral horn cell dendrites and elsewhere in the central nervous system. Conradi (1970a) also described, at the E.M. level, the beaded appearance of thin ventral horn dendrites. In the present work it was difficult to

attribute such beading to damage consequent to the HRP injection. Cells with apparently undamaged somas and proximal dendrites had varicosities on their distal dendrites. Where dendritic swellings appeared on proximal dendrites there was usually evident swelling and bursting of the soma also. The nature of the varicose structures certainly requires further study.

The other, rather uncommonly observed, dendritic structures revealed by HRP injection were the dendritic spine-like projections distributed mainly on the proximal half of the dendritic tree (see Part 7, Fig.11). Spines were located at widely separated sites and consisted of small ($\leq$ 1μm diameter) knobs carried on the end of short and very fine stalks. The paucity of motoneuronal dendritic spines was observed even with larger HRP injections than normally used (> 200 nA min). In all cases the dendrites were intensely stained so the lack of spines is unlikely to be due to restricted staining by the HRP. SCT cells (Brown et al., 1977b) exhibit profuse spinous processes after smaller injections. The present findings agree with ultrastructural analysis of ventral horn neurones (e.g. Conradi, 1970a) where some spines were observed at the proximal parts of dendrites.

To return to a consideration of the organization of the dendrites, Figs. 3 and 4 amply illustrate the enormous span of individual cells. Some of the dendrites terminate 1.5mm or more away from the soma.

Other dendrites, equally long, recurve and end close to the soma. The transverse view is striking - dendrites are not confined to the ventral horn, far less to the motor nuclei. They extend into the ventral and lateral funiculi, sometimes penetrating close to the surface of the cord. Within the grey matter they ramify extensively within laminae VII, VIII and IX, thus overlapping the locations of other motoneurone pools (cf. Sprague & Ha, 1964) as well as interneurone regions. Dorsally directed dendrites often enter the base of the dorsal horn (lateral laminae V and VI) and extend into the dorsolateral funiculus. Dorsomedially, some dendrites approach the level of the central canal. The longitudinally directed dendrites result in rostro-caudal dendritic spreads, for single cells, of up to 3.0mm. Dendrites were often seen to run parallel and very close to the grey-white interface. It should also be noted that there was often subdivision of dendritic branches within the white matter.

Discussion

Intracellular injection of HRP has provided a more complete view of the morphology of motoneuronal dendritic trees in adult cats than did previous anatomical methods.

Triceps surae α - motoneurones have been shown to have between 7 and 18 primary dendritic trunks (mean 11.6) emanating from the soma. Golgi impregnations (Aitken & Bridger, 1961; Gelfan et al., 1970) demonstrated 2 - 14

(mean about 7) dendrites. Intracellular injection of Procion Yellow (Barrett & Crill, 1974a) or tritiated glycine (Lux et al., 1970) demonstrated 8 - 22 dendrites, values more in common with the present results. One implication of these findings is that, contrary to general belief, the Golgi methods do not, at least in adult cats, adequately demonstrate <u>complete</u> neuronal profiles (even when only considering single sections) but fail to stain some dendrites arising from the soma. Verification of this phenomenon would of course require detailed electron microscopical examination of Golgi impregnated cells. An interesting light microscopic observation was that of Somogyi & Smith (1979) who demonstrated (their Figs. 5a, b) striatonigral neurones retrogradely labelled with HRP. Subsequent Golgi stain filled only part of the soma, and dendrites originating from this part of the neurone. Other dendrites, marked with HRP reaction product, were not impregnated. Obviously caution is essential, until the methodologies are clarified, when interpreting results of Golgi studies purporting to show dendritic tree modelling and modification during development (e.g. Conradi & Ronnevi, 1975).

Recently Westbury (1979) has shown by intracellular HRP injections that γ - motoneurones have fewer (4 - 6) main dendrites than α - motoneurones. Total dendritic extents are only slightly smaller, although there was less dendritic branching (cf. the Ia inhibitory

interneurones stained by Jankowska & Lindström, 1974, and in Part 3 of this work). The γ - motoneurone axons entered the ventral roots directly and gave off few collaterals.

The HRP, Procion Yellow and tritiated glycine labelling methods stain similar numbers of dendrites in any neurone type, but conflict in the extent to which the dendrites can be traced towards their terminations. Procion Yellow gave dendritic lengths of 330 - 880 μm. Tritiated glycine (and Golgi stains) gave lengths up to about 1.0mm. In the present study most dendrites were revealed for at least 800 μm and were commonly up to 1.5mm long. It was not surprising that HRP stained dendrites appear almost twice as long as those labelled with Procion Yellow. Brown et al., (1977b) have already shown that HRP labels dendrites of SCT neurones more extensively than did Procion Yellow (Brown et al., 1976). Clearly it is much more difficult to trace fine (< 1 - 2 μm diameter) dendritic profiles, especially when they are cut transversely, against a fluorescing background in the case of the Procion Yellow studies than to trace the demse HRP reaction product deposit. Similarly there may be difficulty in resolving fine profiles by autoradiography as in the studies of Lux et al. (1970), resulting in possible underestimates of dendritic length.

In the circumstances it seems worthwhile to direct some new experiments towards re-evaluating the geometric

parameters of these 'model' neurones. Intracellular HRP staining obviously combines the ability to determine anatomical measurements with a knowledge of many of the electrical parameters of the labelled cell (see also Barrett & Crill, 1974a). The electrical properties of motoneurones have been considered in numerous studies (e.g. Eccles, 1961; Frank & Fuortes, 1956; Ito & Oshima, 1965; Nelson & Frank, 1967; Nelson & Lux, 1970; Jack & Redman, 1971; Lux et al., 1970; Smith, Wuerker & Frank, 1967). There are difficulties in measuring the membrane time constants (Fatt, 1957; Katz & Miledi, 1963; Jack & Redman, 1971; Redman, 1976); electrical uniformity of the soma and dendritic membranes is an essential assumption for the estimation of other cable parameters. For cat motoneurones Iansek & Redman (1973a) suggested that the specific resistivity of the soma membrane is less than the dendritic membrane. The many assumptions involved in these treatments have been discussed at length (Jack et al., 1975; Redman, 1976; Rall, 1977) and modifications and developments of the Rall model can adequately describe variations in anatomy and cable properties and hence resolve problems associated with the production of synaptic locations and interactions. Bearing in mind the restrictions and the different methods used in respect of motoneurone modelling exercises (see Jack et al, 1975) it is perhaps surprising that most workers concur and place the electrotonic length (L) of the equivalent cylinder representing the motoneurone

dendritic tree at about 1.5 space constants. This emphasises the electrical shortness of dendritic branches which extend often for considerable distances.

The studies in which anatomical estimates of L were made, either for cells in which some cable parameters were determined (Lux et al., 1970; Barrett & Crill, 1974a) or for cells where appropriate values were assumed (e.g. Rall, 1964, 1970), further supported the notion that dendrites were electrically quite short and also emphasised the predominance of the dendritic surface over the soma in terms of their respective contribution to membrane conductance and hence synaptic input efficacy. A major mismatch between the present results and earlier anatomy is the total lengths of the dendrites. The electrotonic length of the dendritic tree is composed of components representing individual segments of dendritic branches. The inclusion of the fine callibre distal dendritic branches detected in this study, but not seen in previous attempts to match morphology with electrophysiological data (e.g. Barrett & Crill, 1974a, b), might effectively increase electrotonic length. Estimates of L assume a valid reduction of the whole dendritic tree to an equivalent cylinder; this will represent an average of the values for individual dendrites, which may or may not have equivalent lengths. In this work, it was clear that not all dendrites were the same length. If additionally the cable parameters of some dendrites, say the distal branches, do differ,

then the average L will be weighted more by particular dendrites. It seems important to check these estimates by analysis of HRP labelled motoneurones and compare the values with electrophysiological predictions. On the other hand, from the limited analysis in the present study, it seems that branch points do conform to Rall's 3/2's power law and dendrites do not exhibit much tapering, thus implying that motoneurone dendritic trees may be represented by an equivalent cylinder. Since L values are relatively short, and the dendrite to soma conductance ratio (σ) is large, distal synapses will have an important role to play in the integrative activity of the motoneurone.

There was no relationship apparent between the size of α - motoneurones (estimated from axonal conduction velocity and direct measurement of soma dimensions) and the extent or complexity of the dendritic tree. Proximal dendritic trunks were generally quite short, and the length of branches increased with distance from the soma. Branches ended in different areas. The receptive surface of individual neurones clearly overlaps with many different types of neurone. A single α - motoneurone can extend for up to about 1/4 of the total length of the triceps surae motoneurone cell body pool; some cells will thus overlap with motoneurones in adjacent segments as well as with adjacent cell groups at the same segmental level. The Ia fibre inputs to these cells will be more fully

discussed in the following Part of this work. Many inputs impinge upon the motoneurone dendritic tree. Rose & Richmond (1978) have shown by electron microscopy that synaptic connexions are even made on distal dendrites within the white matter. In the grey matter, the dendrites encompass the terminal areas of a multitude of neurone types. Ia inhibitory interneurones and Renshaw cells (Jankowska & Roberts, 1972b; Burke et al., 1971) tend to synapse on juxtasomatic regions of their target motoneurones. Motoneurone dendrites projecting within the Renshaw cell area may receive direct synaptic connexions from motor axon collaterals (Cullheim & Kellerth, 1978b) which would be assumed to be involved in the activation of the Renshaw cells. Some dorsally directed dendrites ramify in the zone of termination of corticospinal fibres (Kuypers, 1964) whilst many segmental interneurones project and terminate throughout the areas of lamina VII and the intermediate region penetrated by dorsomedially directed dendrites. The detailed organization and weighting of synaptic contacts made on the vast dendritic surface of these 'model' neurones remains to be defined.

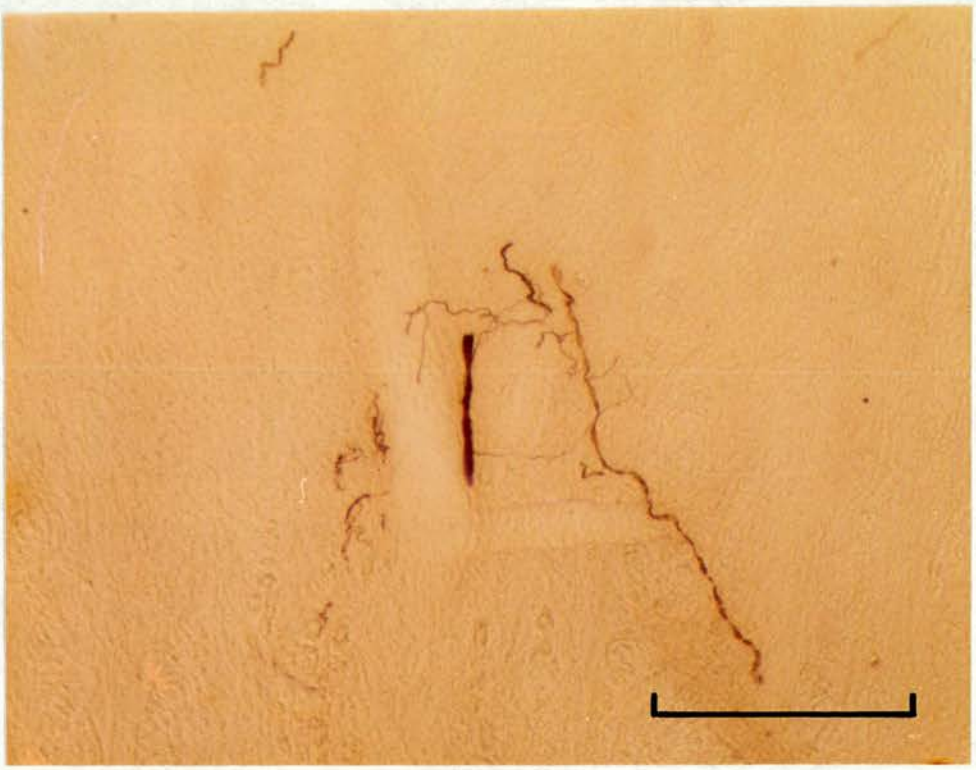
PART 6: Figure 1

Motor axon collaterals.

The upper micrograph shows the origin, 78 μm from the cell body, of a fine recurrent axon collateral.

The lower micrograph, from a single 100 μm thick sagittal section, shows some of the ramifications of a recurrent collateral. A distal dendritic branch from the parent cell is seen at the right of this view. Where collateral branches crossed over that dendrite there was no discernible gap between axons and dendrite; no bouton-like structures were associated with these regions however. The motor axon runs vertically in the centre of this view.

Scale Bars: Upper 50μm
Lower 200μm.



PART 6: Figure 2

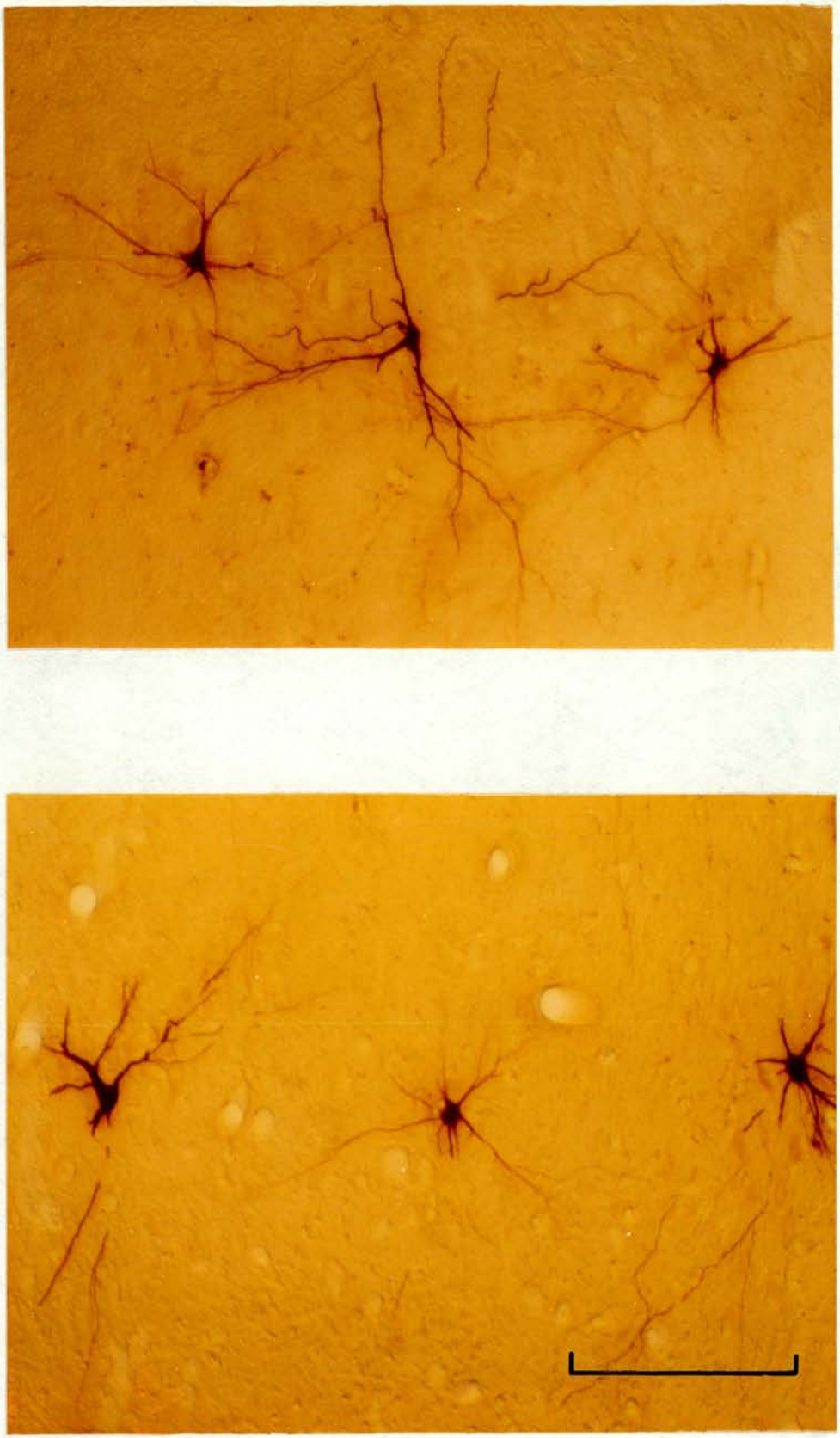
Triceps surae α - motoneurones.

The micrographs (from 100 μm thick sagittal sections) are from different animals in which three motoneurones in each were intracellularly injected with HRP. Electrode tracks were made sequentially at about 500 μm intervals along the rostro-caudal axis of the motor nuclei.

Although only part of each cell's dendritic tree is seen, there clearly is overlap of the dendritic domains.

Dorsal is at the top, rostral to the left.

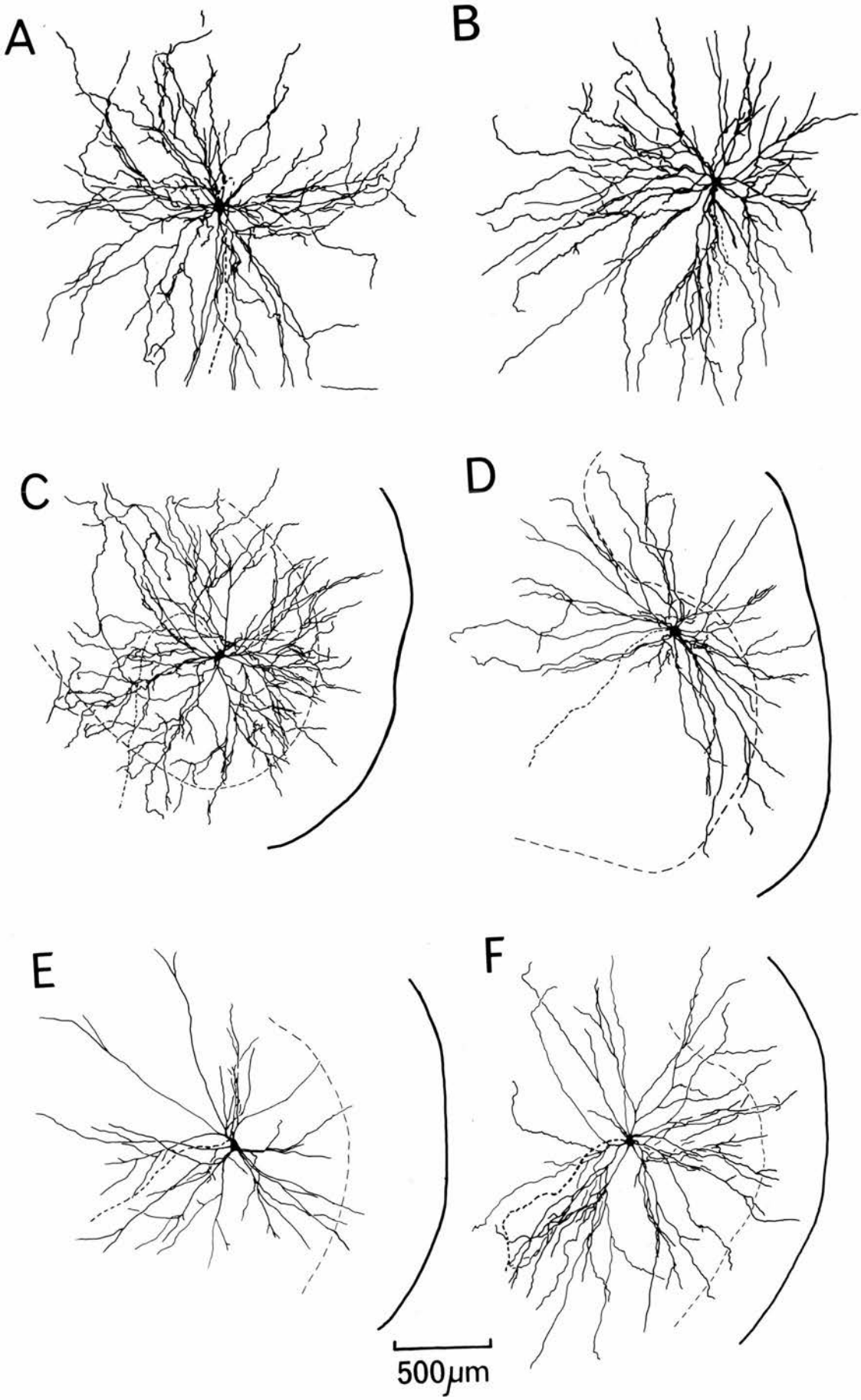
Scale Bar: 500μm.



PART 6 : Figure 3

The morphology of triceps surae α - motoneurones.

Six reconstructions of HRP labelled α - motoneurones are shown. A and B innervate Mg and Lg-S respectively and are shown in sagittal view with rostral to the left. C, D are Lg-S, E, F are Mg motoneurones respectively: all four are reconstructed in the transverse plane with the boundaries of the ventral horn and the surface of the cord indicated. Dashed lines from the somas trace the motor axon trajectories. The extensive branching in all directions and the widespread areas covered by the dendritic trees are apparent. Note that many dendrites enter the white matter. Some additional data is shown in Table 1.


A
B
C
D
E
F
500μm

PART 6: Table 1

The table presents some geometrical and electrophysiological data for the six HRP labelled motoneurones shown in the previous figure (Fig. 3).

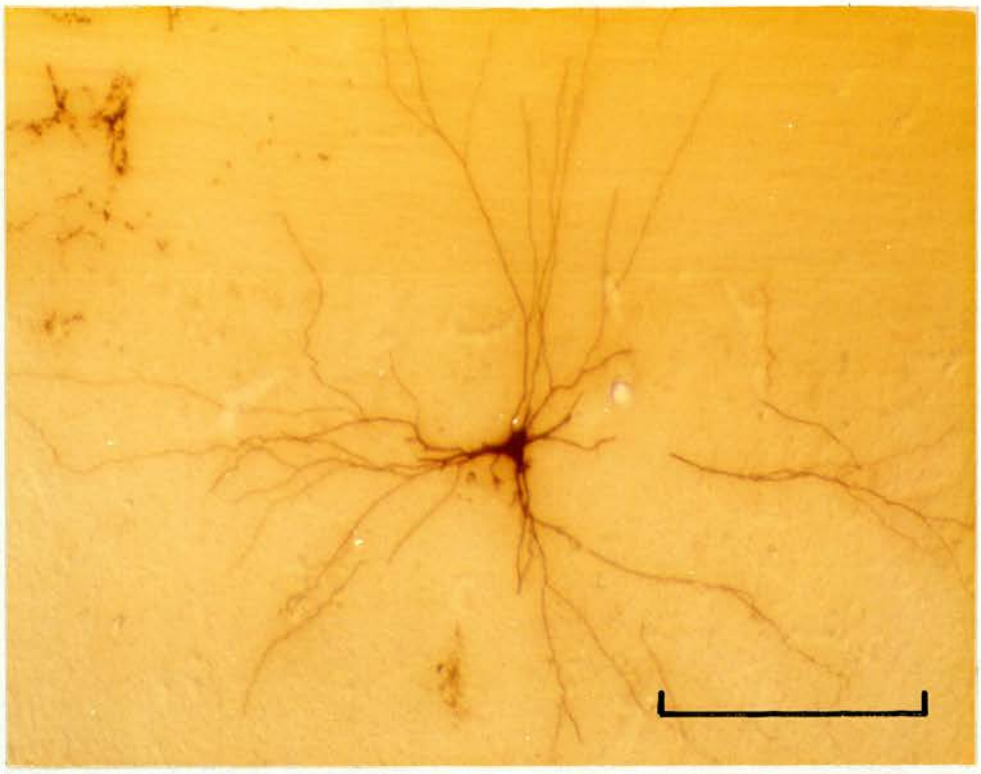
TABLE 1.

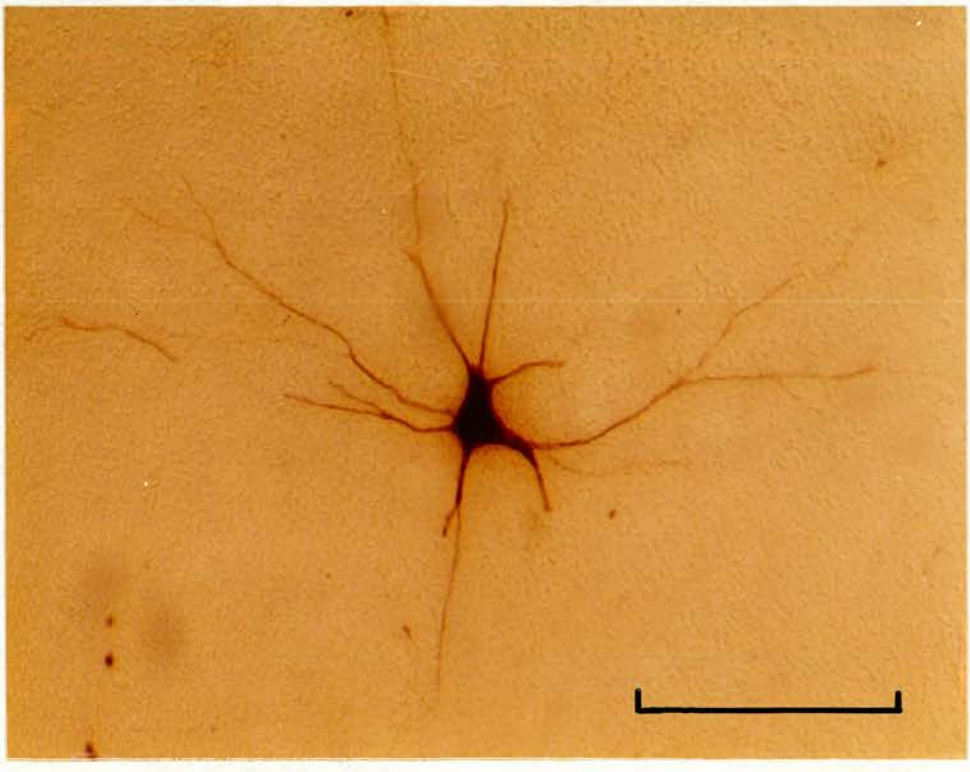
Motoneurone		Soma Diameter (μm)	Conduction Velocity ($m.sec^{-1}$)	No. of 1^{o} Dendrites	Dendritic spread (μm)		
					rostro-caudal	medio-lateral	dorso-ventral
A	Lg-S	60x40	83	11	2131	2000	1938
B	Mg	45x50	90	14	1969	1800	2063
C	Lg-S	48x45	74	8	2600	1720	1915
D	Lg-S	45x50	86	11	2600	1975	2185
E	Mg	75x50	92	9	1100	1688	1625
F	Mg	45x55	76	11	2100	1781	2371

PART 6: Figure 4

These two HRP labelled motoneurones viewed in the sagittal plane, illustrate the diversity of dendritic tree arrangements. Note, in the upper figure, that many of the dorsally (towards the top) directed dendrites penetrate into the white matter of the lateral funiculi.

Scale Bars: Upper 500 μm
Lower 200 μm.





PART 7 : THE DISTRIBUTION OF Ia SYNAPSES UPON α - MOTONEURONES

Introduction

One of the most intensively studied systems in neurophysiology is the monosynaptic reflex pathway from the primary endings in muscle spindles, via the Ia afferent fibres, to the α - motoneurones of the same muscle and its synergists. The Ia-motoneurone synapse and the associated afferent and efferent neuronal pathways have provided the basis for much of our knowledge on integrative function. There have been two kinds of experimental approach: anatomical studies (Szentagothai, 1967; Scheibel & Scheibel, 1969; Conradi, 1969; McLaughlin, 1972a, b; Iles, 1976) suffer from lack of firm identity of the elements examined whilst electrophysiological (Eccles, 1957; Burke, 1967; Mendell & Henneman, 1971; Jack et al., 1971; Kuno, 1964a, b) attempts to define the detailed organization of the pathway are limited by the lack of precise anatomical data.

There are several ways to estimate synaptic location by electrophysiological means (see Jack, Noble & Tsien, 1975), which all rely heavily on the fundamental concepts laid down by Rall's (1959, 1960, 1962, 1964, 1967, 1970, 1977) elegant formulation of mathematical models to describe neurone structure, synaptic events and the consequent flow of electric current throughout the cell

and thereby the state of excitability of the neurone.

However, even the most sophisticated application of these powerful techniques (see Redman, 1976; Burke & Rudomin, 1977) are limited by the reductions made for Rall's simplifying models. In particular, although estimates can be made of the electrotonic sites of synaptic events, the microelectrode recording events at the soma can give no information of the pattern of dendritic branching or even of the number of sites (if all at the same electrotonic distance) at which current is injected into the dendritic tree. Obviously the ideal situation (see later in Discussion) would be to correlate anatomically located synapses of a particular Ia fibre on a motoneurone with physiological estimates (by evaluating the EPSP's of that fibre in the cell) of the synaptic location.

Even without achieving this level of resolution there are other fundamental questions relating to the anatomical organization and fine structure of the Ia-motoneurone system. These can generally be framed in terms of the numbers and distribution of contacts from single afferent fibres. Since it has been shown (e.g. Burke, 1967; Jack et al., 1971; Rall et al., 1967; Iansek & Redman, 1973a) by many workers that Ia synapses have a wide distribution on the motoneurone dendrites, anatomical methods to demonstrate these distal contacts are required, coupled with of course a precise physiological identification of both the pre- and post-

synaptic elements.

The present work uses a combination of the approaches described in previous Parts to label single Ia afferent fibres in the same preparation as an appropriate motoneurone was also intracellularly stained. The results provide direct anatomical information on the connectivity of Ia afferent fibres and the numbers and distribution of terminals upon the dendritic tree of the target motoneurones.

Results

The lamina IX terminal arborizations of Ia afferent fibres. A brief description of the numbers and organization of triceps surae Ia afferent fibre boutons in and around the triceps surae motor nucleus was given in Part 3. The important points to note are that each Ia afferent fibre collateral generates a distinct terminal arborization in the motor nuclei of the appropriate cell groups - the terminals extend for up to 800 μm rostro-caudally and involve some 130 or so synaptic boutons.

The relationships between the Ia collaterals and ventral horn neurones were initially determined by studying counterstained sections containing well stained Ia collateral branches (see for example Figs. 4, 5 & 12 in Part 3). In 100 μm thick transverse sections the motoneurone pools of Romanes (1954) were well defined. The largest cell bodies encompassed in these nuclei were

presumed to be α - motoneurones. These somata had diameters of 40 - 70 μm and, additionally some proximal dendritic stumps (up to about 30 μm from the soma) were also detected on these neurones. These profiles were examined for any association with the HRP labelled Ia collateral boutons.

It was evident that the vast majority of Ia terminals within the confines of the motor nucleus were not in any apparent association or contact with the observable counterstained motoneurones. In six collaterals from different Lg - S Ia afferent fibres 500 boutons were counted within the motor nuclei. Only 45 of these boutons were in close association with the somata and proximal dendrites of motoneurones. A total of 22 motoneurones received these 45 contacts. Fig. 1 shows some of the relationships between Ia afferent fibre terminals and motoneurones. Boutons involved were of both the 'en passant' and 'terminaux' variety. Up to six (mean per cell was 2.05) boutons contacted a single motoneurone - they did not all necessarily arise from the same terminal collateral branch. Each collateral terminated upon, on average 3.66 motoneuronal somata or proximal dendrites. Only 9% of Ia boutons within lamina IX made somatic or proximal dendritic contacts.

The counterstaining approach, although useful in determining the overall projection patterns of Ia fibres to the motor nuclei, suffers from several critical limitations. Firstly, the post-synaptic elements are

not firmly identified as α - motoneurones. Although Romanes (1954) and subsequently others (Burke et al., 1977) have detailed the organization of individual motoneurone pools in the ventral horn it is impossible from counterstained sections to assign a muscle type to the observed structures. There may be further complications because Ia afferent fibres also contact cells of origin of the VSCT (Burke et al., 1971) located in the ventral horn. The dimensions of VSCT neurones are similar to those of α - motoneurones (Jankowska & Lindström, 1970). Boutons seen overlying the soma may be contacting the soma, or perpendicular (to the plane of section) proximal dendrites of the cell, or may indeed not make any contact at all with the juxtasomatic regions of that cell. But the most critical restriction is that distal dendritic contacts were not demonstrable. The value given previously for the number of cells contacted by a single collateral (3.66) is thus a minimum estimate of this parameter. To provide a more reasonable view of the distribution of Ia synapses, the motoneurone dendrites must be visualized along with the afferent collaterals.

To examine the terminations of Ia fibres upon the dendritic trees of α - motoneurones and to provide direct estimates of the locations and frequencies of such contacts, identified α - motoneurones were intracellularly injected with Procion Yellow or HRP in the same preparation as identified Ia axons were injected with HRP.

The study with Procion dyes proved largely unsuccessful in relation to answering these questions. Analysis was made difficult by the alternate use of ultra violet or ordinary light to visualize the fluorescing Procion dye injected dendritic profiles and the HRP stained Ia boutons. As is discussed elsewhere, Procion Yellow does not provide a complete fill of dendritic trees, and the profiles which do contain dye are rather ill-defined at their edges. The only advantage of this technique was that contacts overlying the soma could be easily differentiated. But since 90% of Ia boutons do not end on motoneuronal somata or proximal dendrites this advantage was seldom encountered.

Far more effective was the approach using dual injections of HRP. After a Ia fibre was injected, a motoneurone innervating the same muscle was searched for. The high projection frequency to homonymous motoneurones (Mendell & Henneman, 1971) gave confidence that any α - motoneurone in the appropriate motoneurone pool would receive synaptic contacts. In practice however the neurone had to be impaled within about 1 - 2mm of the level of the axon injection site to maximise the chances of finding adequately labelled Ia collaterals projecting to that cell. The data in Part 6 is drawn from some of the cases where the attempts did not work. In these, the Ia collateral branches were too faintly stained to determine with any assurance whether they made synaptic connexions on the stained cells. In some experiments up

to three motoneurones were injected close to the afferent injection site. Intracellular staining was aimed at determining dendritic tree geometry and to this end the actual injections were limited to 60 - 120nA.min so that although the motor axons were traceable the recurrent collaterals were only faintly stained and therefore did not interfere or intermingle with the primary afferent collateral branches entering the motor nuclei. Even so, the dendritic trees were extensively labelled (see Part 6).

Ia synapses upon α - motoneurone dendritic trees. In the absence of electronmicroscopical examination of the present material the interpretation of results required some assumptions to be made about presumed contacts. All presumed contacts satisfied the following criteria: 1) Only well stained collaterals and cells were analysed; all stained contacts were traced back to the identified Ia axons on the one hand and to the labelled α - motoneurones on the other. 2) Contacts were counted only if the afferent axonal profile exhibited a clear bouton-like swelling of the size range and appearance of terminals described in Part 3. 3) There was no sign, under oil immersion examination at 1000 magnification, of any gap between the two elements.

There were several instances where axon branches seemed to make cross-over contact with a dendrite, but although functional synapses need not necessarily involve

axonal swellings or boutons the present sample is limited to examples where a swelling was apparent. Therefore even the following results may underestimate the number of functional contacts between Ia afferent fibres and motoneurones.

When afferent terminal branches coursed over (or under) densely stained motoneurone cell bodies or proximal dendrites there was difficulty in determining any contacts because of the lack of contrast between the Ia terminal arborization and the cell body. However, there was in fact only one example in the present results (Fig. 8A) where this proved a problem.

A total of ten Ia afferent fibre - motoneurone pairs, from different cats, were suitable for detailed examination in that both the afferent terminal arborizations and the motoneurones were intensely stained, and there were contacts between the two. Details of these are shown in Figs. 2 - 11 and described in detail in the appropriate figure legends. All sections were cut at 100 μm, either in the transverse or sagittal plane. The reconstructions which are presented generally show in full only the motoneurone dendritic branches that received contacts. Other dendrites and motor axons have been omitted or shortened for the sake of clarity in the figures. In Fig. 12 are shown schematic diagrams summarizing the observed contact patterns.

Detailed descriptions of each contact system are presented in the appropriate figure legends. In this

section of Results, only general observations are referred to, together with some comments on the overall patterns. In this respect some specific figures will be referred to.

In the summary diagram (Fig. 12) the identity of the stained afferent and its target motoneurone is indicated. The combinations were:

two Mg afferents contacting two Mg α-motoneurones,
two Mg afferents contacting two Lg-S α-motoneurones,
four Lg-S afferents contacting four Lg-S α-motoneurones,
one Lg-S afferent contacting one Mg α-motoneurone,
one PT afferent contacting one PT α-motoneurone.

Only the Mg-Mg combinations can be assumed to be strictly homonymous.

The contacts in Fig. 12 are indicated by filled triangles. They are placed on schematic dendritic trees such that the distances from the soma to individual dendritic branch points or contacts can be determined from the scale bars. Where possible the presynaptic axonal branching network is indicated, but not to scale. In examples B, E and F the common branch point of the contacting terminal axons was so far back - at a major collateral division in the intermediate region - that representation of all the presynaptic branches would have complicated the diagram too much. Nevertheless it should be emphasised that all the contacts came from only one collateral in each case.

A total of 34 contacts were observed between the 10 pairs of Ia afferent fibres and motoneurones, with a range of 2 to 5. Where more than two contacts were made, the boutons, as well as arising from different terminal branches of the afferent, were often distributed to more than one dendritic branch. The branches contacted were from the same (Fig. 12B, C, D, H, I, J) parent dendrite or (Fig. 12 A, E, F, G) were branches of different trunk dendrites. Dendrites contacted included primary dendrites and second, third and fifth order daughter branches. The contacts were made at widely ranging geometric distances from the soma. In some cases (Fig. 12A - D) all the contacts were within 50 - 70 μm of the soma. Where multiple contacts were made on more distal dendritic regions there was usually relatively wider separation of the contact sites. In example E of Fig. 12 four contacts were made - three 'en passant' contacts were grouped about 330 - 380 μm from the soma on a second order dendrite; the remaining contact was on a third order branch of another dendrite and was 765 μm from the soma. In G, five contacts were made on three branches (second and third order) of two dendrites. They were located at 190 - 365 μm from the soma.

It should also be noted that in other cases where multiple contacts were made on different dendritic branches (Fig. 12F, H) they were located at essentially similar distances. The most distal contacts observed

were on a third order dendrite (Fig. 12I) where two 'en passant' boutons terminated 820 μm from the soma.

There was no dendritic orientation which received a predominant projection from the afferents. Contacts were seen rostral, caudal, dorsal, ventral, medial and lateral to the position of the soma. Although in most cases the dendritic tree of the α - motoneurone, which frequently extended for more than 2mm in the rostro-caudal direction, was penetrated by terminal arborizations of up to three adjacent Ia afferent collaterals, the contacts arose from only one of these arborizations.

Eleven of the contacts were made by boutons 'terminaux'. The majority (69%) involved 'en passant' contacts either of the climbing type (Fig. 12C, D, E, I) or, commonly, of the cross-over type where subsequent boutons on the terminal axon were not involved in contacts on the same dendritic branch.

In one example (Fig. 8A and Fig. 12D) there was some difficulty in determining the exact number and type of contacts. Two 'en passant' climbing contacts were made on a trunk dendrite at 20 - 30 μm from the soma. The fine collateral branch giving rise to these contacts extended over the motoneurone soma but no further contacts were observed. It was impossible to differentiate the two elements due to the intense staining of the soma. This particular Ia - motoneurone system might have been more readily investigated had

Procion Yellow been the marker of choice for intracellular staining. Interestingly, this type of contact system - one or two 'en passant' boutons along a proximal dendrite plus perhaps one further somal contact - was observed also in the Ia afferent counterstained material and has also been described by Szentagothai (1958) and Iles (1976).

Contacts located upon fine distal dendrites involved boutons which were considerably larger than the diameter of the dendrite. This was particularly apparent (see Figs. 3, 11E) when the bouton made an 'en passant' cross-over contact. Ia boutons were sometimes associated with beaded dendritic regions (see Figs. 5, 11G). In these cases extreme care was taken when selecting presumed contacts because 1) the varicosities were often densely stained and large (up to 10 μm diameter) so that the same problems arose as with juxtasomatic contacts and 2) other fine beaded dendrites could be confused with terminal bouton carrying collateral branches. No Ia afferent boutons made contact with α - motoneurone dendritic spines.

One final observation was that all the contacts, regardless of their actual distance from the motoneurone soma, were made within the confines (as well as they can be defined by cytoarchitectonic criteria) of the lamina IX motor nuclei. No contacts were made on dendrites in the white matter (occasionally Ia collateral branches extend slightly into the white matter) or on the portions

of dendrites passing through laminae VI and VII regions. All of the boutons involved recognizably belonged to the 'lamina IX' terminal arborizations of the Ia afferent fibre collaterals.

Discussion

The distribution of Ia afferent fibre collaterals to α - motoneurones. The present experiments go some way to answering the questions posed by electrophysiological observations. In the first instance we can consider the anatomy of the Ia afferent projections in relation to their known motoneurone connectivity. Signal averaging techniques have provided precise information on the projection frequency of Ia afferent fibres to different populations of α - motoneurones. Beginning with the spike-triggered averaging studies of Mendell & Henneman (1968, 1971) it was recognized that each Ia afferent projected to at least 90%, if not all, of the motoneurones supplying the muscle of origin and that a single motoneurone must receive terminals from almost all of the Ia fibres of the muscles it innervates. These findings were essentially confirmed in subsequent investigations (Watt et al., 1976; Scott & Mendell, 1976; Nelson & Mendell, 1978; Munson & Sypert, 1979a). The projection of Ia afferent fibres to α - motoneurones of heteronymous motor pools is rather less, being of the order of 50 - 60% (Mendell & Henneman, 1971; Munson & Sypert, 1979a).

Earlier anatomical studies present information on the likely numbers of afferents and motoneurones involved. For the triceps surae muscles in adult cats there are about 150 or so muscle spindle primary endings (Boyd & Davey, 1968) giving around 56 S, 62 Mg and 35 Lg Ia afferent fibres. The numbers and extent of α - motoneurones in the triceps surae motoneurone pool have also been determined. There are about 725 α - motoneurones (290 Mg; 280 Lg and 155 S) occupying a length of up to 10mm in the lumbosacral spinal cord (Boyd & Davey, 1968; Romanes, 1951; Van Buren & Frank, 1965; Sprague, 1958; Burke et al., 1977).

Ia afferent fibres issue collaterals every millimetre or so (see Part 3). Thus about 10 collaterals will supply the triceps surae motor pool. Considering, say, a Mg Ia fibre; this afferent would be expected to project to 290 Mg and 273 Lg + S α - motoneurones (total 563 cells). Each Ia afferent fibre collateral must then contact 50 - 60 triceps α - motoneurones (as well as other indirect synergists). Since each collateral arborization ramifies over a rostro-caudal distance of up to 800 μm in lamina IX, and motoneurone dendrites extend for up to 3.00mm in that plane, a single collateral could reasonably make that number of monosynaptic connexions. A single collateral may contact up to seven (average about four) motoneurones (juxtasomatic terminals only - Ishizuka et al. (1979) have one Mg collateral contacting 17 motoneurones). But

only about 10% of the lamina IX boutons are associated with these cells. On this basis there are enough boutons remaining for each collateral to contact another 50 or so motoneurones.

That a single collateral might synapse on 50 - 60 motoneurones indicates that Scheibel & Scheibel (1969) and Iles (1976) underestimated this aspect of Ia - motoneurone synaptic organization. These groups estimated that each collateral supplied about 10 motoneurones. The discrepancy probably arises because the Scheibels (1969) studied Golgi stained spinal cord from young cats. They showed collaterals arising every 200 μm from large dorsal root axons. Iles (1976) used this figure in his analysis. The present work (Part 3) and other recent independent anatomical and electrophysiological studies (Ishizuka et al., 1979; Munson & Sypert, 1979a) have clearly demonstrated that in the adult cat Ia collaterals are spaced at intervals of about 1,000 μm. The present results (see also Part 3) have not provided any evidence that Ia fibres give off more collaterals and/or more boutons in the regions close to their entry into the spinal cord and thereby make more frequent contacts upon motoneurones in that area.

While discussing the general distribution of Ia terminals to the motor nuclei it is also worth noting that, given the average number of lamina IX boutons per collateral (127, ranging up to about 200), each axon delivers 1,200 - 2,000 terminals to the triceps surae

motor nuclei. It will be shown later that this value is in good agreement with the expectations from electrophysiological results and from the more exact determination of the likely number of contacts made by single afferents on to individual motoneurones as elucidated by the dual injections of HRP.

Interpretation of dual labelling experiments. The parameters for identification of a presumed synapse between a physiologically defined Ia afferent and a target motoneurone have already been detailed. The criteria are rather more rigid than those of Burke et al. (1979) who included contacts with no pre-synaptic swelling in their sample. Functional synapses neet not involve pre-synaptic swellings (Waxman, 1975) but until electronmicroscope examination of the present type of material is carried out these possibilities must remain possibilities. The criteria suitable for our light-microscope analysis might therefore still tend to give underestimates of the numbers of contacts per cell. This error is likely to be less serious than in the experiments where Ia - motoneurone contacts were determined from counterstained material, in which only the dendritic trunks were visible.

On occasions, when well stained Ia collateral branches arborized amongst the dendrites of a labelled motoneurone, no possible contacts were observed. This negative type of finding can most readily be explained on

the basis of the accepted projection frequencies - even for strictly homonymous pairs the generalization that each axon projects to every motoneurone is clearly a simplistic view. In fact the available data indicates values of 85 - 95% connectivity.

One of the assumptions made in the interpretation of the results is that the method allowed complete visualization of the Ia terminals. Since the injections were made at roughly the same level in the rostro-caudal axis of the cord, collaterals in that region were well stained. The analysis was only performed if the Ia arborizations were densely labelled, preferably if adjacent collaterals on either side were well stained too. Burke et al. (1979) used cobalt intensification (Adams, 1977) of their material. In our hands this method did not reveal any 'extra' terminals.

The final assumption is that synapses were present at boutons on the terminal axons. There is the possibility that pre-synaptic swellings themselves may be wrongly identified as boutons. Experience and careful examination allows one to differentiate between axonal bends or twisting and bouton-like swellings. All the presumed contacts involved axonal swellings in the size range of Ia boutons described in light (e.g. Iles, 1976; Brown & Fyffe, 1978a) and electron microscope (Conradi, 1969; Mclaughlin, 1972a, b) investigations. Finally some swellings may not have synaptic specializations. Some axon swellings in HRP stained primary afferent

fibres (A.G. Brown, R.E.W. Fyffe & D.J. Maxwell, unpublished observations) are not synaptic but filled with mitochondria. These are rarely seen in the electron microscope and are therefore unlikely to confuse the present issues.

Distribution of Ia synapses on motoneurone dendritic trees. This work (also Brown & Fyffe, 1978c) has provided the first direct anatomical information of the numbers and locations of synapses made by single Ia afferent fibres on α - motoneurones. On the basis of the analysis of extracellular field potentials, Fatt (1957) proposed that the excitatory postsynaptic potential (EPSP) evoked in α - motoneurones by a synchronous Ia afferent volley was generated primarily in the motoneuronal dendrites. Subsequently a large body of evidence has been built up to support the suggestion that Ia EPSP's are generated by synapses distributed over much of the motoneuronal surface including the distal parts of dendrites. Electrophysiological experiments have shown a wide range of Ia EPSP shapes in motoneurones, consistent with widespread distribution of Ia boutons (Kuno, 1964a, b; Burke, 1967; Rall et al., 1967; Jack et al., 1970, 1971; Iansek & Redman, 1973b; Nelson & Mendell, 1978; Mendell & Weiner, 1975) and single Ia fibres can generate EPSP's with vastly different time courses in different motoneurones (Mendell & Henneman, 1971).

Jack, Noble & Tsien (1975) discuss in detail the methods for determining synaptic location. One of the most developed methods involves analysis of the time course of the synaptic potential to predict synaptic location and allow calculation of the synaptic current. The motoneurone models used assume a brief synaptic current and a point synaptic location. The electrical or cable properties of the motoneurone should preferably be measured from each motoneurone. There have now been several studies in which synaptic location has been estimated, and in which EPSP time courses have been compared with theoretical predictions (Rall et al., 1967; Jack et al., 1971; Iles, 1976; Iansek & Redman, 1973a, b). The studies estimated that Ia synapses were situated for the most part between 0 to 1.25 space constants from the soma. Some EPSP shapes indicate that boutons from a particular Ia afferent fibre are probably sited within a restricted electrotonic locus on the motoneurone (Jack et al., 1971; Mendell & Henneman, 1971; Munson & Sypert, 1979b) thus providing support for the point synaptic location assumption. But other studies (Rall et al., 1967; Munson & Sypert, 1979b) demonstrate EPSP shapes more consistent with boutons from the Ia afferent being located at distinct and separate electrotonic positions.

The present work allows some comments to be made on the assumption of a single locus for the Ia-motoneurone contact. The actual number of contacts and the

relevance to previous estimates will be discussed later. Figure 12 summarizes the distribution of contacts. In agreement with electrophysiological studies, Ia synapses have been shown to occur at considerable distances, up to 800 μm from the soma, i.e. on the proximal geometrical half of the dendritic tree. A striking feature of some of the results was that there was a tendency for contacts to be 'clustered' at a particular distance on the dendritic tree even when more than one primary dendrite and its branches were involved. Especially 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. In other examples there is wider dispersal of bouton locations (Fig. 12E, G). These are on relatively distal regions and the analytical methods (Jack & Redman, 1971; Jack et al., 1971; Iansek & Redman, 1973a, b; Redman, 1976) might not detect some of the arrangements in Fig. 12G. The pattern in Fig. 12E however would be expected to generate an EPSP time course with a half life in excess of that compatible with its rise time. In the example in Fig. 12D, if a somatic contact was also made, then a composite EPSP might be recognized.

It is concluded that not only do Ia fibres terminate on motoneurone dendrites with more than one synapse, but that these contacts may often be located at different sites. The assumption of a single electrotonic locus for boutons from a particular afferent fibre did seem

applicable in a number of instances - and since the previous electrophysiological studies (Kuno & Miyahara, 1969a; Jack et al., 1971; Iansek & Redman, 1973b) tended to 'select' rather simple time courses for analysis, it is likely that in these studies the assumption was valid.

What we now have however is direct evidence (see also Iles, 1976) that boutons from a single fibre do not always end in a single electrotonic compartment of the motoneurone dendritic tree. Burke et al. (1979) also describe Ia-motoneurone contact systems with bouton dispersal compatible with some previously described non-simple EPSP shapes. Factors other than actual synaptic locations may also influence the EPSP shape and hence any prediction of the (mean) synaptic location made from it. Iles (1976) has recently shown that afferent conduction velocity is an important determining parameter when considering multiple synapses and that the behaviour of the synaptic arrangement deviates from 'point location' EPSP shapes as conduction velocity is decreased. Where terminals arise from a single collateral branch and conduction in the afferent is towards the soma (Fig. 12D) then introduction of a conduction delay would increase the rise time. In Fig. 12C, I, J, conduction in the terminal branch is away from the soma and a conduction delay would increase rise time and half width. The resultant EPSP's would tend to predict more distal synaptic locations than actually occur. In the other

examples the situation is even more complicated. Boutons contact different branches of different dendrites and arise from separate and distinct pre-terminal axons. The pre-terminal branching patterns are complex but, in this study, all boutons contacting any motoneurone come from the same Ia collateral. Thus, factors other than conduction delays may also be significant in these arrangements.

The number of Ia synapses upon motoneurones. It is now evident that each Ia afferent fibre terminates with more than one bouton on its target motoneurones. The data provides interesting information on the number of Ia synapses on individual motoneurones. Several workers (Wyckoff & Young, 1956; Rall, 1964; Conradi, 1969; Ellis, 1967; Koziol & Tuckwell, 1978) have estimated the total number of synapses on motoneurones. The values presented range from 2000 to over 20000 boutons per cell. The most recent estimates (Koziol & Tuckwell, 1978) use the data of Barrett & Crill (1974a) on motoneurone surface area to estimate the number of cells in particular regions. Since (see Part 6) most motoneurone dendrites are longer than indicated by Barrett & Crill (1974a) these estimates may be undervalued. Very detailed morphological analysis remains to be done on HRP labelled motoneurones.

Conradi (1969) and McLaughlin (1972a, b) have presented detailed electronmicroscopic analysis of

presumed dorsal root boutons (M type) on motoneurones. The bouton identity was inferred since the M type boutons disappear following rhizotomy. These workers noted that the distribution of these boutons was not uniform on the motoneurone surface - very few were found on the soma, their density was maximal some 50 - 100 μm out on the dendrites and grew progressively less towards the distal dendrites. The number of M type boutons is only about 0.5% of the total; thus the dat indicates that there are fewer than 100 dorsal root boutons per cell (Jack et al., 1971; see also Szentagothai, 1958; Koziol & Tuckwell, 1978).

The functional connectivity of Ia afferents and motoneurones is known (Mendell & Henneman, 1971; Watt et al., 1976; Munson & Sypert, 1979b). Each Ia fibre contacts approximately 90% of homonymous motoneurones and 60% of heteronymous motoneurones. Conversely each motoneurone receives contacts from almost all the Ia fibres innervating its muscle as well as from about 60% of Ia fibres from the heteronymous muscles. Taking Mg motoneurones as an example, each cell will be supplied by ((62x100%) + (91x60%)) = 116 Ia fibres from triceps surae muscles. If each fibre made a single contact then the total would indeed range around 100 Ia boutons per cell. Pooling the HRP data for somatic (counterstaining) and dendritic (dual labelling) contacts each afferent makes 2.72 contacts on average. Thus each motoneurone should have at least 315 Ia afferent boutons. This value is much higher than was estimated from

previous light and electron-microscopical investigations. When one considers (see also Iles, 1976) that a proportion of spindle group II afferents will also make monosynaptic connexious on to α - motoneurones the estimate for 'dorsal root boutons' should be even higher.

The conflict in these results is likely to have arisen from the anatomical studies, because the electrophysiological information has long indicated that Ia boutons were not only widespread on the motoneurone surface, but more numerous than the work of Conradi and others had implied. There are actually some excellent correlations between the previous anatomy and the electrophysiological approaches. The distribution of Ia synapses to different dendritic locations (Jack et al., 1971) match the density distribution of presumed dorsal root boutons (see Koziol & Tuckwell, 1978). Jack et al. (1971) demonstrated that Ia synaptic density was greatest at 0.2 - 0.6 from the soma, a result confirmed by other workers using even more refined methods for determining synaptic location (e.g. Iansek & Redman, 1973b). The present sample of 34 dendritic contacts is too small to infer a spatial distribution pattern, but almost all of the contacts are on the proximal geometric half of the dendritic tree (see also Iansek & Redman, 1973b; Redman, 1976). But on the basis of quantal studies (Kuno, 1964a, b; Kuno & Miyahara, 1969b), EPSP amplitudes (Mendell & Henneman, 1971) and charge transfer at dendritic synapses (Iansek & Redman, 1973b) there is

evidence that Ia fibres make multiple contacts with up to 10 synapses per afferent per motoneurone. The present work confirms that each fibre makes multiple contacts on single motoneurones. Indeed, very recently Burke and his co-workers (Burke, 1980; Burke, Pinter, Tov & O'Donovan, 1980) have extended their small (Burke et al., 1979) sample of Ia-motoneurone contacts and have noted up to 30 contacts from a Ia afferent to a single motoneurone. In this maximal case, the Ia and motoneurone innervated plantaris muscle. Iles' (1976) results cannot be directly compared at this point because he only investigated juxtasomatic contacts.

It seems that the conclusions of electrophysiological works have been restricted to a great extent by the concepts of one bouton per afferent per cell and the likelihood of a rather small total number of boutons. The anatomical data can now be revised, both in terms of the directly observed multiple contact systems and also in terms of the larger known extent of the motoneurone dendritic tree. Even assuming that the density of boutons previously determined is correct, this latter parameter would increase the absolute numbers of any particular bouton type. Critical testing of the ultrasturctural interpretations can in future be carried out by electronmicroscopical examination of HRP labelled Ia afferent terminals in contact with motoneurones. A further advantage of a study at that level is that degeneration techniques could be dispensed with.

What then are the implications for the interpretation of electrophysiological studies? In the first instance, the data indicates that the assumption of a point location for synaptic input is not valid in a considerable number of cases. If it were, the methods described by Jack, Noble & Tsien (1975), Jack & Redman, (1971), Iansek & Redman (1973a, b), Redman (1976), Rall (1977) would, even for multiple contact systems, give a good estimate of the mean synaptic distance from the soma. There are of course other parameters affecting this type of calculation. Notably the electrotonic length (L) of the different dendrites comprising the equivalent cylinder model might be different from the assigned value for the whole dendritic tree. If the synapses are situated on a dendrite with longer L then an underestimate of synaptic location distance will be made from measurement of EPSP half width. Conversely, if an estimate of synaptic location can be made using the reversal potential technique (see Coombs et al., 1955), the deviation in a similar situation will be in the opposite direction, i.e. an overestimate of distance will be made. Iansek & Redman (1973a) give a full discussion of the importance of other motoneurone membrane properties, cable parameters and modelling assumptions.

Wide dispersal on the dendrites of terminals from a single afferent would result in EPSP half widths which would classify mean synaptic location with a bias towards the electrotonic location of the more distal boutons.

Furthermore if the dispersal pattern involved a somatic contact (with resulting composite waveform) the EPSP would not be analysed in terms of the models used by Jack et al. (1971), Rall (1967) and Iansek & Redman (1973b) although Iles (1976) has presented calculations to account for EPSP time courses like that (examples of which have been often described, e.g. Burke, 1967; Rall et al., 1967; Mendell & Henneman, 1971; Munson & Sypert, 1979b).

In the cases where multiple synapses are located at a restricted locus, particularly on a single dendritic branch, then the single synaptic location assumption will be applicable. But where the terminals of a Ia fibre are very close together and are activated almost simultaneously then the resulting charge transfer, or EPSP, is not the linear sum of the charge transfer or EPSP generated by each synapse alone (Rall, 1962a, b; Burke, 1967; Edwards, Redman & Walmsley, 1974; Redman, 1976; Kuno & Miyahara, 1969a; Rinzel & Rall, 1973). Similarly a non-linear effect occurs when terminals from two different afferents contact a restricted locus. Some of the individual patterns observed in the present work have contacts in such close proximity that the EPSP components should add non-linearly; other arrangements where the contacts are at similar electrotonic locations but on different dendrites should produce linear additions of the EPSP components.

The influence of conduction delays on the expected

time courses of EPSP's generated by multiple contacts has already been mentioned. Iles (1976) has shown that the introduction of a temporal delay in bouton activation can significantly alter EPSP configuration, and indeed can explain some experimentally observed shapes. Iansek & Redman (1973b) concluded however that conduction delays or differences in the terminal axon were unlikely to be the mechanism by which EPSP's which were not accounted for by the cable model were generated. They suggested that prolonged synaptic current, or a brief synaptic current followed by a voltage dependent membrane response determined these time courses.

Terminal branches connecting boutons are extremely thin, often less than 1.0 μm diameter. Assuming a conduction velocity comparable to small C fibres ($0.5 msec^{-1}$, Iggo, 1958) there could be a conduction delay of 0.1msec for the invasion of the three boutons comprising the proximal contacts in Fig. 12E even although the three boutons are carried on the same terminal branch. This might significantly increase the rise time and half width and lead to a prediction of a more distal synaptic location than actually occurs. In the particular example of Fig. 12E the distal contact on another dendrite would further bias the synaptic location estimate to a more distal site. In other examples conduction along a terminal chain of boutons was 'away' from the soma. Temporal dispersion of synaptic activation under this type of arrangement would again

generate EPSP's indicating a mean synaptic location further from the soma than the actual electrotonic locus.

The results indicate a further complicating factor relevant to determination of synaptic location by electrophysiological methods. In most cases, even those involving juxtasomatic contacts (cf. Szentagothai, 1958; Sterling & Kuypers, 1967), the contacts are not only distributed to different dendrites (whether or not at the same estimated electrotonic locus) but arise from different end branches of the particular Ia collateral generating the contacts. The pre-terminal branching patterns are complex and lead to a variety of ways in which the Ia EPSP might be fractionated due to failure of, or reduced, invasion of certain terminal branches and boutons.

It has long been known that the EPSP's evoked by single Ia fibres can be fractionated (see Burke & Rudomin (1977) for a discussion of the hierarchy of EPSP components). Amplitude fluctuations (Kuno, 1964a, b; Burke, 1967; Kuno & Miyahara, 1969a; Jack et al., 1971; Iansek & Redman, 1973b; Zucker, 1973; Mendell & Weiner, 1975; Edwards, Redman & Walmsley, 1976a, b) are now interpreted in terms of transmitter release from several sites. Jack, Redman & Wong (1980) and Redman (1980) have suggested, following deconvolution noise removal procedures which allowed analysis of amplitude distribution probabilities, that the amplitude fluctuations are quantised (see also Kuno 1964a, b) and

that one unit of transmitter is released per impulse for each bouton. The probability distributions do not fit any classical single statistical model (Edwards et al., 1976a, b). It seems likely that failure of action potential invasion at branch points in the Ia collateral arborization will make a contribution to the observed patterns of single fibre EPSP amplitude distribution.

There is a method which would go a long way to resolving some of these issues. If the single Ia fibre and the motoneurone in which EPSP's are evoked by activation of that afferent can both be labelled after the electrophysiological analysis then not only the number and spatial distribution of contacts can be determined but these parameters can be directly compared to the recorded EPSP time course and amplitude fluctuations. During the present work attempts were made to record simultaneously from a single Ia afferent fibre and one of its target motoneurones. Motoneurones were impaled in the motor nucleus, Ia axons at their dorsal root entry zone. The Ia axon could be directly stimulated by the intra-axonal microelectrode and the spike triggered averaging technique was used to monitor the evoked single fibre EPSP's. The aim was to record the post-synaptic response to single afferent action potentials, then to label both elements with HRP. The experiments were unsuccessful. Short duration (up to 10 minutes) simultaneous recordings were made, and some correlations made between the Ia impulses and resultant post-synaptic potentials. There

was never enough time however to complete the dual HRP injections. There were many technical problems - the movement of either microelectrode frequently displaced the other; searching for afferents monosynaptically linked with the recorded motoneurone took some time, during which intracellular recording characteristics deteriorated; furthermore, because the microelectrodes were in such close proximity there was a great deal of capacitative 'cross talk' between them - shielding the electrodes to near their tips reduced some, but not all of this artifact. Very recently exciting advances were reported by Redman & Walmsley (1980) who overcame the stability and microelectrode problems by using only one HRP filled microelectrode - penetrating the motoneurone. The Ia fibre was isolated in a dorsal root filament and after responses were measured HRP was applied to the cut end of the dorsal root filament; the motoneurone was labelled by standard ionophoretic injection of HRP. Reconstruction of the contact systems confirmed the electrophysiological methods (Jack et al., 1971; Rall, 1977) and, although the sample size is yet small, supported the hypotheses (see Redman, 1979) presently describing EPSP amplitude fluctuations.

The number of terminals distributed by a single Ia afferent to the motor nuclei can also be determined. Each fibre will provide 1,000 - 2,000 boutons over a 10mm length of motor cell columns. This distance corresponds to the length of the triceps surae motor

nuclei (Romanes, 1951; Burke et al., 1977). Mendell & Henneman (1971) estimated that the largest Mg Ia fibres would supply at least 4,000 endings to Mg and Lg motoneurones. This implies an average of about 7 endings per cell from that fibre. The average value in the present work is from 2.0 - 3.4 endings per cell. This of course is a minimum value (see also Burke, 1980) and the sample size, even including Burke's results, is very small. When one considers the range of EPSP mean amplitudes described by many workers (see Burke & Rudomin, 1977) and remembering that peak amplitude is independent of the site of origin of the EPSP (Iansek & Redman, 1973b) the physiological data relate rather well to the morphological determinations of numbers of Ia boutons and the number of contacts made by a single afferent on a single motoneurone.

The specificity of Ia monosynaptic connexions. There are conclusions which can now be made on the general organization of the Ia-motoneurone contact systems. Unfortunately as yet there is not enough anatomical information to address directly the questions posed by Henneman's size principle (Henneman et al., 1965; Lüscher et al., 1979, 1980) and its relationships with the functional identity of motoneurones (Burke et al., 1968, 1973) and the size of Ia afferents (see also Mendell & Henneman, 1971). It is clear that most Ia afferent fibres make more than one synapse on any

motoneurone. The total complement of synapses is distributed over much of the motoneurone's receptive surface - indeed synapses from a single axon may also be widespread. There are some indications that predominantly distally located contact systems may involve greater numbers of synapses. Certainly the average number of contacts on the whole soma - dendritic tree (3.4) is greater than the number of juxtasomatic contacts (2.05) although the whole extent of the latter systems (from counterstaining) may not be visualized. However, the available material from this work shows no systematic change of the number of boutons per synaptic structure with mean distances from the soma. The problem becomes a statistical one because of the variables involved, including the size and type of the motoneurones as well as the number and location of the synapses. Furthermore the number and physical distance of the synapses may not be as important as possible underlying electrical variables including greater efficacy at distal synapses because of receptor saturation or other post-synaptic mechanisms.

Spatial weighting of synapses occurs in other systems. Inhibitory synapses from Ia inhibitory interneurones and Renshaw cells (see Jankowska & Roberts, 1972a, b; Burke et al., 1971) are located on the soma and proximal dendrites of motoneurones with the terminals of Ia inhibitory interneurones generally being closer to or on the soma.

Ia synapses on dendritic appendages. Rall (1970) has discussed the implications of synaptic contacts upon dendritic spines in terms of the possibility of changing the relative weights of synaptic inputs from various afferent sources. No Ia synapses were observed on spines in this material. Nevertheless several contacts were made on varicose regions of dendrites, particularly at distances greater than 300 μm from the soma. The function of the varicosities is unclear. It may be that they serve effectively to isolate fragments of the finer dendrites. Thus, whereas several terminations on the same fine dendritic shaft might interact non-linearly, synapses on a dendrite, contacting, or separated by varicosities or expansions might provide linear summation. On the other hand, these varicosities will also introduce a loading factor into the cable properties of the dendrites and lead to more rapid attenuation of post-synaptic potentials.

The mode of termination of Ia fibres upon motoneurones. Scheibel & Scheibel (1969) proposed that the geometrical arrangement of primary afferent fibres was such that different afferent domains make synaptic contacts at loci which, for any one afferent, will be at a restricted distance (on one or more dendrites) from the soma. Their hypothesis was based on a) Ia afferent fibre collaterals having very limited rostro-caudal spread and b) predominantly longitudinally oriented dendrites. For triceps surae Ia afferents and

motoneurones we have demonstrated that the collateral terminals may extend over 800 μm and that motoneurone dendrites have a radiative pattern.

There were however some general points of agreement with the Scheibels' studies. Firstly, even when contacts were on different dendrites, they were often at similar distances from the soma. Secondly, each Ia afferent collateral arborization will cut across the dendritic domains of many, possibly more than one hundred, triceps surae motoneurones. Thirdly, because of the great extent of motoneurone dendrites, the dendritic tree will be intersected by more than one collateral arborization (usually two or three) from a single afferent fibre.

Basically the first point concurs with much of the electrophysiological evidence (e.g. Jack et al., 1971). The exceptions (see Fig. 12) might also be expected from previous data (Rall et al., 1967). The second point is supportive of the calculations made earlier of the number of cells contacted by a single collateral (50-60). Incidentally the possibility remains (and requires ultrastructural and electrophysiological testing, although the latter would be particularly difficult) that single boutons may synapse on more than one motoneurone or even on two locations on the same motoneurone. The anatomical basis exists - boutons are large in relation to some dendrites and thus have the available surface to contact more than one post-synaptic element.

Furthermore dendrites from different cells overlap greatly (see Part 6). If failure of invasion of such a terminal occurred the post-synaptic cells would be affected simultaneously. The second possibility above is much less likely because, despite the complexity of the motoneurone dendritic tree, branches seldom come in such close apposition that they could be spanned by a single bouton. The third point is interesting because although several of an axon's collaterals might pass through a motoneurone's dendritic tree, contacts will be made by terminals from only one of the collaterals even although they may be located at different distances from the soma. It is not necessarily the most caudal (or rostral) of the overlapping collaterals which makes contact. In one case contacts were made only by the middle of three overlapping arborizations. This suggests some selective process occurs during the initial formation of Ia-motoneurone contacts, perhaps akin to the process of neuromuscular junction innervation (see Purves, 1976). In Burke's most recent work (Burke et al., 1980) there are descriptions of motoneurones (plantaris) receiving contacts from up to three adjacent collaterals of a single Ia afferent. This emphasises that the present samples are very small and that definitive answers to the problems of specificity and any possible systematic differences in contact numbers and location with afferent or motoneurone identity must await further study.

The nature of Ia-motoneurone synaptic transmission. At the light level the present morphological data gives no indication of the actual synaptic mechanisms. There has long been controversy (see Burke & Rudomin, 1977) over the involvement of chemical or electrotonic mediation at this synapse. Many investigators (Rall et al., 1967; Shapovalov & Kurchavyi, 1974; Edwards et al., 1976a, b; Werman & Carlen, 1976) have been unable to demonstrate a reversal potential for the Ia-motoneurone EPSP. Munson & Sypert (1979b) have however demonstrated a 'true' synaptic delay in the system and conclude a chemical transmission mechanism at the Ia-motoneurone synapse. Krnjevic et al. (1979) also suggest that there is an absence of electrotonic coupling.

In the present study 62% of dendritic contacts were made by 'en passant' boutons. Burke et al. (1979) suggest that if action potentials propagate efficiently to the ends of the axonal branches they will be present in the preceding 'en passant' boutons. This arrangement would be suitable, and necessary, for stable electrotonic EPSP's to be generated. Detailed ultrastructural analysis of identified Ia boutons on motoneurones may provide further clues to this problem.

It should also be noted that 'climbing' 'en passant' contacts are not always lined along the dendrite in the direction of the soma as would be expected to maximise synaptic efficacy. Again however the significance of this sort of pattern is unclear.

Concluding remarks. The Ia-motoneurone monosynaptic reflex arc is recognized at the anatomical and physiological level. HRP labelling through recording microelectrodes now allows us to establish some of the fine organizational details of the system. Each Ia fibre makes, via one of its collaterals, several synaptic connexions with each of its target motoneurones. The synapses from a collateral may be at the same site or distributed to different locations - the overall distribution, when taking all Ia synapses on a cell into account, will probably consist of few somatic contacts, with bouton density high on proximal dendrites but decreasing on distal branches in agreement with electrophysiological analyses. Models can be used to estimate or predict synaptic events - the models can now and in the near future be matched with precise anatomical data coupled with direct electrophysiological measurements. We know that even distal synapses can, and probably will under certain circumstances, influence integrative events occurring at the soma-initial segment zone of the motoneurone. There is no doubt that events at single boutons, dependent on true synaptic area, charge injection and post-synaptic response properties, require detailed examination to allow firmer conclusions to be made about the distribution of synapses and their effectiveness in particular systems. Pre-synaptic events also influence motoneurone activity and the anatomical organization (branching patterns) of the

afferent fibre collaterals reflects one aspect of this.

PART 7: Figure 1

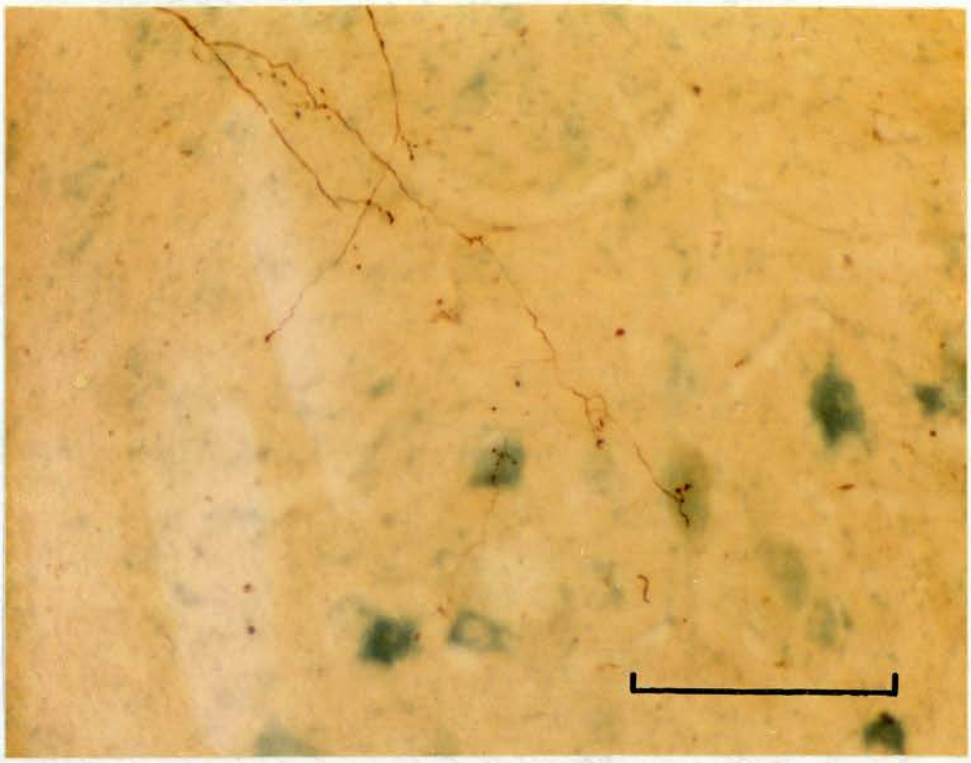
Ventral horn connexions of Ia afferent fibre collaterals.

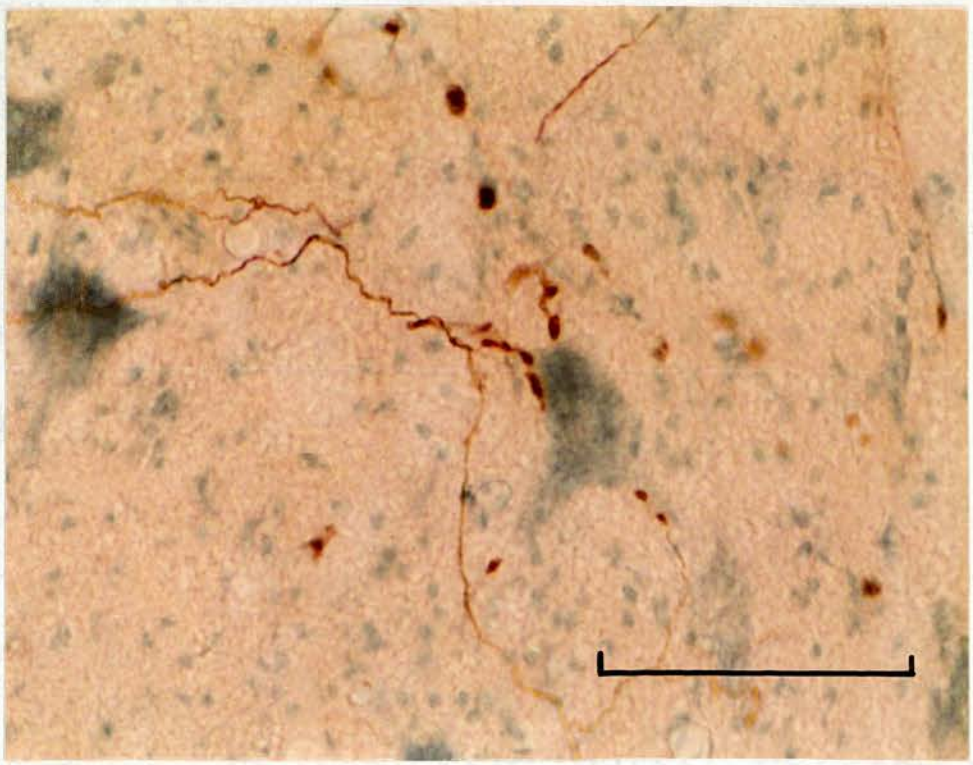
Both micrographs are from single 100 μm thick transverse sections of cord, counterstained with methylene green.

The upper figure shows Ia collateral branches projecting into the lamina IX motor nuclei. At top left are some of the lamina VII terminals. Within the motor nuclei two neurones receive apparent axo-somatic connexions. Some boutons do not contact any of the demonstrable motoneurone profiles.

The lower micrograph shows some possible axo-dendritic and axo-somatic contacts on a motoneurone. These 5 or 6 juxtasomatic terminals arise from different branches of the collateral.

Scale Bars: Upper 200 μm
Lower 100 μm.



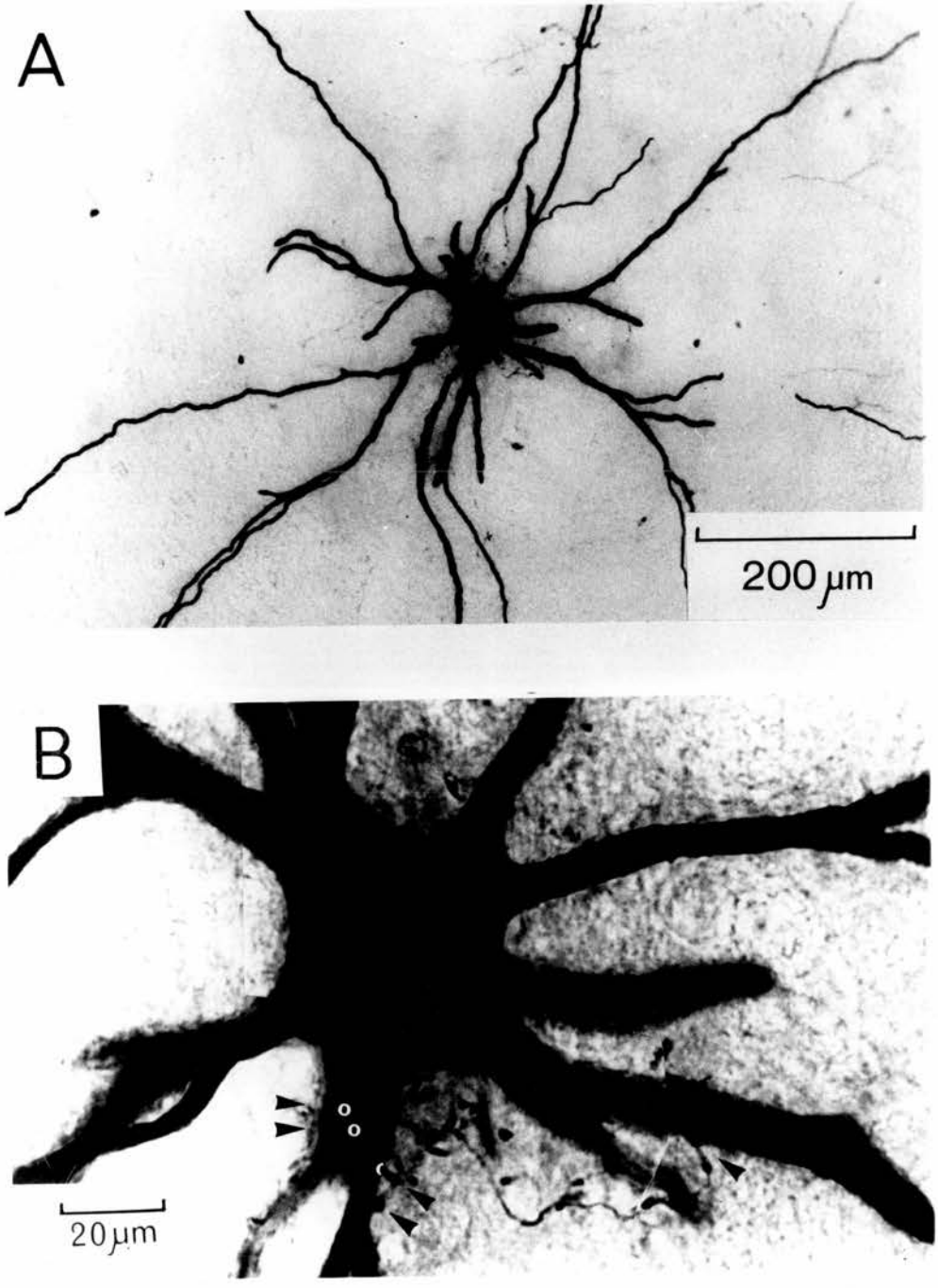


PART 7: Figure 2

Juxtasomatic Ia synapses made by a Mg Ia on a homonymous α - motoneurone.

A. Low power view (single 100 μm thick sagittal section: rostral to left, dorsal to top) of the HRP labelled motoneurone. Note the dendritic branching pattern, including occasional trifurcations and the uniform diameter of most of the dendrites. Some of the very fine Ia terminal arborizations can just be seen amongst the motoneurone profiles.

B. Is a high power photographic reconstruction of the juxtasomatic region. Some Ia fibres and boutons are evident, especially in the area just ventral to the soma. Five synaptic contacts were determined (see also Fig. 12A) on three different dendritic trunks. They are indicated by arrowheads and some, in which the boutons largely overlie the stained dendrites, have their positions emphasised by circles. The contacts on these primary dendrites are within 45 μm of the soma.

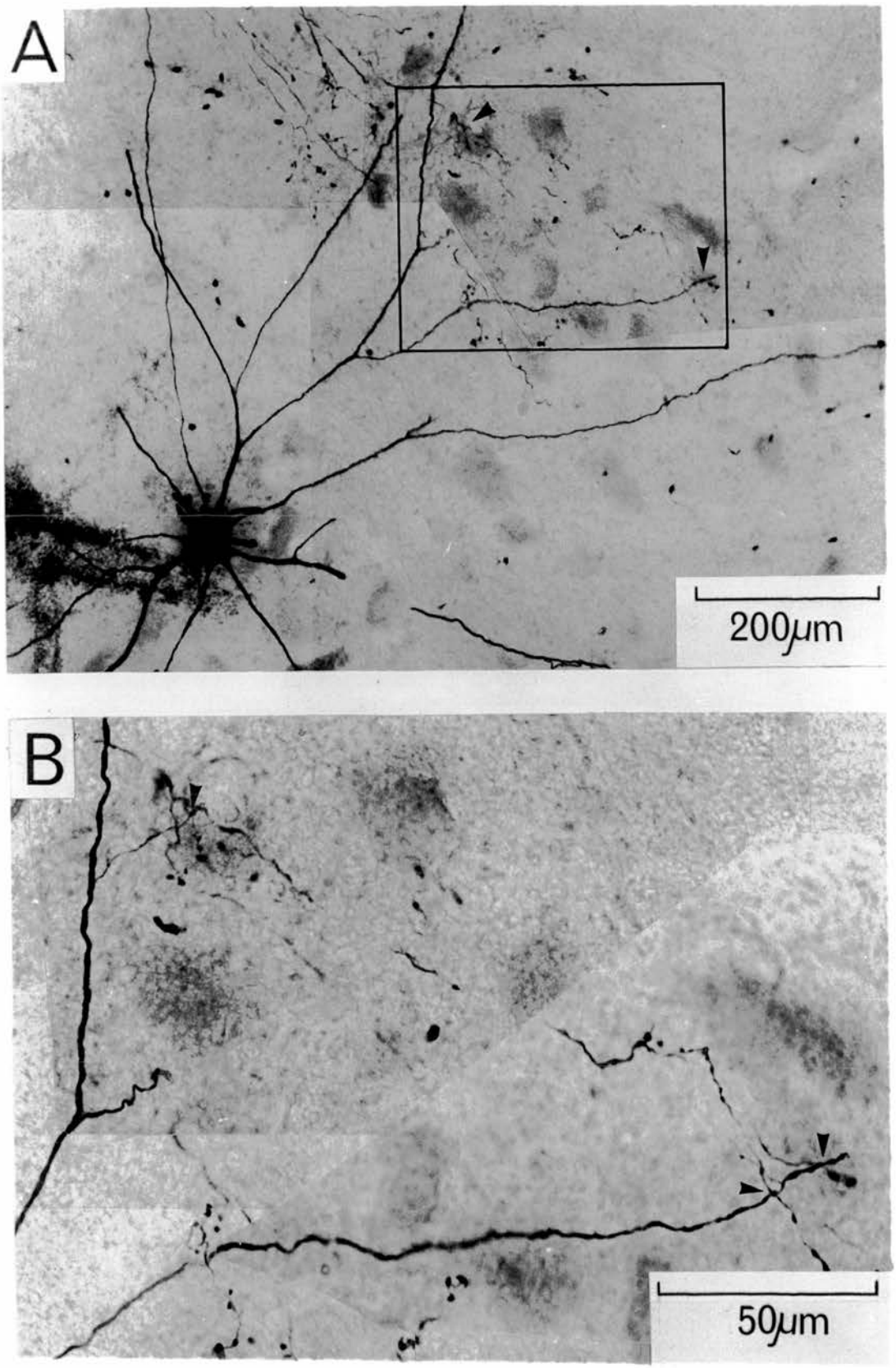

A
200 µm
B
20µm

PART 7: Figure 3

Distal synapses between a Lg-S Ia afferent fibre and a Mg α - motoneurone.

A. A low power micrograph showing part of the motoneurone dendritic tree (single 100 μm thick transverse section). Other presumed motoneurone cell bodies can be seen lightly counterstained with methylene green. At the top, Ia afferent collateral branches course ventro-laterally into the motor nuclei and intersect the dorso-laterally directed stained dendrites. Several boutons are located close to counterstained cell bodies.

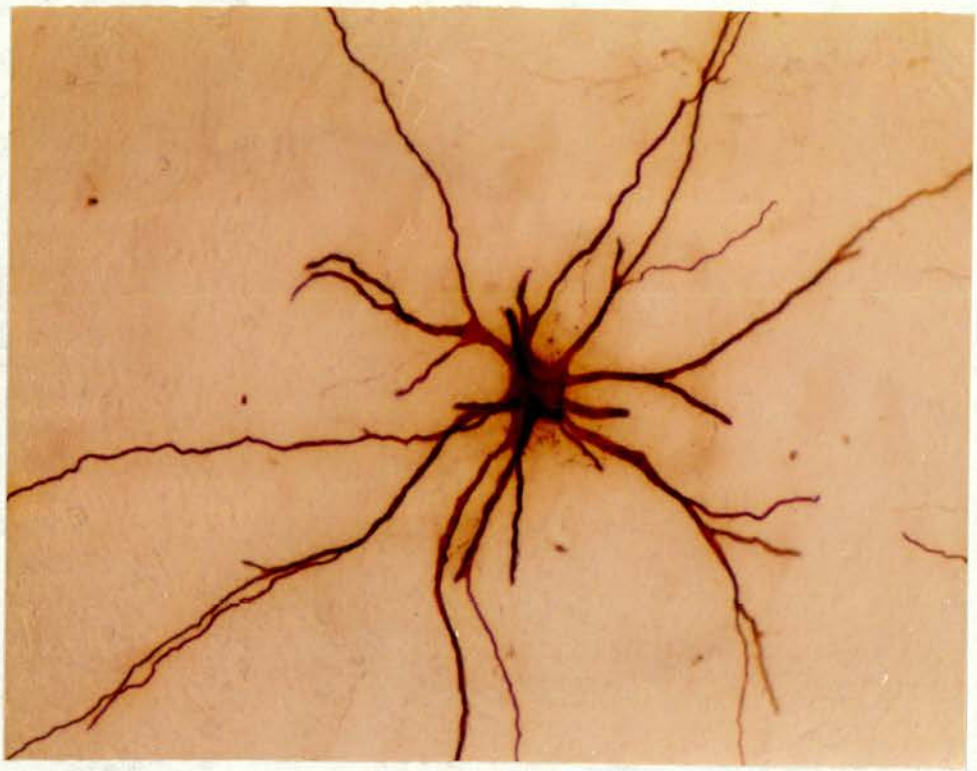
B. The region of A which is outlined by the solid rectangle is illustrated at higher magnification. Three contacts (arrowheads) are made on two branches (3rd and 5th order) of a single trunk dendrite. All three involve crossing over 'en passant' connexions. Although dendritic order is different, the synapses are all at similar distances from the soma (see also Fig. 12H) at around 600 μm.

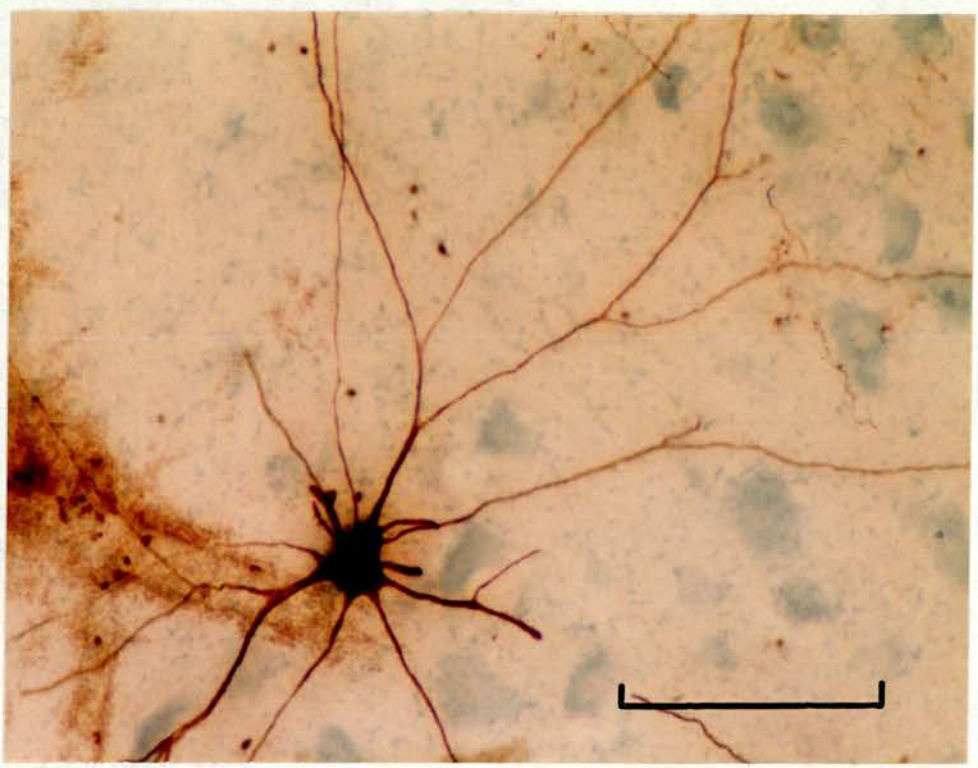

A
200μm
B
50μm

PART 7: Figure 4

The colour micrographs (single 100 μm thick sections) delineate more clearly some aspects of the systems described in the preceding two figures (2 and 3). In particular the homogeneous staining of the motoneurone dendrites is seen and there is more contrast between the stained afferent terminals and the motoneurone profiles.

Scale Bar: 200 μm.

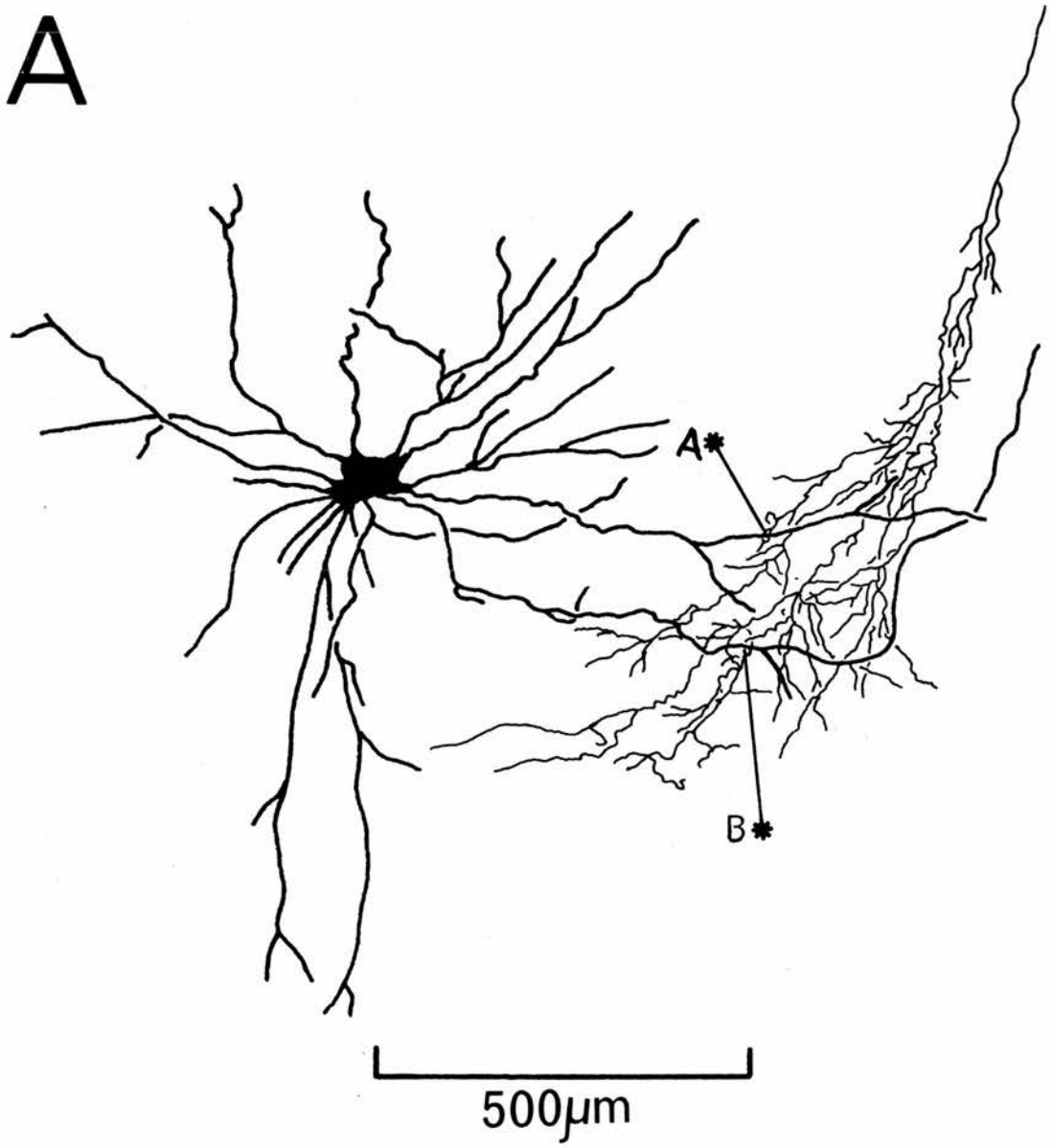


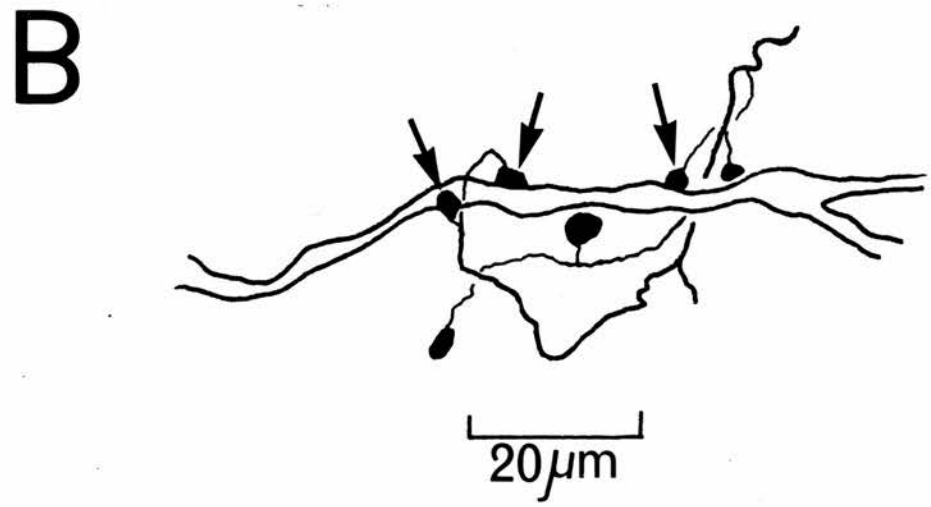


PART 7: Figure 5

A. Sagittal reconstruction of a PT motoneurone contacted by a PT Ia afferent. Four contacts were observed (see Fig. 12F) on 2nd and 3rd order dendrites at a mean synaptic distance of about 500 μm. At point A* there was a single contact involving an 'en passant' bouton. The synaptic location indicated by B* is shown in greater detail below in B.

The three connecting boutons (arrows) were 'terminal' and contacted a 25 μm length of rather varicose dendrite. The dendrite divided a few μm distal to the contacts. Other boutons were in very close proximity to the stained dendrite but on very detailed examination did not appear to make contact. This synaptic region is also shown in Fig. 11F.


A
A
B
500μm

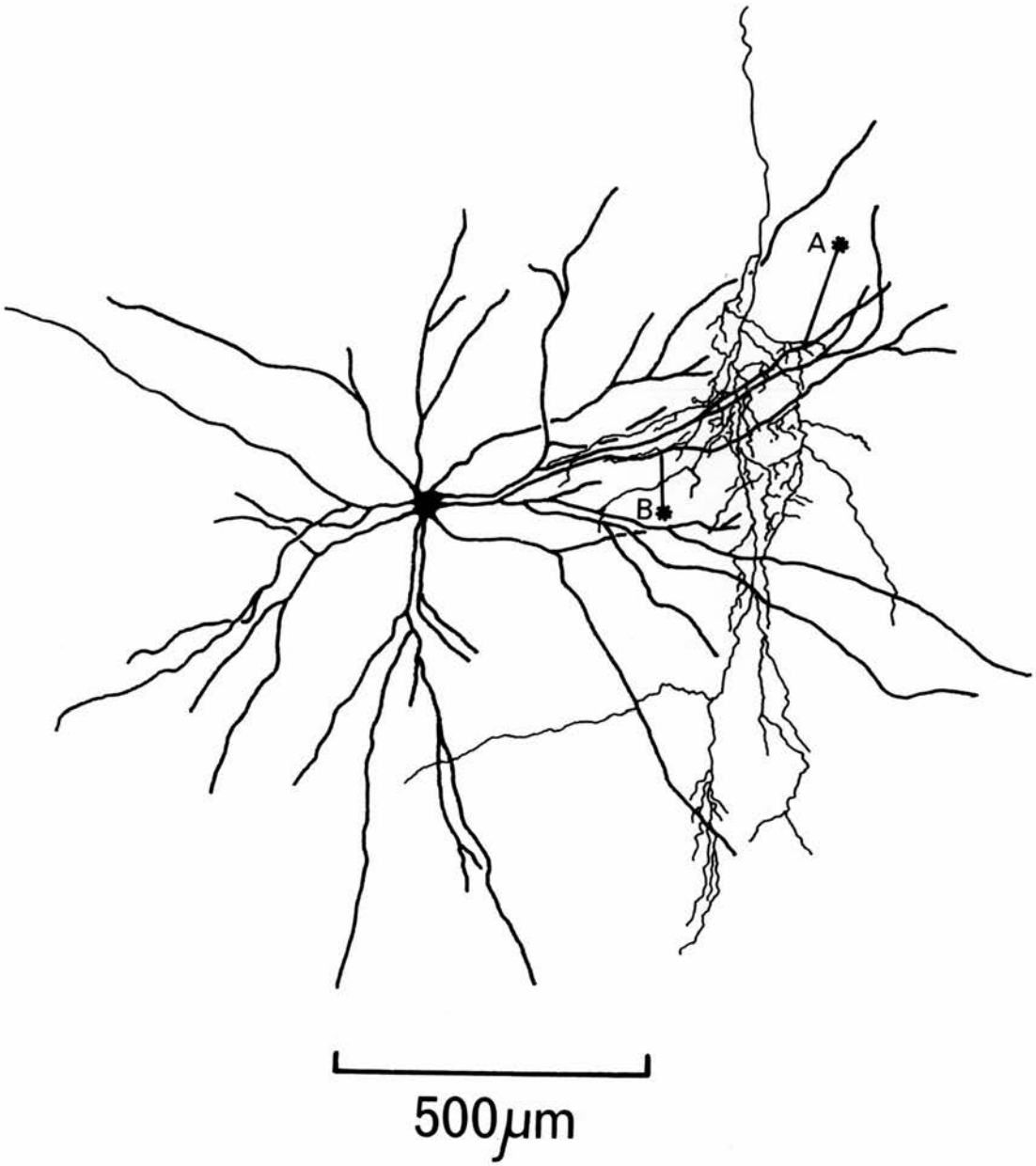

B
20μm

PART 7: Figure 6

The reconstruction shows another arrangement which is composed of distal contacts. The afferent and motoneurone both innervate Lg-S (see Fig. 12E). The four contacts are made at two distinct locations, one about 370, the other about 800 μm from the soma, on a different dendrite. The distal contact region (A*) had one synapse, from an 'en passant' bouton crossing over a fine distal dendrite.

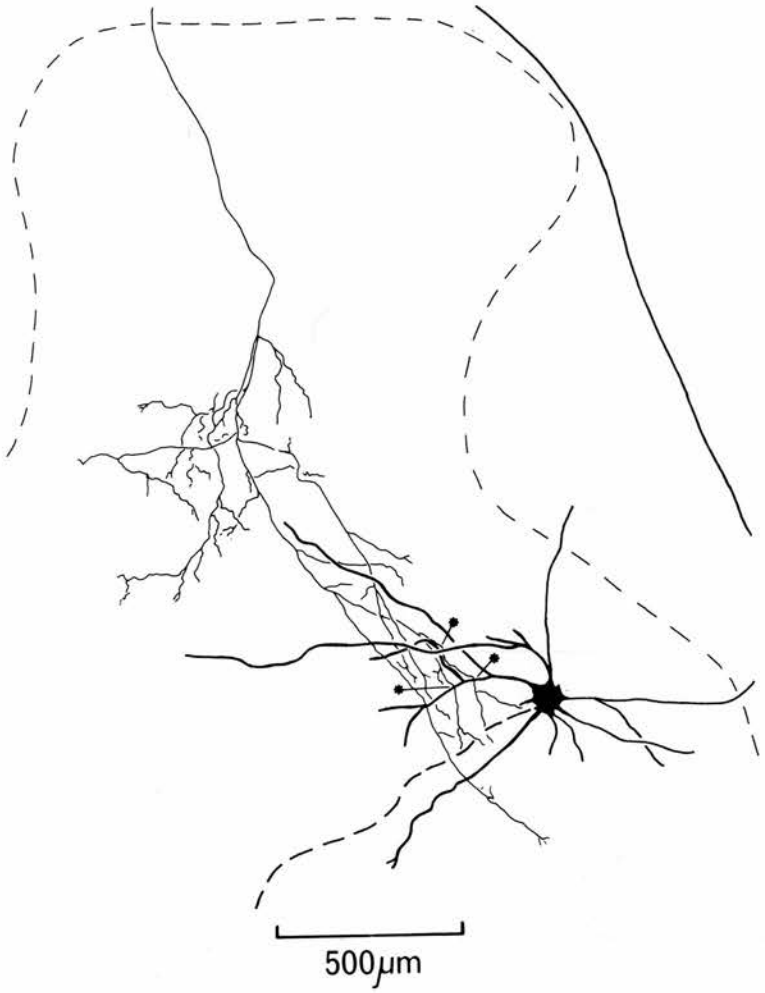
At region B* three 'en passant' boutons make climbing contacts along a 2nd order dendrite.

Region A* is also shown in Fig. 11E; B* is shown in Fig. 11G.


A
B
500μm

PART 7: Figure 7

This figure shows reconstructions, from transverse sections, of a Lg-S Ia which made 5 contacts on second and third order dendrites of a Lg-S motoneurone. The contact region (* mark the locations in the upper drawing) is detailed in the lower figure. The contacts are a variety of 'en passant' and terminal type synapses - 3 are grouped at one location, the other 2 are at separate locations on different dendritic branches. See also Fig. 12G.

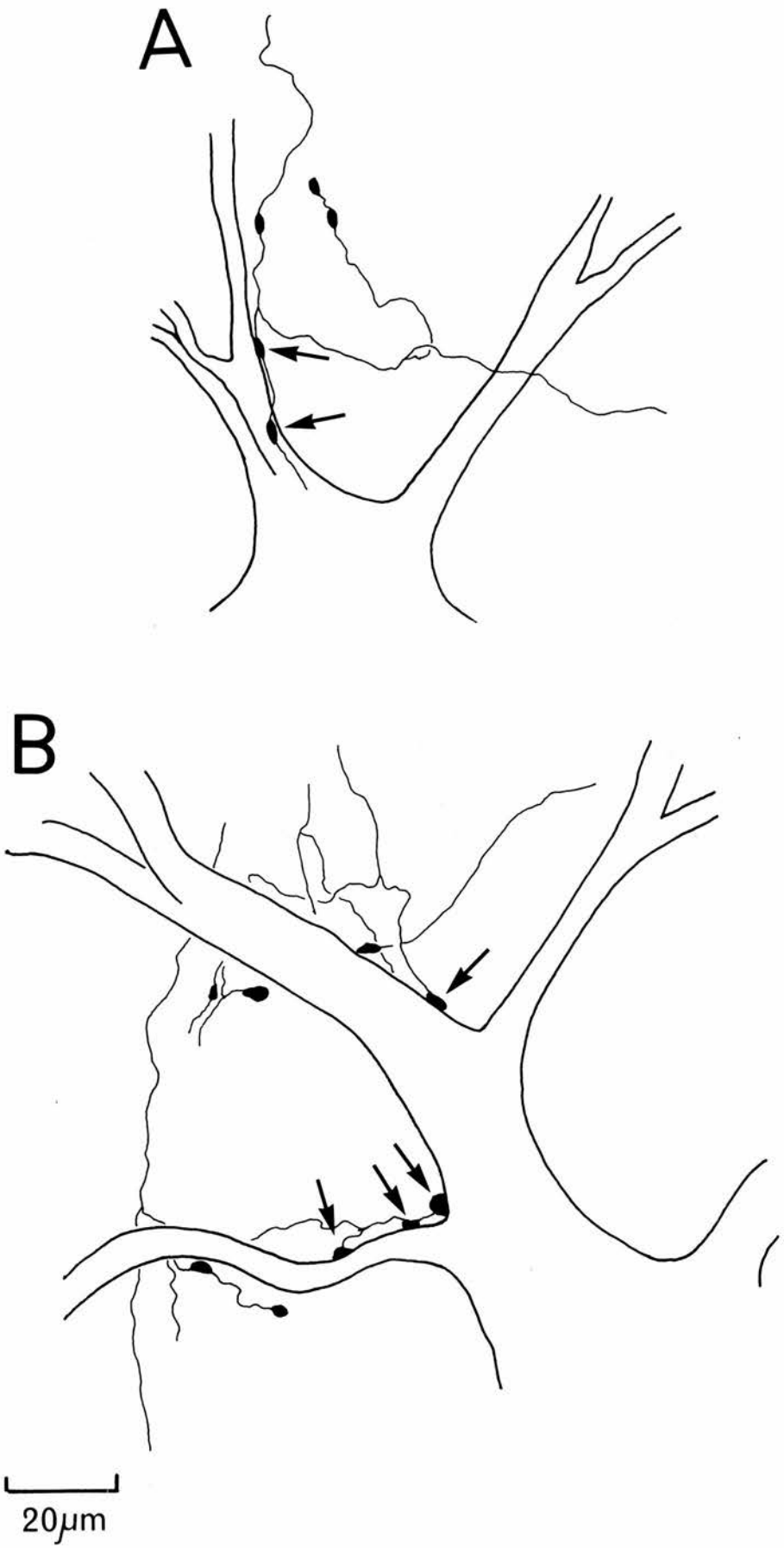

500μm


20μm

PART 7: Figure 8

Juxtasomatic contacts.

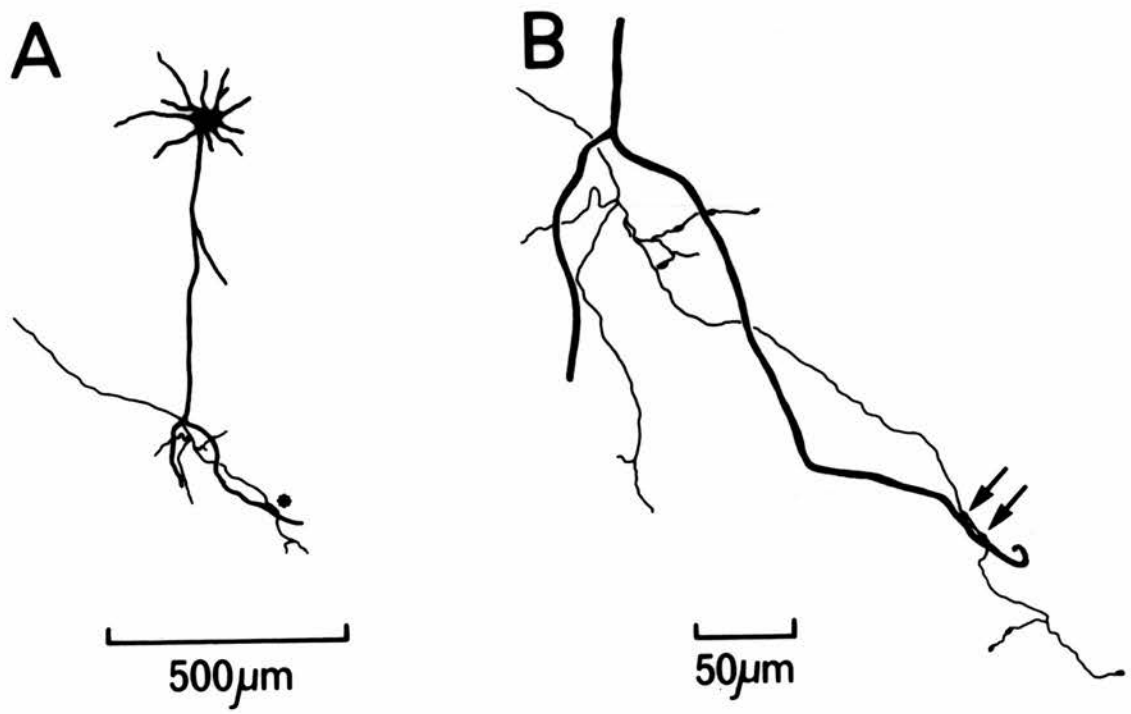
Both arrangements involve Lg-S Ia afferents and Lg-S motoneurones (see also Fig. 11B, D). Reconstruction A posed some interpretative difficulties because although the two 'en passant' contacts were clear, the thin axonal branch was 'lost' as it coursed across the surface of the densely stained motoneurone soma. In B 4 contacts were made, on different branches of a trunk dendrite.


A
B
20μm

PART 7: Figure 9

Distal Contacts.

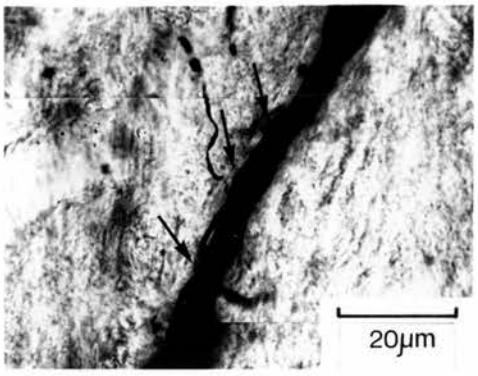
The reconstructions, from transverse sections, show (see also Fig. 12I) the location of two contacts made by a Mg Ia fibre on a distal dendrite of a Lg-S motoneurone.

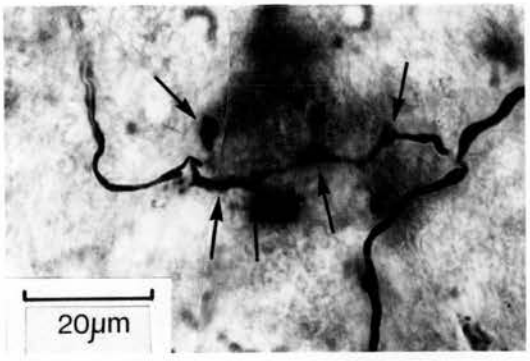

A
500μm
B
50μm

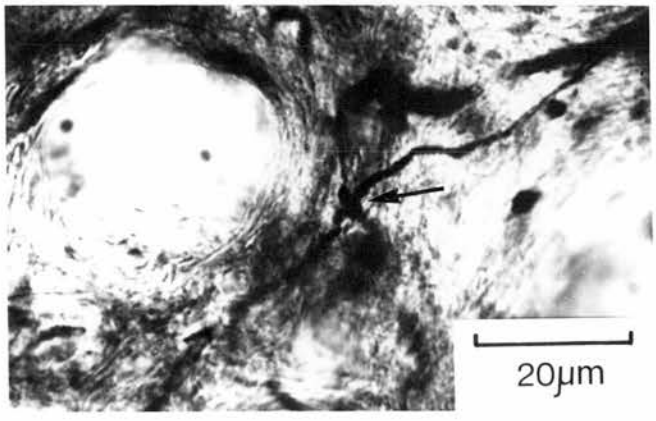
PART 7: Figure 10

Ia synapses on motoneurones.

The micrographs show some of the HRP stained profiles. The upper figure shows a terminal Ia axon meeting a dendrite, dividing, and forming 3 synaptic connexions. The middle figure shows contacts on a counterstained neuronal soma. The lower figure shows the distal contact described in Fig. 6.


20μm


20μm


20μm

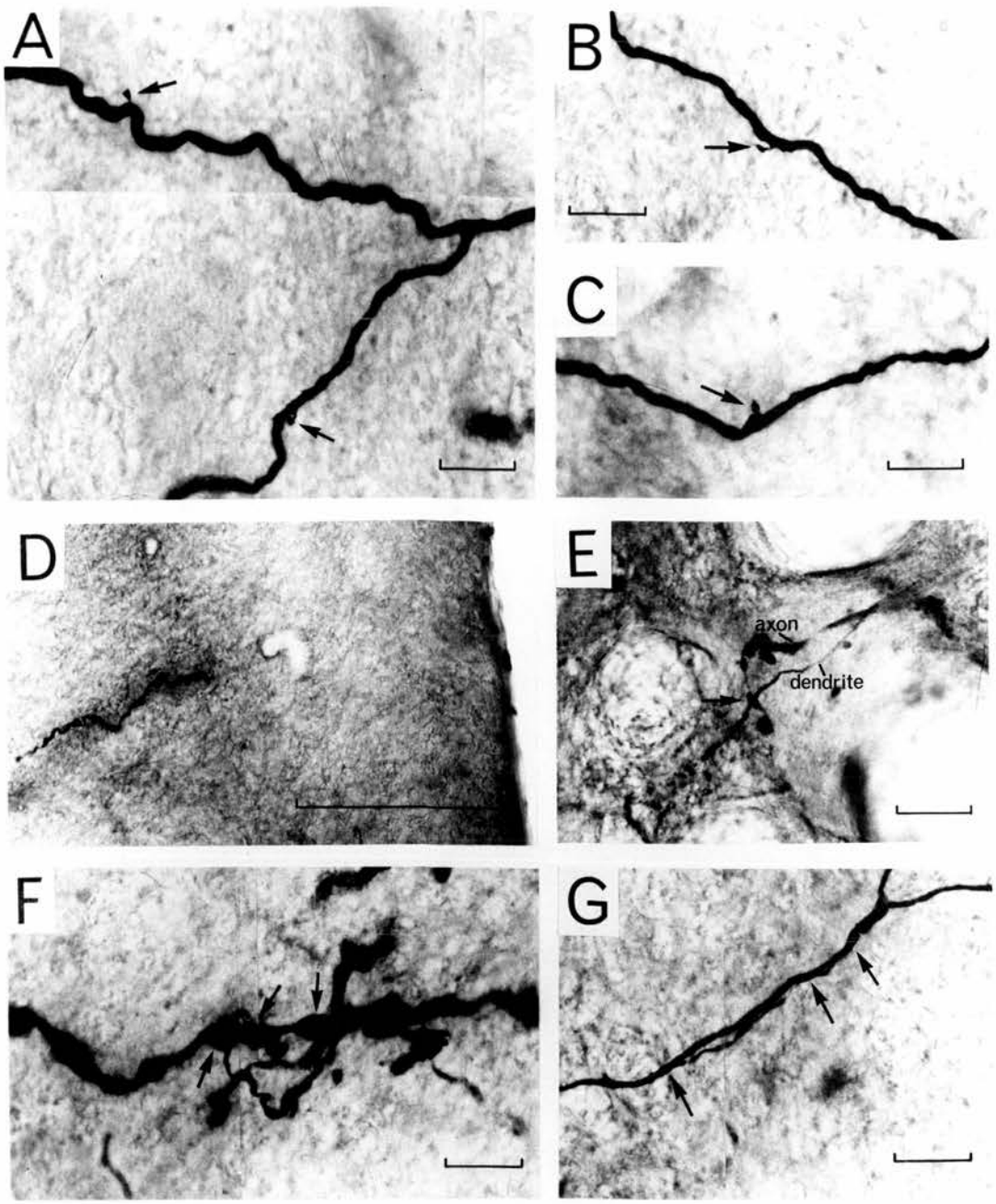
PART 7: Figure 11

A - C. Dendritic spine-like structures on motoneurone dendrites. Note how short the spine stalk is, and how widely spaced these structures are on the dendrites. Scale bars: 20 μm.

D. A fine, beaded, dendrite in the lateral white matter projecting close to the surface of the cord (at right). Scale: 100 μm.

E. The synaptic contact described in Fig. 6 on a fine distal dendrite. The middle of 3 boutons on a short terminal axon makes contact with the dendrite. The bouton is several times larger than the dendritic diameter. Scale bar: 20 μm.

F, G. Further examples of synapses on motoneurone dendrites. F is the region shown in Fig. 5B whilst G is the contact area indicated at B* in Fig. 6. In both these cases the synapses are at mean distances of about 500 μm from the soma and the terminal arrangements mean that the boutons end in fairly restricted electrotonic compartments. Scale bars: 20 μm.


A
B
C
D
E
axon
dendrite
F
G

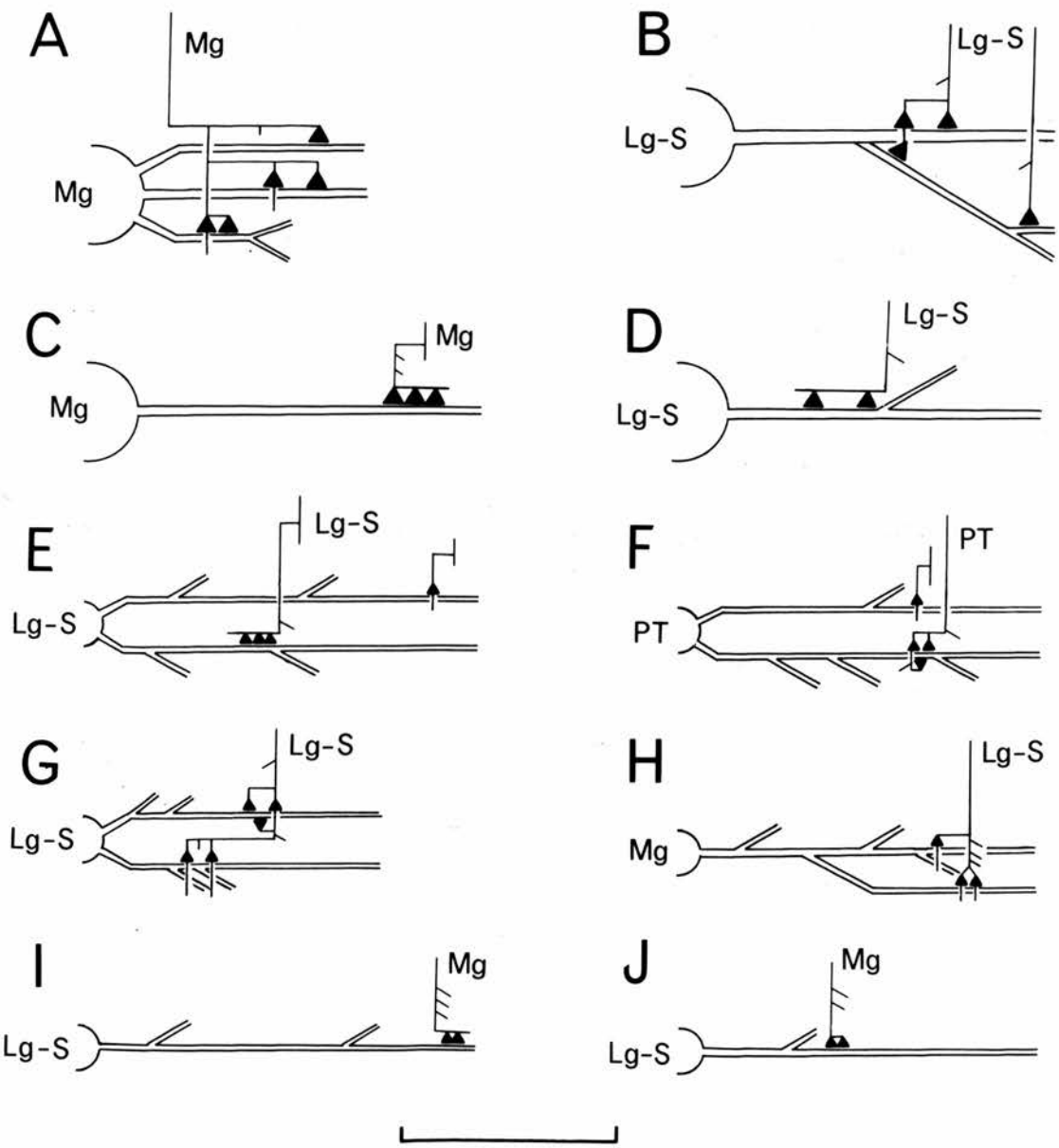
PART 7 : Figure 12

Summary 'wiring' diagrams of ten Ia - motoneurone contact arrangements.

Most of these synaptic arrangements are shown in detail in subsequent reconstructions and micrographs. In this figure numerical and spatial distributions are represented. In each case the identity of the motoneurone and Ia afferent fibre is indicated. Synaptic contacts are shown by filled triangles. The scale bar is 50 μm for the juxtasomatic systems A - D and 500 μm for E - J. It refers only to dendritic distances from the soma or between synapses. The length of pre-synaptic axons is not to scale except for those making climbing 'en passant' connections (e.g. C, D, E, I) where boutons arise from the same terminal axon. Dendritic branch points are shown at appropriate distances. The pre-terminal branching is indicated although in some cases (B, E, F) the common branch point of the boutons involved was too far back and thereby too complex to represent here.

Variable numbers of boutons, terminal axons and dendritic branches are involved in these arrangements. In some boutons are located close together (e.g. C, I, J), others have synapses at comparable distances from the soma although on different dendrites or branches (e.g. A, F, H) whilst some (e.g. B, E, G) have relatively widespread distribution.

The greatest mean synaptic distance was over 800 μm (I). Although complete dendritic trees are not shown here, almost all the contacts were in fact on the proximal geometric half of the dendrites.


A
Mg
Mg
B
Lg-S
Lg-S
C
Mg
Mg
D
Lg-S
Lg-S
E
Lg-S
Lg-S
F
PT
PT
G
Lg-S
Lg-S
H
Lg-S
Mg
I
Mg
Lg-S
J
Mg
Lg-S

PART 8 : GENERAL DISCUSSION

Identification of muscle afferents and spinal neurones. The validity of the anatomical conclusions rests on proper physiological identification of the stained neurones. The criteria for defining the Ia, Ib and spindle group II axons were based on a rather subjective usage of the wide body of precise information obtained during studies of muscle afferent responses to passive stretch and muscle contraction, etc. (Matthews, 1972) and may be contentious. This type of approach was necessitated because identification had to be performed rapidly and with minimum disturbance of the preparation. The primary consideration was to gain stable intra-axonal penetration and to inject as much HRP as possible. Interestingly, development in the last two years of techniques for pulling very fine tipped microelectrodes (e.g. Brown & Flaming, 1977; Ensor, 1979; Molony, 1978), use of thin walled micropipettes and the introduction of high voltage electrometers (D. Smith, unpublished; see Light & Perl, 1979a) have so improved the recording and dye injection procedures that, provided the preparation is maintained rigid, more time could be spent in initially characterizing the impaled unit. Unfortunately these methods could not be used in this work. Nevertheless, some firm identity testing was performed. Firstly, peripheral conduction velocities were accurately measured. This presented a clear cut

division of axons into Group I (conducting above $72msec^{-1}$) and Group II (conducting below $72msec^{-1}$). With the electrodes used, no axons conducting below about 35 - $40msec^{-1}$ were satisfactorily penetrated. In fact, axons were only included in the Group I if the conduction velocity was above $75msec^{-1}$ and in Group II if less than $65msec^{-1}$. Clearly, using such a 'safety factor' precludes investigation of the possibly interesting borderline axons - these require study in conjunction with precise analysis of response properties. The subdivision of Group I into Ia and Ib fibres was a more difficult problem. Unlike thigh muscle afferents (Bradley & Eccles, 1953) the Group I fibres from triceps surae cannot be differentiated on the basis of threshold to electrical stimulation or conduction velocity. But the hind limb was stretched to a degree where spindle primary endings are discharging. Under these conditions all Ia fibres penetrated had ongoing regular discharge and could be further excited by light manual extension of the joint. In some cases, the muscle was stimulated directly and during contraction a pause in the Ia discharge was observed. Ia axons also responded to light tapping of the muscle or tendon. Ib axons had no ongoing activity when isolated and required noticeable stretch of muscle to excite them. Occasionally they could be made to fire by stimulating the muscle directly. In the final analyses, the anatomy of the injected fibre always confirmed the tentative electrophysiological

classification.

The Group II fibres innervated muscle spindle secondary endings. Their response to light stretch of the muscle was characteristic and current evidence indicates few if any Group II axons innervating non-spindle receptors in triceps surae muscles (Boyd & Davey, 1968), although this is not so in other muscles (e.g. Barker, 1967).

α - motoneurone recordings were confirmed by antidromic activation from the appropriate muscle nerve and by measurements of axon conduction velocity and threshold to stimulation. No systematic tests were carried out to determine motor unit type. Flaxedil immobilization precluded motoneurone evoked twitch contractions. It may now be possible, due to intensive studies on the intrinsic properties of motoneurones to base a tentative classification on features such as input resistance and duration of the afterhyperpolarization (see Burke et al., 1973; Burke & Rudomin, 1977). Continuation of the work described in Part 7 of this thesis is being performed by R.E. Burke who is identifying the target motoneurones in respect of twitch type characteristics (Burke et al., 1979, 1980). The work will require a considerable sample size to determine whether Ia synaptic distribution is related to motoneurone functional characteristics.

But this approach is worthwhile in a wider sense. Many studies of central neurones are concerned simply

with describing the response properties of the cells to some stimulus parameters without attempting detailed investigation of the output of the neurone, in particular the destination(s) of its axon. Even knowledge of the initial path of the axon (from anatomical or physiological work (see Fyffe, 1981)) does not convey sufficient information as to its termination. The electrophysiological developments mentioned briefly earlier will hopefully encourage experimental approaches in which identification (physiological and anatomical) will be more adequately performed. Perhaps more general 'models' will derive from detailed study of spinal neurones other than motoneurones.

HRP Staining. In the last five years, HRP has proved itself not only to be a very useful retrograde anatomical tracer but, when injected into single neurones, to provide exciting information on neuronal structure (see Brown, 1981). Intracellular deposition of HRP provides a Golgi-like histological picture but with many advantages. First and most important is the fact that the physiological characteristics of the stained neurone are defined. The experimenter determines the selectivity of the material by his own approach, identification procedures and the number of injections made. Secondly, the technique is equally effective in new born and adult preparations. This feature will have useful applications in developmental studies. Thirdly, serial sections can be easily examined. Thus extensive

dendritic trees can be fully analysed, axonal trajectories followed and detailed three-dimensional reconstructions of neuronal morphology produced. Finally, the reaction product in both the DAB and Hanker Yates HRP demonstration procedures is electron dense. Thus physiologically identified elements can be analysed at the ultrastructural level (Jankowska et al., 1976). This has had useful implications. Several groups have now verified that the axonal swellings considered under light microscopy as 'boutons' do indeed contain synaptic vesicles and make specialized contacts upon other (as yet undefined) neuronal elements (see e.g. Brown, Fyffe & Maxwell, 1981a). Rastaad (1978) has pursued this approach assiduously and has analysed serial E.M. sections through a single HRP labelled spinocervical tract neurone (see also Brown et al., 1981a) and obtained detailed information on its synaptic connexions at the segmental level. Furthermore, very recent work by Light & Perl (1979b) and Gobel et al. (1980) on identified small neurones of the superficial layers of the dorsal horn of the cat has amply demonstrated a spectrum of possible inter-neuronal connexions, viz. axo-somatic; axo-dendritic; axo-axonic and dendro-dendritic and dendro-axonic. The effect of these studies on our understanding of spinal cord organization is likely to be profound. HRP - E.M. studies may also be combined in the near future with transmitter histochemical techniques to give insights into possible

synaptic mechanisms.

Comparisons with other intracellular dye marking methods were discussed in other Parts of this thesis. Of particular interest was the observation that HRP injections allowed visualization of more dendritic trunks and greater dendritic lengths than the commonly used silver stains. That sort of information casts doubt on the suitability of comparing new born and adult Golgi material in developmental studies. Indeed the indications, as far as motoneurones are concerned, are that silver staining does not necessarily label every dendrite of a neurone. Intra-axonal HRP staining at the same time clarifies and complicates our analysis of primary afferent projections. It clarifies because single, identified axons are studied and their finest branches and terminations detected. This allows us to study all the projections made by its collaterals in a particular region. It complicates because each axon has so many terminations and projects perhaps to three or more distinct zones of cord. It is as yet impossible to define all the target neurones adequately and so we must look on the information as only the first step in determining the microcircuitry of the spinal cord. Further information requires approaches such as are described in Part 7.

The HRP technique has its limitations. The use of glass microelectrodes restricts the application to systems in which the neurones or axons are large enough to

be penetrated and stabilized without damage. Even in the spinal cord these difficulties are being overcome. Light & Perl (1979a, b) have successfully labelled single Aδ axons and single neurones (of sizes down to 10 x 5 μm) of the substantia gelatinosa.

More critical in some respects is the limitation imposed by the spread of HRP within the neurone. It seems likely that intracellular injections label adequately the complete dendritic tree. But good staining of the axon and its collateral branches is only achieved for up to 10mm from the soma, with maximal extent of axon traced for about 20mm. When HRP is injected intra-axonally, it migrates bidirectionally but again only for a maximum end to end extent of about 15 - 20mm. The difficulties of intra-axonal injections became apparent early in this work. Brown et al. (1977b) had stained hair follicle afferent fibre collaterals in the dorsal horn with total injections of about 150nA.min. With similar electrodes such an injection into a Ia fibre - even though the axon was larger and penetration more stable - resulted only in staining of the main axon and the initial parts of the collaterals, perhaps to the level of branching in the intermediate region. Clearly to stain the long Ia collaterals (perhaps 2 - 3mm from origin in the dorsal columns to lamina IX terminations) required larger injections. Even so, total length of stained axon remained similar. So in effect, to study axonal

projections over more than one or two spinal segments, e.g. the Ia projections to Clarke's column, a series of experiments would be necessary, with injections of fibres being made at different levels.

The question of pressure versus iontophoretic applications of HRP then arises for axonal injections. Ishizuka et al. (1979) preferred to use pressure. Their only published micrograph of an axonal injection site exhibited quite extensive spillage of HRP. In our experiments, particularly the later ones, it was usually quite difficult even to find the injection site despite having placed a contralateral marker electrode. Pressure injections have not yet been consistently tested using fine microelectrodes or for injections of small neurones.

Finally emphasis must be placed on attaining a good perfusion and fixation of the nervous tissue. It is likely that we are approaching the limits of the amount of HRP that can be injected without causing damage and that this amount determines the extent of staining. Sensitive processing coupled with 'clean' sections thus become critical factors for producing quality material under the present circumstances.

Some basic principles of muscle afferent collateral organization in the spinal cord. Figures 1 - 3 diagrammatically summarize the branching patterns and zones of termination of Ia, Ib and Group II afferent

fibres. As a first stage in this discussion it is worthwhile to consider the zonal termination sites of afferent axons in relation to their possible target neurones. Spinal neurones are organized in a laminar fashion in different areas of the cord (Rexed, 1952). In many cases, particular functional groups of cells are arranged in topographically based longitudinally and transversely organized nuclear columns (Romanes, 1951). Recent intracellular labelling approaches, which provide the most accurate localization of cell body position indicate that Romanes' outstanding work on the nuclear organization of spinal motoneurones may equally apply to specific groups of interneurones which are found in discrete regions of the ventral horn (e.g. Ia inhibitory interneurones, Renshaw cells and Group Ia monosynaptically activated cells in the intermediate region) and rarely overlap with the positions of other types of cells (see Jankowska & Lindström, 1974). The dendritic tree patterns of spinal neurones however do not always adhere to laminar or nuclear boundary restraints (see also Fyffe, 1981). In this work for example the enormous extent of a single α - motoneurone's dendrites is clear. The soma-dendritic membrane in its entirety acts as a receptive integrating centre for the synaptic inputs to the cell.

One question posed by this work is, "Knowing the terminal arborization area of particular afferents, what (groups of) neurones are likely to receive (important)

monosynaptic connexions from the afferent?".

The important aspects obviously are a consideration of the position of the target cell body and its dendrites and whether they overlap with the afferent terminal region. On this basis, motoneurones for instance could receive inputs directly from many sources including Ib and Group II afferent fibres, corticospinal fibres, etc. But when we look at the connexions made on motoneurones by Ia collaterals we find that no contacts are made by the intermediate region or lamina VII arborization boutons. All the contacts, even those made 800 μm from the soma, were made by boutons which were part of the lamina IX or motor nuclei arborizations of the Ia fibres, and were made within the confines of the motor nuclei. So even although motoneurone dendrites extend into lamina VI, the intermediate region and the surrounding white matter, the Ia afferent fibre specifically makes its contacts within lamina IX, the region of the cell bodies and densest network of intertwining motoneurone dendrites. Likewise the monosynaptic connexions from Group II axons will probably be made by the collateral branches which penetrate the motor nuclei.

To extend the argument to other muscle afferent terminal regions we can imply that lamina VII Ia terminations contact specifically the Ia inhibitory interneurones; Ia's and Ib's excite interneurones in the base of the dorsal horn and Group II collaterals activate

dorsal horn cells and cells in the motor nuclei. But while it seems reasonable to state that if an axonal system projects to a defined (nuclear) region of cord then this represents a monosynaptic projection primarily to the neurones intrinsic to that region or nucleus, we must not forget the significance of dendritic contacts, not just in terms of their functional role in individual cells, but in terms of cord organization. Indeed recent work in this laboratory (reviewed by Brown, 1981) has shown that spinocervical tract cells, which are largely arranged in a sheet across lamina IV, receive very powerful monosynaptic excitation from hair follicle afferent fibres which end mainly in lamina III. The anatomical basis for the connexions (Brown & Noble, 1979) is that contacts are frequently made on the dorsally oriented dendrites of the cells. So, we can extend the argument to include as significant targets of a terminal zone dendrites specifically directed into the region as well as the intrinsic neurones. Further studies are required, at light and EM levels to resolve whether synaptic contacts are made on cells located within the area of terminal branching and on cells which are located further away and extend only their dendrites towards the terminal zone. Thus pathways which converge on to a group of neurones need not terminate in the same area - the actual arrangement will be relevant to the weighting of synaptic effects from these pathways.

Since the primary afferent terminals themselves

occupy specific and restricted volumes of cord the argument equally applies to these terminals as the targets for other systems. The mechanisms for generation of primary afferent depolarization (PAD) (see Burke & Rudomin, 1977; Rudomin, 1980) are still under discussion. Particularly if PAD was due to a non-specific mechanism, e.g. increase in extracellular K+ due to activation of nearby neuronal elements, the localized grouping of terminal branches and boutons would maximize the effects. If PAD was due to release of a transmitter substance by interneurones making axo-axonic contacts with the afferent fibre terminals the arrangement would again be suitable. Many interneurones terminate in the regions of muscle afferent terminal arborizations (e.g. Czarkowska et al., 1976).

The anatomical specificity of muscle afferents is striking. Each type of axon generates collaterals which are characteristic of and dependent upon the physiological identity of the fibre. The specificity extends to gross morphology, axon trajectory, terminal branching patterns and bouton distribution.

Some principles can be extracted:

I) The significance of the bouton distributions to specific zones has been discussed previously.

II) Each muscle afferent axon, on entering the cord through the dorsal roots, divides and sends major branches for considerable distances up and down the cord.

Electrophysiological evidence indicates that monosynaptic connexions are made over several segments. The present anatomic descriptions support these findings because all afferents had central collaterals arising from their ascending and descending branches. Issuing of collaterals did not a priori lead to a reduction in diameter of the parent axon.

III) Collaterals are generated every 700 - 1000 μm or so along each axon. Collateral morphology can and does alter somewhat, depending on segmental level i.e. upon the location of target neurones.

IV) Each collateral arborizes and terminates in well-defined areas. Collaterals of an axon are lined up with each other in the longitudinal axis of the spinal cord. They thus form sagittal columns of terminals extending for long distances along the cord. These individual columns may be almost continuous or have gaps between the terminal fields of individual collaterals. It seems likely that any gaps would be 'filled in' by collaterals from other like axons. Thus, the nervous signal of a single afferent axon is transmitted to a very large volume of cord.

V) Pre-terminal branching is complex. We do not know if an action potential invades all the terminal branches but it is clear that there are many possibilities for fractionation of effects on post-synaptic targets.

VI) Some collaterals, e.g. from the Ia and Group II

fibres, have terminals in separate and distinct regions of the cord. This indicates specific monosynaptic activation of different groups of spinal neurones.

VII) Motoneurone dendrites have massive spread into surrounding areas - although Ia collaterals pass through these areas they only connect with motoneurones within the motor nuclei.

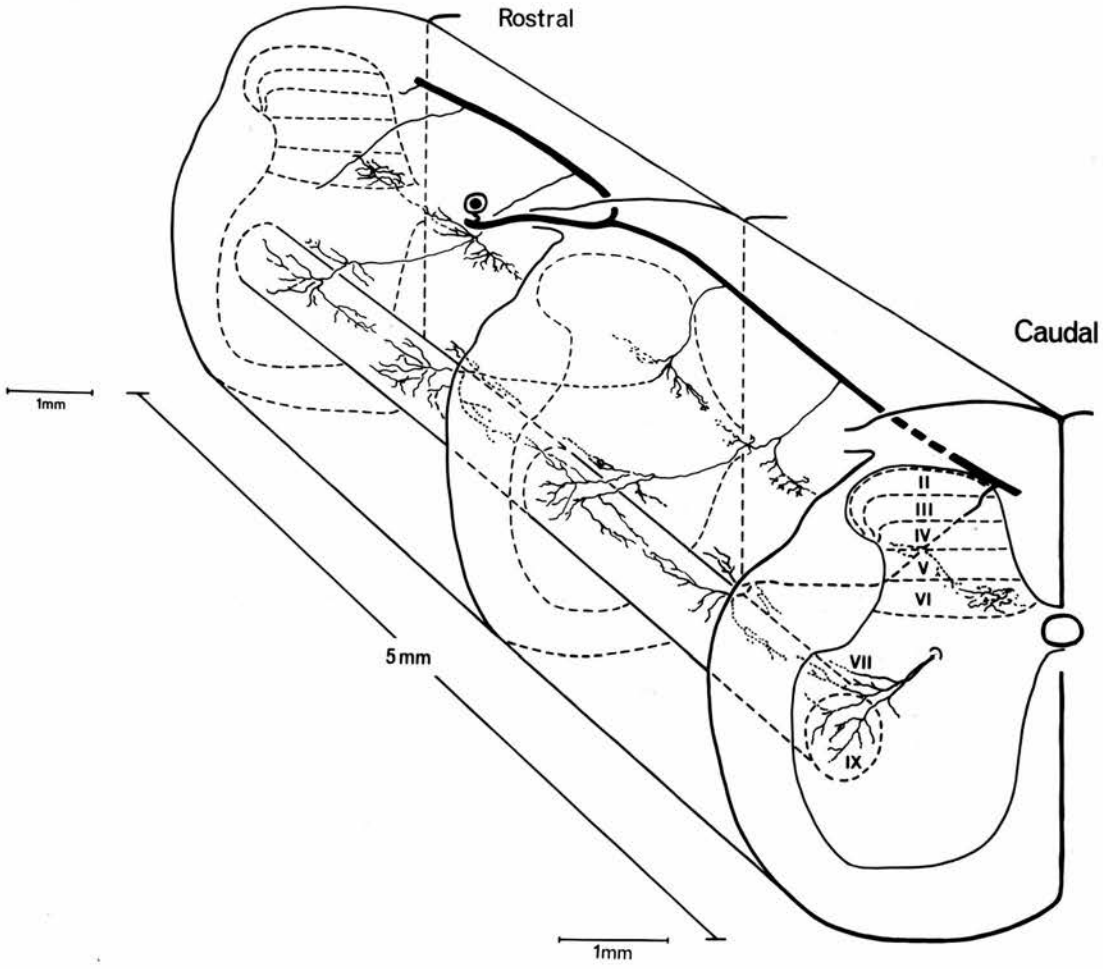
VIII) Ia contacts are distributed over the soma, proximal dendrites and on more distal dendrites up to 800 μm from the cell body. Even these distal synapses are electrically quite close to the soma.

IX) Only one collateral from an axon establishes direct contacts on any one cell. The contacts may be on different regions of the cell and be from different terminal branches of the collateral.

PART 8: Figure 1

The arrangement of Ia afferent fibre collaterals.

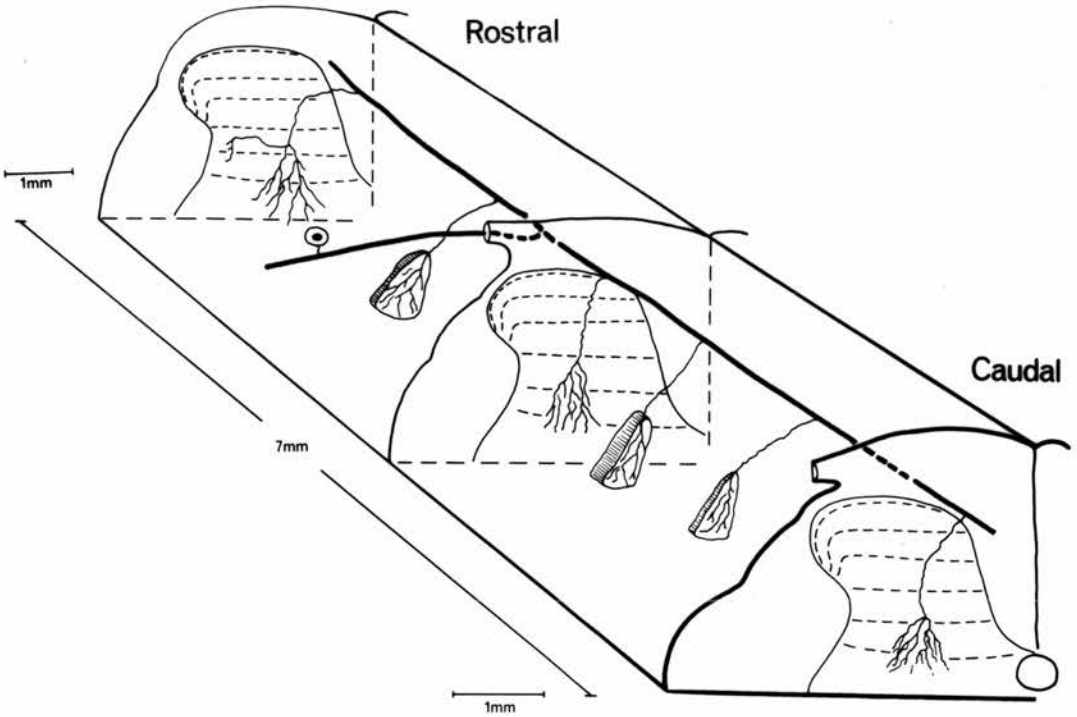
The figure is a schematic representation of a Ia afferent in the lumbosacral spinal cord. The salient points indicated are: bifurcation of the main axon; collateral spacing (average 1mm); laminar distribution of terminals; the relationship of adjacent arborizations viz. the almost continuous distribution of boutons along the length of the motor nucleus.

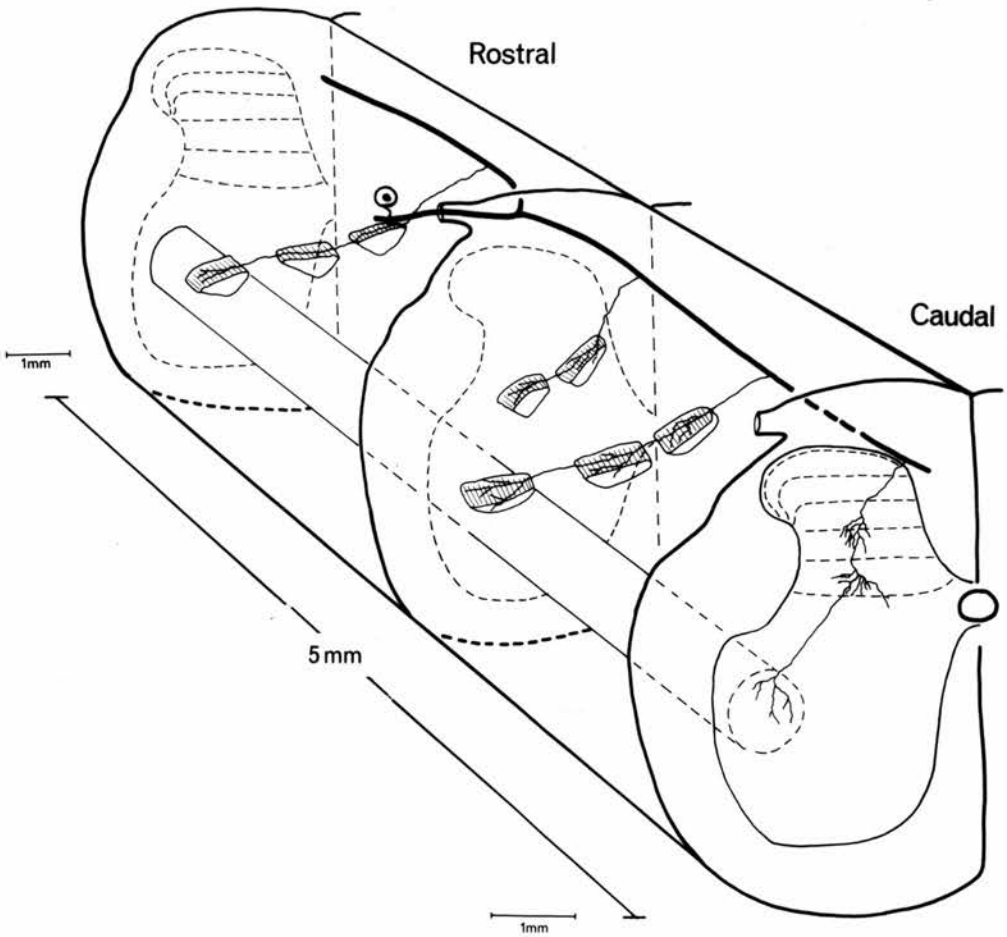

Rostral
Caudal
1mm
5mm
1mm
II
III
IV
V
VI
VII
IX

PART 8: Figure 2

Ib and group II axon projection patterns.

The upper schematic diagram represents a Ib afferent whilst the lower diagram shows the arrangements of group II axons. Both types of axon have little overlap between adjacent collaterals.

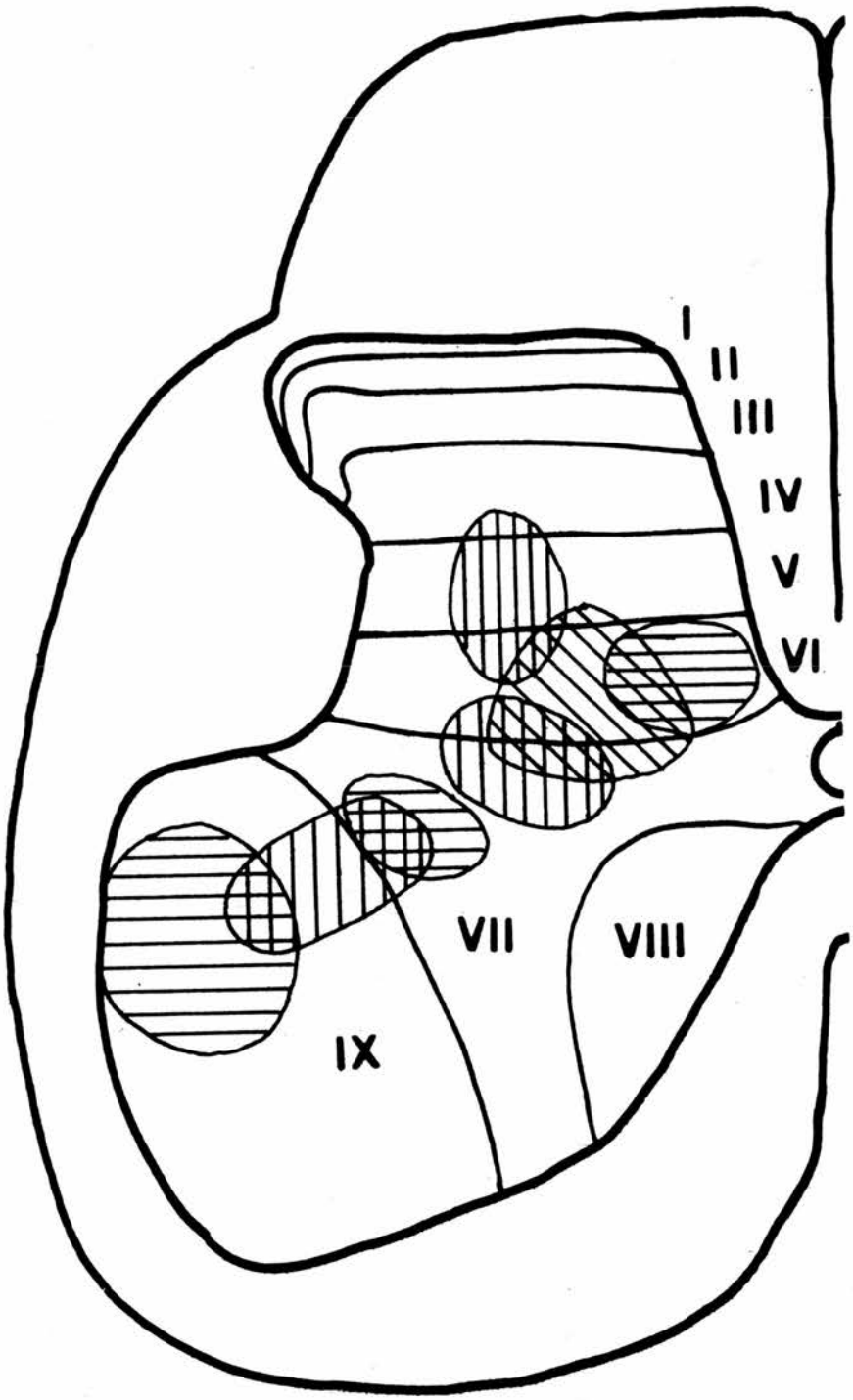

Rostral
1mm
Caudal
7mm
1mm


Rostral
Caudal
1mm
5mm
1mm

PART 8: Figure 3

Laminar distribution of muscle afferent terminals in the lumbosacral spinal cord.

Ib fibres terminate predominantly in one area - in the intermediate region. The spindle afferents each have three discrete terminal zones although these are not quite coincident. Group II collaterals arborize dorsal and lateral to the most dorsal group of Ia terminals. Both types also project to lamina VII and IX, where the Ia collaterals have more extensive ramifications.



Ia terminals

Ib terminals

II terminals

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APPENDIX

Some of the work reported in this thesis has been published in The Journal of Physiology:

J. Physiol. 274, 111-127 (1978)

J. Physiol. 277, 44-45P (1978)

J. Physiol. 284, 43P (1978)

J. Physiol. 296, 39-40P (1979)

J. Physiol. 296, 215-228 (1979)

and was communicated to meetings of the Physiological Society at University College London, March 1977; Bristol, January 1978; Cambridge, July 1978 and Oxford, July 1979.

J. Physiol. (1978), **274**, *pp.* 111-127
With 2 plates and 8 text-figures
Printed in Great Britain

THE MORPHOLOGY OF GROUP Ia AFFERENT FIBRE COLLATERALS IN THE SPINAL CORD OF THE CAT

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SUMMARY

1. The enzyme horseradish peroxidase (HRP) was injected into single Ia muscle afferent fibres in anaesthetized cats. Subsequent histochemistry allowed the morphology of the axons and their collaterals in the lumbosacral spinal cord to be determined.

2. Fifteen Ia axons were stained, four from medial gastrocnemius, four from lateral gastrocnemius-soleus and seven from muscles innervated by the posterior tibial nerve. All thirteen axons that could be traced into the dorsal roots bifurcated upon entering the cord. Between 4 and 11 mm of axons were stained and they gave off eighty seven collaterals over distances between 3 and 9 mm. Collaterals were given off at intervals of 100–2600 μm at an average spacing of about 1000 μm.

3. All Ia collaterals had a characteristic morphology. After leaving the parent axon they ran ventrally to lamina VI and then ventrolaterally to the motor nuclei. The collaterals coursed cranially from their point of origin to the motor nuclei so that their lamina VI terminations were about 100–300 μm caudal to their terminations in motor nuclei. Terminal arborizations were limited to three sites; lamina VI (the intermediate region), lamina VII (the Ia inhibitory interneurone region) and lamina IX (the motor nuclei). The three sets of terminals had characteristic arborizations and bouton arrangements.

4. The results are discussed in relation to previous anatomical studies. In particular the present results suggest that a single Ia collateral makes contact with many more motoneurones than has previously been suggested. in fact with fifty to sixty rather than with about ten.

INTRODUCTION

One of the most intensively studied systems in neurophysiology is the monosynaptic reflex pathway from the primary endings in muscle spindles, via the Ia afferent fibres, to the motoneurones of the same muscle and its synergists. And yet there are still some major deficiencies in our knowledge of the morphology of the system, even at the level of light microscopy. Iles (1976) has recently drawn attention to some of these deficiencies and has listed them as (1) there is inadequate information on the gross morphology, branching pattern and extent of myelination of collaterals reaching the ventral horn in the adult cat, (2) we do not know how many synaptic contacts are made by a single Ia fibre on a motoneurone, and (3) there is no detailed knowledge of how the monosynaptic terminals are distributed to the soma-dendritic

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membrane of motoneurones. Using the electrophoretic application of cobaltous chloride into the cut ends of dorsal roots, Iles (1976) was able to provide evidence towards making good some of these deficiencies.

The recent introduction of intra-axonal staining of electrophysiologically identified axons with horseradish peroxidase (HRP) now allows these sorts of problems to be attacked directly (Brown, Rose & Snow, 1977). The particular advantages of the HRP method (Snow, Rose & Brown, 1976; Jankowska, Rastad & Westman, 1976) are that the electrophysiological properties of the neuronal elements may be recorded, staining of soma, dendrites and up to 2 cm of axon with its collaterals is achieved, preterminal axons and boutons are stained and the reaction product is electron dense so that ultrastructural studies may be carried out in addition to light microscopy.

The experiments to be reported in the present paper form part of a series in which all the larger cutaneous and muscle afferent fibres are being studied (see Brown, 1977; Brown *et al.* 1977). This report describes the gross morphology of collaterals of Group Ia muscle afferent fibres, including the morphology of their terminal arborizations in the intermediate region (lamina VI of Rexed, 1952), the Ia inhibitory interneurone region (lamina VII, see Jankowska & Lindström, 1972) and in the motor nuclei (lamina IX). Some of the results have been demonstrated to the Physiological Society at the University College London meeting, March, 1977.

METHODS

The experiments were performed on eleven cats anaethetized with chloralose (70 mg.kg^{-1}) after initial anaesthesia with a mixture of halothane in nitrous oxide:oxygen. Full details of the methods used in this laboratory for maintenance of the preparation, intra-axonal recording, injection of HRP and histology have been published previously (Snow *et al.* 1976; Brown *et al.* 1977). Transverse sections of spinal cord were cut at 100 μm; some of the sections containing HRP-stained synaptic boutons were counterstained with cresyl violet.

For the present experiments on muscle afferent fibres the nerves to medial gastrocnemius, lateral gastrocnemius and soleus and the posterior tibial nerve were exposed in both hind limbs and mounted on bipolar platinum or silver–silver chloride electrodes for stimulation in continuity. Ingoing volleys were monitored at the dorsal root entrance zone with a monopolar silver ball electrode. Conduction distances from peripheral nerve to spinal cord were carefully measured at the end of each experiment.

RESULTS

Fifteen Ia afferent fibres were stained in the present experiments, four from medial gastrocnemius, four from lateral gastrocnemius-soleus and seven from muscles with axons in the posterior tibial nerve. All axons had peripheral conduction velocities greater than 80 m.sec^{-1} and therefore the sample was not contaminated with Group II fibres (Matthews, 1963). No systematic tests were performed to differentiate Ia fibres from Ib (Golgi tendon organ) afferents. All fibres in the present sample had a regular discharge when isolated. In fact the final differentiation into Ia and Ib fibres was made on the basis of the histological results; Ia fibres had collaterals reaching the motor nuclei, whereas Ib fibres had collaterals that arborized widely in the intermediate region (see Fig. 5*A* in Réthelyi & Szentágothai, 1973). Tentative classifications into Ia and Ib were made during the electrophysiological recording; Ib fibres classed in this way usually had no ongoing activity when isolated and nearly always required noticeable stretch of muscle (manual extension or flexion of joints)

to excite them. In fact the tentative classifications made during recording always agreed with the histological results.

Surprisingly, Group Ia muscle afferent collaterals were more difficult to stain with HRP than cutaneous afferents even though they have larger diameters. In order to stain collaterals to the level of their terminal boutons in the motor nuclei at least 200 nA.min of change usually had to be passed through the micro-electrode and this required intra-axonal impalements lasting about 30 min. Furthermore, we had to increase the HRP concentration in the micro-electrode from 4 to 9%. These difficulties were presumably due, in part at least, to the greater distance muscle afferent collaterals have to travel to their terminal arborizations compared with cutaneous collaterals. Even greater difficulties have been experienced in staining Ia collaterals in the cervical spinal cord which have to travel further than those in lumbosacral cord (P. K. Rose, personal communication).

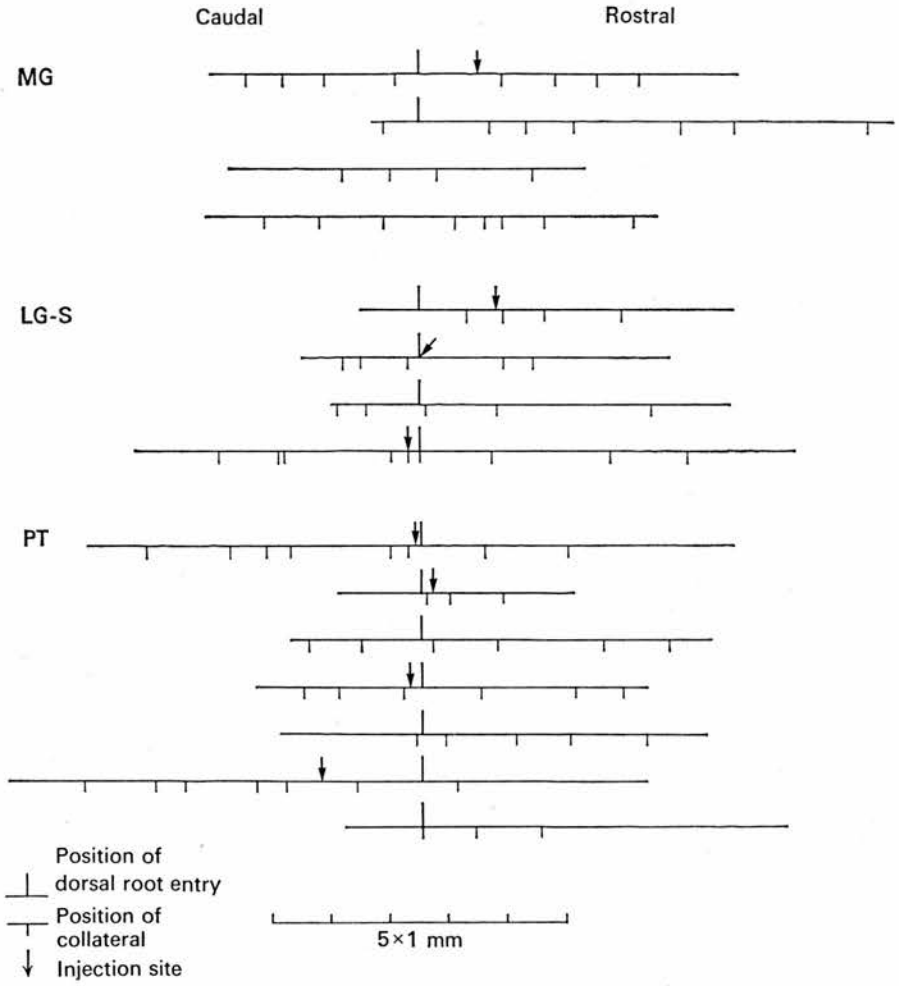
As in our other primary afferent material the most intensely stained collaterals were near the site of HRP injection and only these showed terminal boutons in the motor nuclei and lamina VII. Collaterals further away from the injection site showed the main branching pattern and boutons in lamina VI and those farthest away only showed their initial parts.

Density and distribution of Ia collaterals

Text-fig. 1 shows, in diagrammatic form, the main features of the stained sample of Ia axons and their collaterals. For each axon the following information is shown: the total rostrocaudal length of axon stained, whether or not it could be traced into the dorsal root, whether it bifurcated into rostral and caudal branches upon entering the cord, the number and spacing of collaterals and, where possible, the site of injection with HRP. Thirteen of the fifteen Ia axons could be seen entering the cord through the dorsal roots and all of them bifurcated into rostral and caudal branches as they left the root. The total lengths of axon (rostral plus caudal branches) stained in the present sample ranged from 4 to 11 mm ($7{\cdot}8 \pm 2{\cdot}0$ mm; mean $\pm$ S.D.). These figures are similar to those from cutaneous and other muscle afferent fibres we have stained. None of the caudal branches in the present sample terminated in a collateral; presumably the caudal branches were longer than indicated and gave off further collaterals.

The rostral and caudal branches moved medially within 1–2 mm of the bifurcation and ascended or descended the cord in the dorsal columns. A total of eighty seven collaterals arose from the fifteen Ia axons, with a range of 3–9 ($5{\cdot}8 \pm 1{\cdot}9$; mean $\pm$ S.D.). Obviously only a fraction of all the collaterals arising from a single axon was stained, but on the assumption that all collaterals between the most rostral and most caudal ones on any axon were stained then some useful quantitative data is available. We have no evidence to suggest that collaterals between the most rostral and caudal ones stained were not revealed by the method; near the injection site collaterals were most densely stained and they became progressivly fainter with distance from the injection site, and faintly stained collaterals were not intermingled with more densely stained ones as might be expected if some collaterals were not stained at all. The distance between the most rostral and most caudal collaterals ranged from 1·3 to 8·2 mm ($5{\cdot}0 \pm 2{\cdot}16$ mm; mean $\pm$ S.D.) and collaterals were spaced at intervals of 100–2600 μm

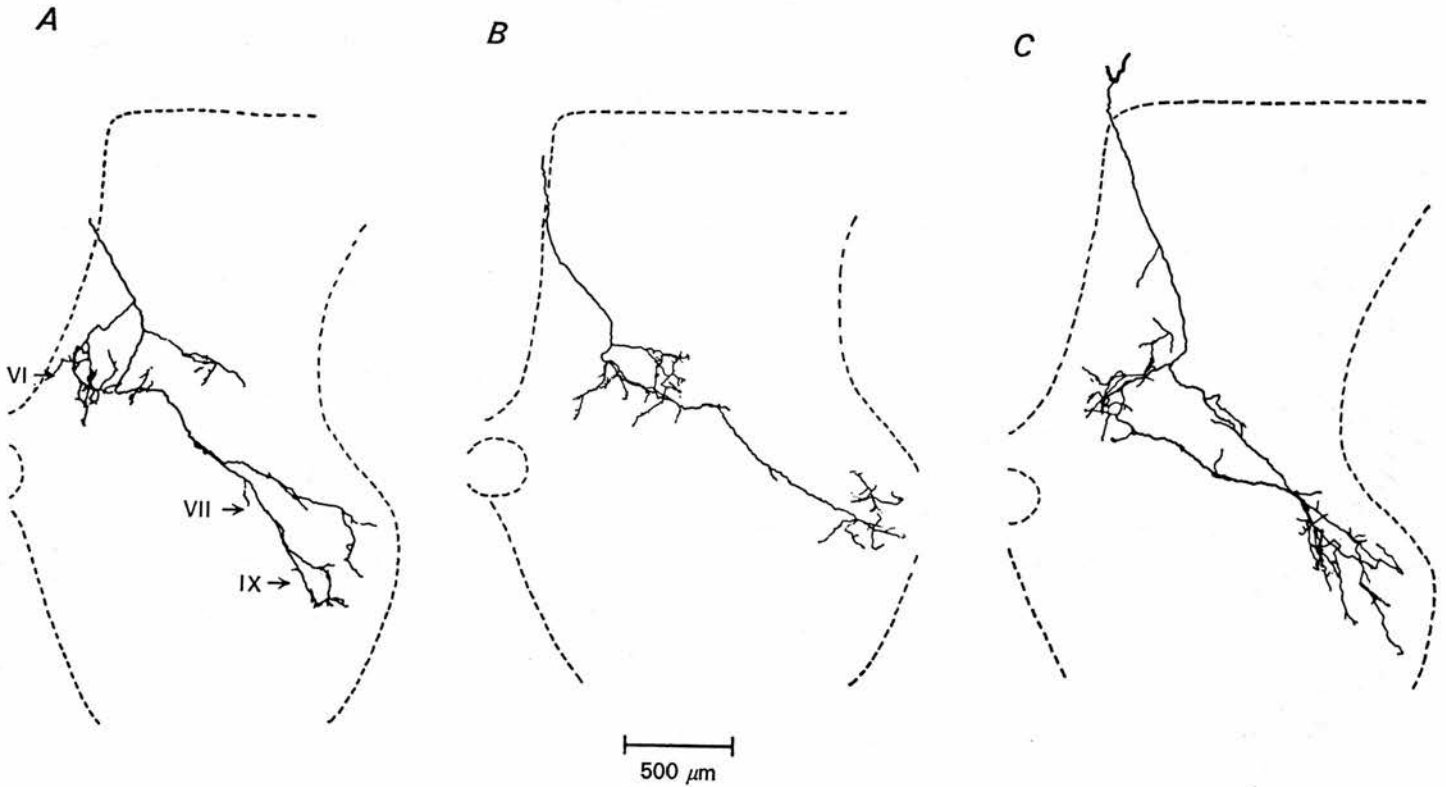
(1040 ± 513 μm; mean ± S.D. n = 73). Therefore, on the average, a collateral arose about every mm along the axon. Examination of Text-fig. 1 reveals that there was no obvious tendency for Ia collaterals to be spaced closer together near the dorsal root entrance of the axon. This is in contrast to the hair follicle afferent collaterals (Brown *et al.* 1977).



Text-fig. 1. Diagrammatic representation of the branching pattern of Ia afferent fibres in the spinal cord. The total stained length of each axon is shown, together with (where possible) the position of its entry to the cord through the dorsal root, the origin of stained collaterals and the site of injection of HRP. All axons that could be traced into the dorsal root can be seen to bifurcate upon entering the cord. For further description see the text.

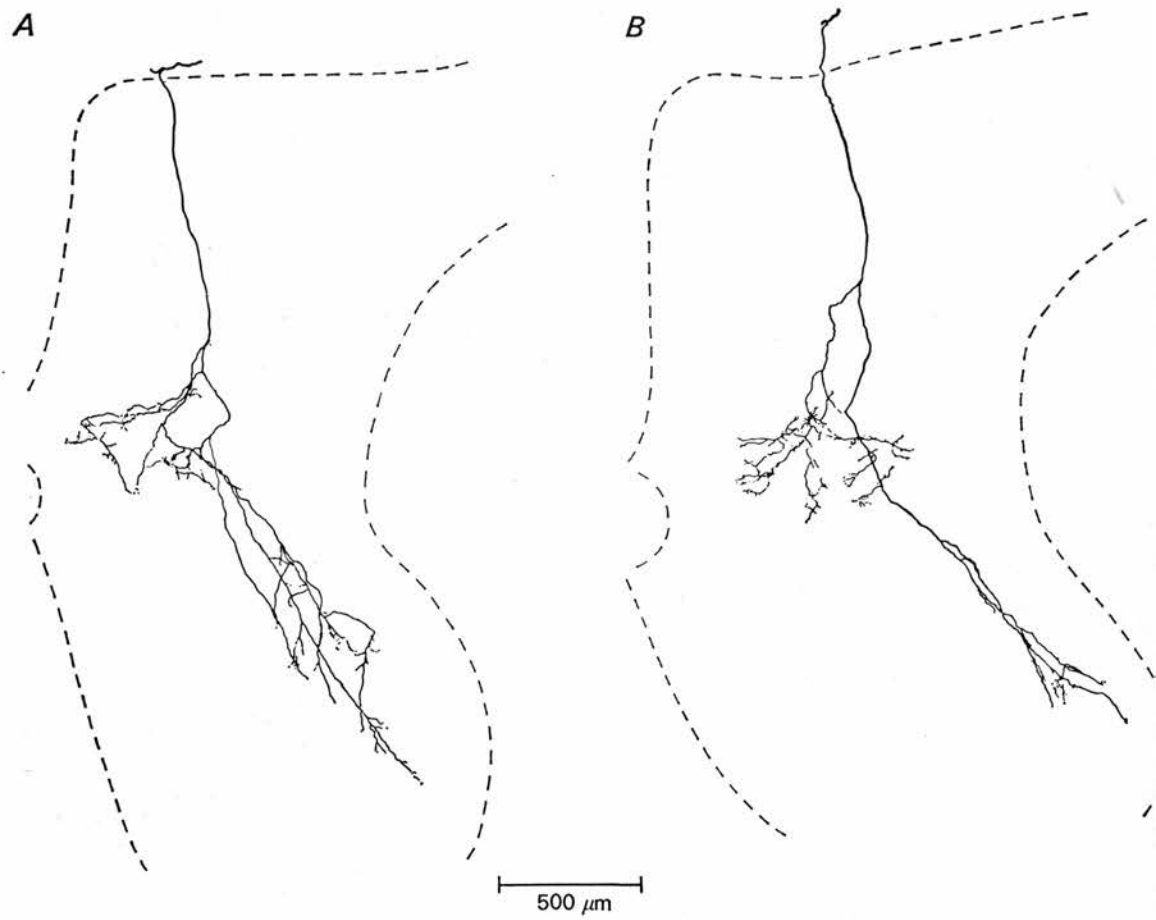
Morphology of Ia collaterals

All collaterals belonging to Ia afferent fibres from the same muscle were strikingly similar in their gross morphology (branching pattern). This was so, not only for collaterals from a single axon, but also for collaterals from different axons from the



Text-fig. 2. Reconstructions, in the transverse plane, of three adjacent collaterals from a medial gastrocnemius Ia afferent fibre. *A* is the most caudal and *C* the most rostral of the three. The similarities in the three collaterals, with regard to their gross morphology, is striking. Each collateral gives branches which arborise profusely in the medial intermediary region (lamina VI), then moves laterally across the cord to break up into arborizations in the Ia inhibitory interneurone region (lamina VII) and the motor nucleus (lamina IX). The three regions are labelled VI, VII and IX respectively in *A*.

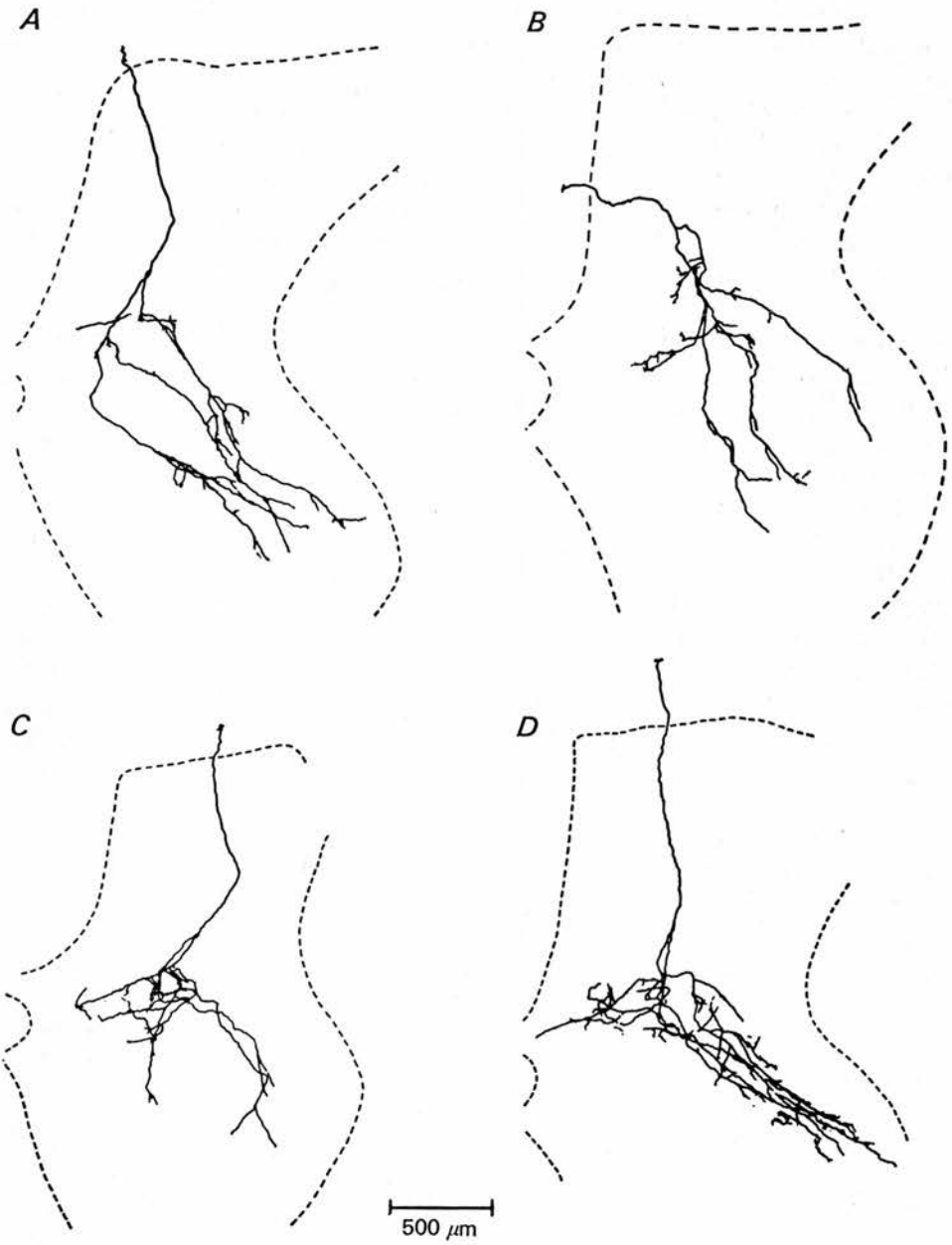
same muscle in different cats. Indeed the similarity extended to collaterals from Ia fibres of muscles in the functional group triceps surae. These similarities are shown in Text-figs. 2–4. Text-fig. 2 consists of reconstructions of three adjacent collaterals from a medial gastrocnemius Ia axon, Text-fig. 3 is of two adjacent collaterals from lateral gastrocnemius-soleus and Text-fig. 4 shows collaterals from medial gastrocnemius, lateral gastrocnemius-soleus (different cats to those of Text-figs. 2, 3) and also from muscles innervated by the posterior tibial nerve.



Text-fig. 3. Reconstructions, in the transverse plane, of two adjacent lateral gastrocnemius-soleus Ia afferent collaterals. This Figure should be compared with Text-figs. 2 and 4*A*, *B*. The similarities between Ia collaterals from triceps surae is obvious.

All medial gastrocnemius and lateral gastrocnemius-soleus Ia collaterals had a similar morphology to the examples shown in Text-figs. 2–4 and Pl. 1. The collaterals usually entered the dorsal horn at its medial or the medial part of its dorsal edge and descended to lamina V directly before branching (branching sometimes occurred in lamina IV, see Text-fig. 2*C*). Collaterals arising close to the bifurcation of the parent axon often ran medially along the dorsal edge of the grey matter before descending towards lamina VI. The first set of terminal arborizations were in the medial half of

lamina VI (intermediate region) with occasionally less well developed arborizations in the middle third of lamina VI (Text-figs. 2*A*, 4*B*). The collaterals then moved ventrolaterally at about 45° towards the region of the triceps surae motor nucleus (Romanes, 1951). Before reaching the nucleus the collaterals subdivided and the



Text-fig. 4. Reconstructions, in the transverse plane, of Ia afferent collaterals from *A*, medial gastrocnemius, *B* lateral gastrocnemius-soleus, *C*, *D* muscles innervated by the posterior tibial nerve.

final arborizations were in the nucleus and also dorsal to it in lamina VII, now known to be the site of the Ia inhibitory interneurones responsible for 'direct inhibition'.

Ia collaterals of axons from the posterior tibial nerve had a somewhat different morphology (Text-fig. 4*C*, *D*). They, in our sample, entered the dorsal horn through its dorsal border. The terminal arborization in lamina VI spread more widely in the transverse plane than collaterals from triceps surae, but were still mainly confined to the medial half of the horn. The terminal arborizations in laminae VII and IX were in ventrolateral regions in the ventral horn.

A feature of most Ia collaterals in the present material and one not mentioned by the Scheibels (1969) who studied sagittal sections of cord in the kitten, was that the collaterals ran rostrally as they descended through the grey matter. In other words they gave off their branches to the intermediate region caudal to the position at which they reached the motor nucleus. Generally the terminal boutons in lamina VI were some 100–300 μm caudal to those in the motor nucleus from the same collateral.

Terminal arborizations and synaptic boutons of Ia collaterals

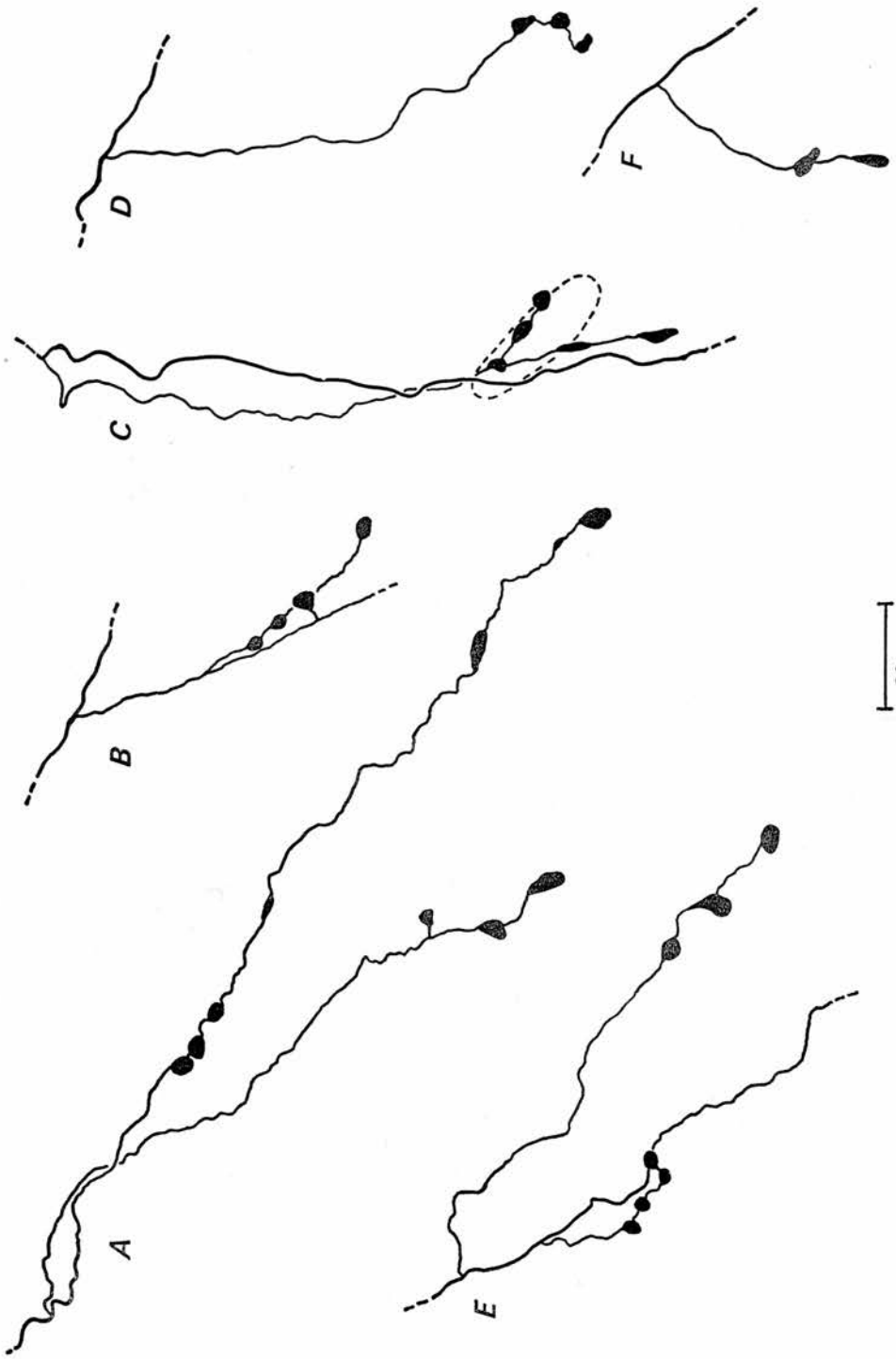
Terminal arborizations and synaptic boutons were often stained in collaterals near the injection site. Because the motor nuclei were further from the injection sites than lamina VI many more collaterals had stained terminal arborizations and boutons in the latter than the former. A feature of Ia afferent terminal arborizations in comparison with most cutaneous afferent terminals (Brown, 1977; Brown *et al.* 1977 and unpublished observations) was the relative simplicity of the muscle afferent terminal arborizations and the relative paucity of synaptic boutons.

Lamina VI. The terminal arborizations were more complex and the density of synaptic boutons was higher in the intermediate region than in either lamina VII or IX (Text-figs. 5–7, Pl. 2). For axons in which adjacent collaterals were well-stained (e.g. those of Text-figs. 2 and 3) the terminal arborizations of adjacent collaterals in lamina VI formed a continuous, or almost continuous, sagittal column of terminals with gaps between adjacent collaterals of 100–200 μm at the most. For the three collaterals of Text-fig. 2 this column of terminal arborizations extended for 1300 μm continuously in the sagittal plane; for the two collaterals in Text-fig. 3 the terminals extended for 1100 μm with a gap of about 150 μm between them.

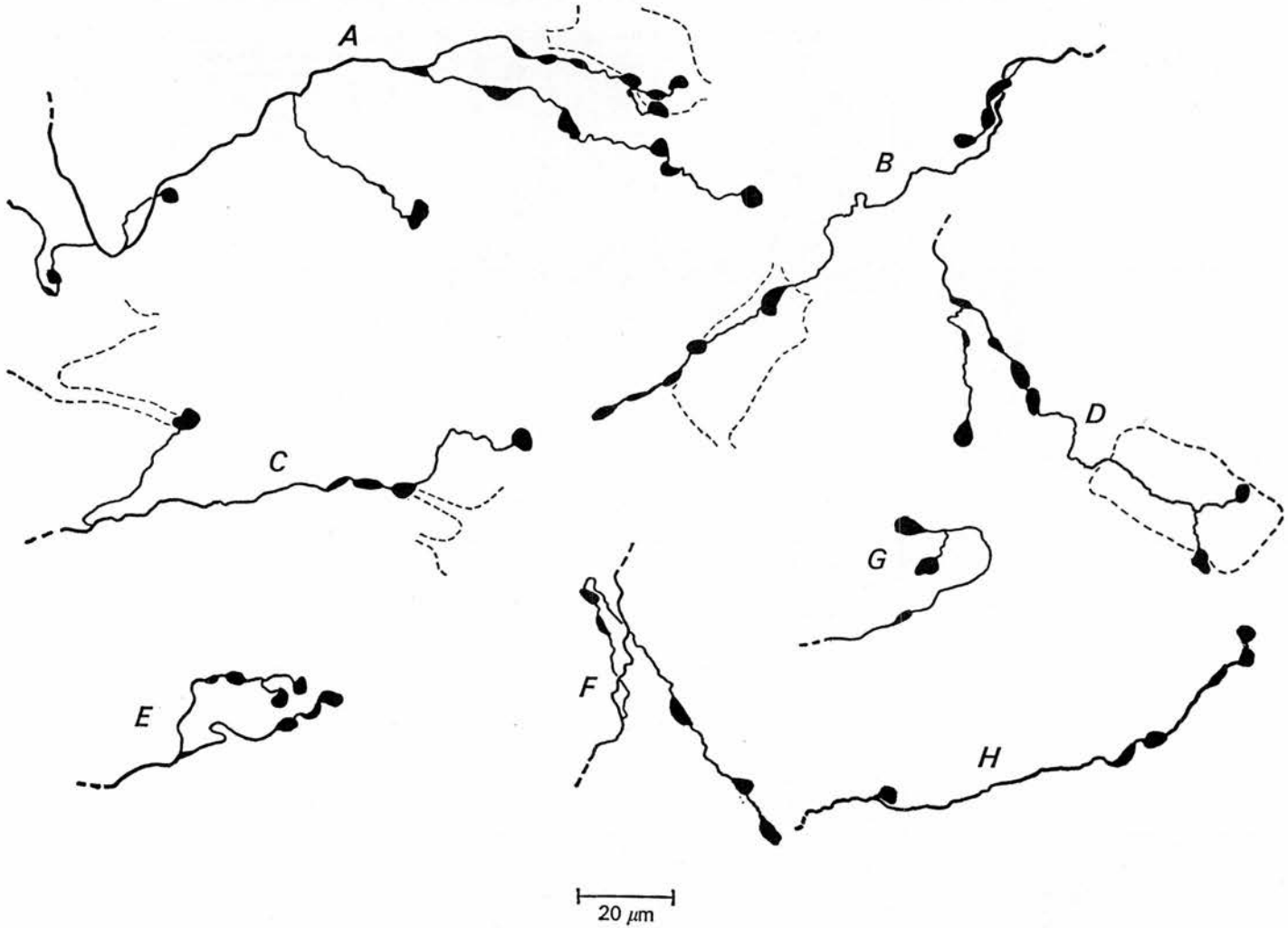
Camera lucida drawings of lamina VI endings are shown in Text-fig. 5 and photomicrographs in Pl. 2*A*. In lamina VI the most common type of terminal axon carried boutons '*de passage*' along its last 20 to 100 μm. Usually 4–5, but as many as six to seven boutons '*en passant*' were situated along the terminal axon which then terminated in a '*bouton terminal*' (Text-fig. 5*E*, *J*; Pl. 2*B*). Another feature of terminals in lamina VI, not seen in VII or often in IX, was that terminal axons often divided into two or three short (20 μm) branches each carrying boutons '*en passant*' and '*terminal*', so that there was a cluster of seven to eight boutons in a small area of cord (Text-fig.5*F*, *G*); these small areas were about 20×20 μm in 100 μm thick sections.

In sections counterstained with cresyl violet these clusters of boutons could be seen to be arranged in relation to the somata and proximal dendrites of lamina VI neurones and were, presumably, making synaptic contact onto these neurones. The outlines of some lamina VI neurones are indicated in Text-fig. 5 and may also be seen in Pl. 2*C*.

Text-fig. 5. Camera lucida drawings of terminal arborizations and synaptic boutons of Ia afferent fibres in lamina VI. The somata and proximal dendrites of neurones with which the boutons appear to make contact in counterstained sections are indicated by dashed lines. This Figure should be compared with Text-figs. 6 and 7. Note the boutons '*en passant*' and the clusters of boutons on neurones.



Text-fig. 6. As Text-fig. 5, but terminal arborizations are in lamina VII. To be compared with Text-figs. 5 and 7. Note the simple types of terminals.



Text-fig. 7. As Text-fig. 5, but terminal arborizations are in lamina IX (motor nuclei). To be compared with Text-figs. 5 and 6. Note the boutons on somata and proximal dendrites of motoneurones.

Boutons in lamina VI ranged from $2{\cdot}5 \times 2{\cdot}5$ to $6{\cdot}5 \times 3{\cdot}5$ μm in size ($4{\cdot}5 \pm 1{\cdot}18 \times 2{\cdot}76 \pm 0{\cdot}69$ μm; means $\pm$ S.D., $n = 62$).

Lamina VII. The terminal arborizations in lamina VII, which is the site of the interneurones on the direct Ia inhibitory pathway (Jankowska & Lindström, 1972) were simpler than those in either lamina VI or in the motor nuclei. As in the other regions they formed an almost uninterrupted sagittal column of endings where adjacent collaterals were well stained. Camera lucida drawings of lamina VII terminal arborizations are shown in Text-fig. 6 and photomicrographs in Pl. 2*D–F*. The terminal axon branches to lamina VII usually arose as fine branches from the main collateral as it ran to the motor nucleus and carried one to three boutons '*de-passage*' and a terminal bouton over a length of 100 μm. Sometimes only a single terminal bouton was carried with no boutons '*de passage*'. In cresyl violet counterstained sections terminals could be seen overlying cell bodies of presumed Ia inhibitory interneurones (Text-fig. 6). Boutons in this region ranged from $3 \times 2{\cdot}5$ to $7{\cdot}5 \times 3{\cdot}5$ μm ($4{\cdot}71 \pm 1{\cdot}53 \times 3{\cdot}03 \pm 0{\cdot}77$ μm; means $\pm$ S.D., $n = 29$; these values were not significantly different from those in laminae VI and IX).

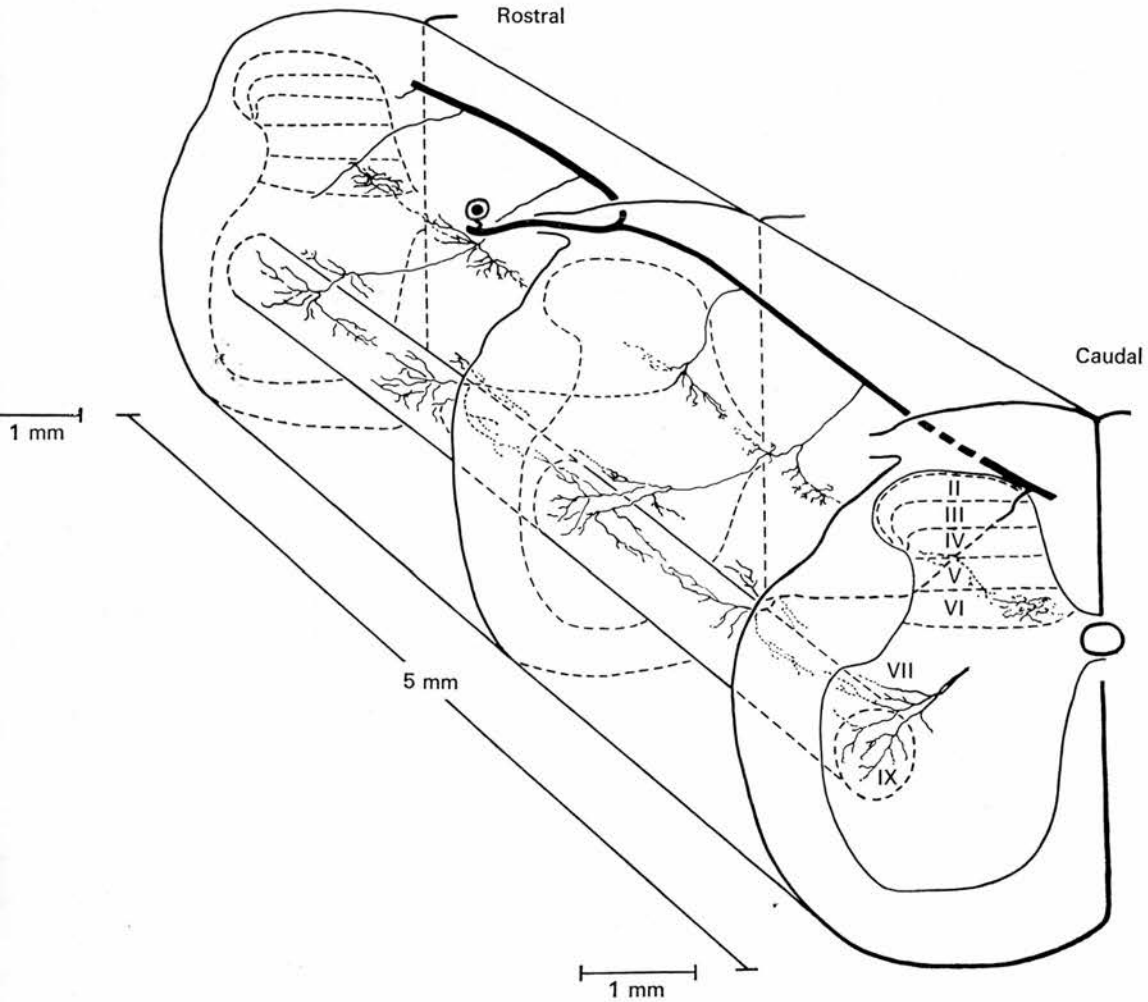
Lamina IX (*motor nuclei*). Terminal arborizations in the motor nuclei were intermediate in complexity to those in laminae VI and VII. In adjacent well stained collaterals they also formed a relatively uninterrupted column of terminals; each collateral had terminals spread over at least 300–500 μm in the sagittal plane and where collaterals arose within 600 μm of each other then there were usually no gaps in the columns of terminals. A variety of terminal arborization patterns was seen in the motor nuclei. At one extreme some were very simple and similar to those in lamina VII (Text-fig.7*B*, *F*) with isolated boutons at the ends of, or in the course of, terminal axons. At the other extreme more complex terminals were observed including ones with four to five boutons '*en passant*' plus a terminal bouton (Text-fig. 7) or branched terminal axons with small clusters of boutons (Text-fig. 7*A*, *E*); but clusters were not a common feature and did not have as many boutons as the clusters seen in lamina VI. In counterstained material many boutons appeared to be situated on motoneurones, on either somata or proximal dendrites (which are the only structures satisfactorily stained with cresyl violet); some motoneuronal outlines are indicated in Text-fig. 7*A–D*. Boutons in the motonuclei ranged in size from $3{\cdot}5 \times 3$ to $7 \times 3{\cdot}5$ μm ($5 \pm 1{\cdot}06 \times 2{\cdot}76 \pm 0{\cdot}63$ μm means $\pm$ S.D., $n = 49$; these values were not significantly different from those in laminae VI and VII).

DISCUSSION

The intra-axonal injection of HRP has proved to be as suitable for studying the morphology of Group Ia muscle fibres as it is for cutaneous afferent fibres (Brown, 1977; Brown *et al.* 1977). But higher concentrations of HRP were needed and more charge had to be passed through the micro-electrode. Presumably this was mainly because the terminal arborizations of Ia afferents in the motor nucleus are a mm or more further from the parent axon than the terminals of cutaneous afferents.

The present report describes the morphology of Ia afferent fibres from triceps surae and from muscles innervated by the posterior tibial nerve. All axons in the sample had peripheral conduction velocities greater than 80 m.sec^{-1} and were, therefore,

Group I fibres. Group II fibres, with conduction velocities less than 70 m.sec^{-1} (Matthews, 1963), have monosynaptic excitatory connexions to motoneurones according to electrophysiological evidence (Kirkwood & Sears, 1974; Stauffer, Watt, Taylor, Reinking & Stuart, 1976) and it is important that they were excluded since the final differentiation into Ia and Ib fibres was made on the basis of collateral morphology. We are confident that our differentiation of Group I fibres into muscle spindle and tendon organ units was correct. As described in Results a tentative



Text-fig. 8. Schematic representation of the arrangement of Ia afferent fibre collaterals (from triceps surae) in the lumbosacral cord of the cat. The Figure is drawn to scale and shows the main features of the collateral morphology as described in the text.

classification was made during the recording session on the basis of the presence, or absence, of 'resting' discharge, regularity of the response and subjective thresholds to muscle stretch. This tentative classification always correlated with the morphology; units classed as Ia always had collaterals reaching the motor nuclei whereas units classed as Ib had collaterals which only reached the intermediate region.

The morphology of Ia afferent fibre collaterals

It has been shown many times (most recently by Iles, 1976) that the large diameter axons with collaterals reaching the motor nuclei bifurcate into rostral and caudal branches upon entering the spinal cord. We have confirmed this for identified Ia fibres and indeed all Ia axons which could be traced into the dorsal roots showed this bifurcation. This is in marked contrast to the hair follicle afferent fibres conducting at above 40 $m.sec^{-1}$; two thirds of them do not bifurcate (Brown *et al.* 1977).

As we gain more experience of the morphology of collaterals from different types of primary afferent fibre in the adult cat it becomes more and more obvious that each type of primary afferent unit has axons that have collaterals with a morphology characteristic of the receptor type. This has now been confirmed for Ia afferents from the primary endings in muscle spindles. Indeed the similarities extend to all Ia afferents from triceps surae and not just to one component muscle of the group. Text-fig. 8 summarizes the results and forms the basis of the following discussion.

A significant feature of all Ia collaterals in the present sample from triceps surae and muscles innervated by the posterior tibial nerve was that the collaterals ran rostrally through the grey matter, so that terminal arborizations were given to lamina VI some 100–300 μm caudal of those in the motor nucleus. It is surprising that this was not mentioned by Scheibel & Scheibel (1969) who examined sagittal sections of the lumbosacral cord in the kitten. They show collaterals dropping vertically to the motor nuclei (see their Figs. 6, 8, 9, 12). It is possible that the cranial trajectory of the collaterals develops as the kitten grows.

All well stained Ia collaterals had three areas of terminal arborization and these agreed with expectations from both anatomical and electrophysiological results gathered by many workers over many years (for review see Réthelyi & Szentágothai, 1973). The first set of terminal arborizations was in lamina VI. No terminals were seen dorsal to lamina VI. In the present sample from ankle extensor and toe plantar flexor muscles the lamina VI arborization was in the medial half of the grey matter. The terminal arborizations in lamina VI were the densest and most complex of all of the Ia arborizations, but they failed to reach the complexity or density of most cutaneous afferent fibre collaterals in more superficial laminae (see Text-figs. 5 and 6 and Pl. 2 in Brown *et al.* 1977). A characteristic feature of Ia terminals in lamina VI was the clustering of boutons on short terminal branches of the axon, often seen in counterstained sections to be arranged on neuronal cell bodies. They would presumably form the anatomical basis for the well known secure transmission from Ia afferents to the lamina VI cells (Eccles, Fatt & Landgren, 1956).

After giving branches to lamina VI the Ia collaterals ran ventrolaterally towards the motor nuclei in lamina IX. As they traversed the region dorsomedial to the motor nuclei they gave off branches which terminated dorsomedial and dorsal to the motoneurones in Rexed's lamina VII, now known to be the site of Ia inhibitory interneurones (Hultborn, Jankowska & Lindström, 1971; Jankowska & Lindström, 1972). The terminal arborizations in lamina VII were the simplest yet seen for primary afferent fibres and consisted, for the most part, of either single terminal boutons at the end of a fine axonal branch or up to 4 boutons strung out on the terminal 100 μm of axon.

The terminal arborizations in the motor nuclei were intermediate in complexity between those in lamina VI and those in lamina VII. There was a variety of terminal structures including boutons '*en passant*' (up to six boutons along the last 80 μm of axons; certainly more than the three described by Szentágothai, 1958) and occasional clusters of boutons, similar to, but less well developed, than those in lamina VI.

The distribution of Ia afferent fibre collaterals to motoneurones

The HRP method gives little information, except a lower limit, on the lengths of axon giving rise to collaterals. In the present sample these ranged from 1300 to 8200 μm, but according to Sprague (1958) monosynaptic connexions spread over five segments of the lumbosacral cord in the cat, about 30,000 μm in the adult animal. Triceps surae motoneurones alone occupy a length of about 10,000 μm in the adult (Romanes, 1951; Sprague, 1958) so our values are obviously too low. But the data on the spacing of collaterals is useful. Collaterals of Ia axons were given off at intervals ranging from 100 to 2600 μm with a mean spacing of about 1000 μm. Scheibel & Scheibel (1969) in their study on the kitten state that collaterals arise within 2–10 mm of the dorsal root entrance at intervals of 100–200 μm, each axon giving rise to between ten and 100 collaterals. If the differences in length of the cord in new-born and adult cats is taken into account then the two sets of data may not be too different. But extrapolation of our data leads to the conclusion that for collaterals to triceps surae a 10 mm length of axon would give only ten collaterals (average of one every millimetre). This value is much less than that suggested by Iles (1976) who concluded that about fifty collaterals were given off each Ia axon to triceps sura emotoneurones. Since the triceps surae motoneurone pool contains some 725 motoneurones (Boyd & Davey, 1968; Iles, 1976) and each Ia afferent from triceps surae should have monosynaptic connexions with about 550 motoneurones (Mendell & Henneman, 1971; Iles, 1976) then our figures indicate that each collateral should contact fifty to sixty motoneurones, about 5 times more than suggested by either the Scheibels (1969) or Iles (1976).

These differences are initially somewhat disturbing. As mentioned in the Results we do not think that the HRP method misses or stains collaterals in an all-or-none fashion, but stains all collaterals between the most rostral and the most caudal ones stained on an individual axon. If it is accepted that Ia afferents give off collaterals at a rate of about one every millimetre then obviously each collateral will have to contact considerably more than ten motoneurones. A single motoneurone has dendrites up to about 1 mm in length (Barrett & Crill, 1974; see Fig. 1 in Cullheim & Kellerth, 1976) and until the percentage of Ia contacts on dendrites are fully described calculations based on somatic and proximal dendritic contacts are of limited value. The figures of the Scheibels (1969) and Iles (1976) are based on such somatic and proximal dendritic contacts. Conradi (1969) has observed presumed Ia synaptic boutons (his M type) on occasion to make more than one synaptic contact, that is, on more than one post-synaptic element, perhaps on a distal dendrite of one motoneurone and on the soma of another (Conradi, 1969, p. 100). But the evidence from the present results is strong that the values suggested by the Scheibels (1969) and Iles (1976) underestimate the true state of affairs. In a 100 μm thick cresyl violet stained section through the triceps surae motor nucleus about ten motoneuronal

somata are seen. The terminal arborizations of Ia afferent collaterals in the nucleus run for at least about 400 μm in the sagittal plane therefore could contact about forty motoneurone cell bodies. Our suggestion that a single Ia collateral from a triceps surae muscle should have monosynaptic connexions to between fifty and sixty triceps surae motoneurones seems quite reasonable if dendritic contacts are taken into account.

We wish to thank Mr R. B. Hume for continued excellent assistance. Animals were held in the Wellcome Animal Research Laboratories, Faculty of Veterinary Medicine.

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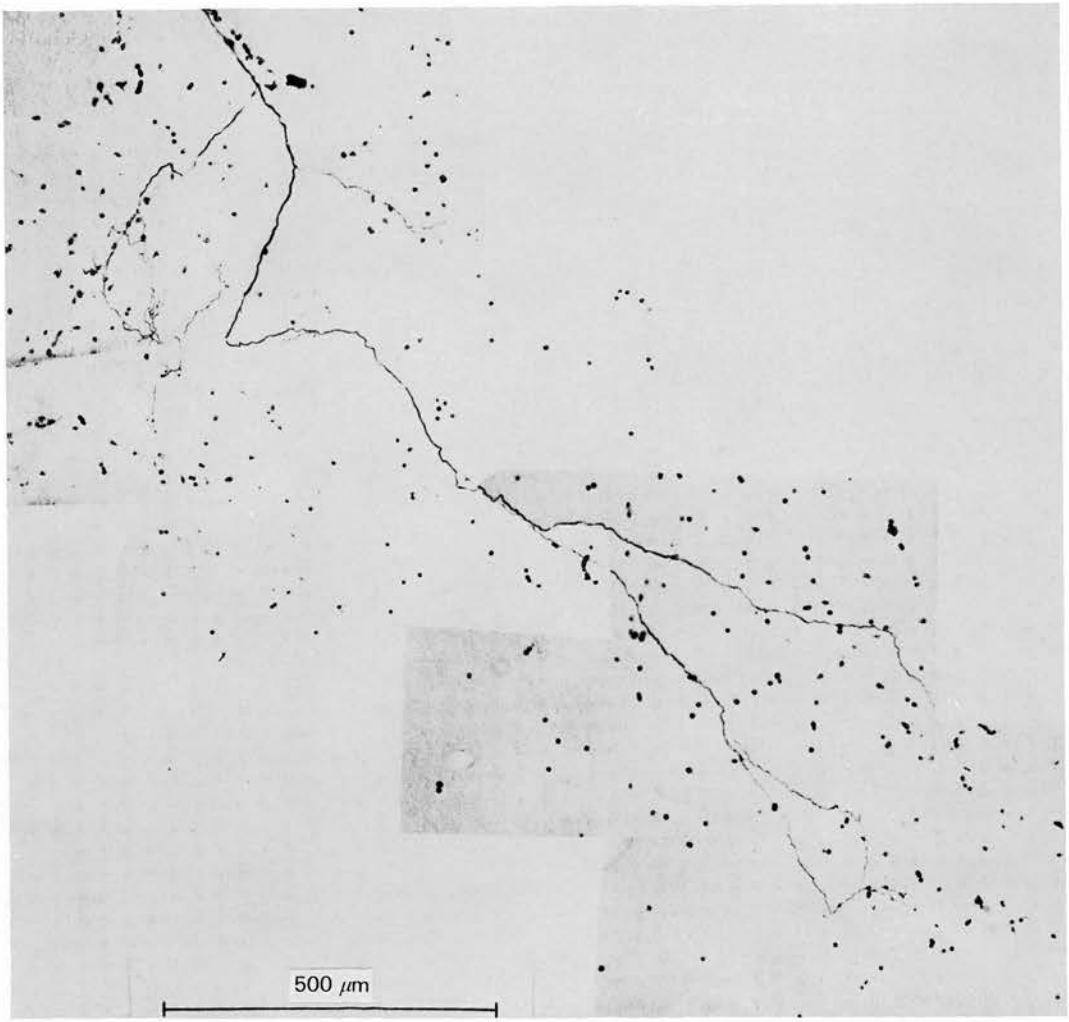
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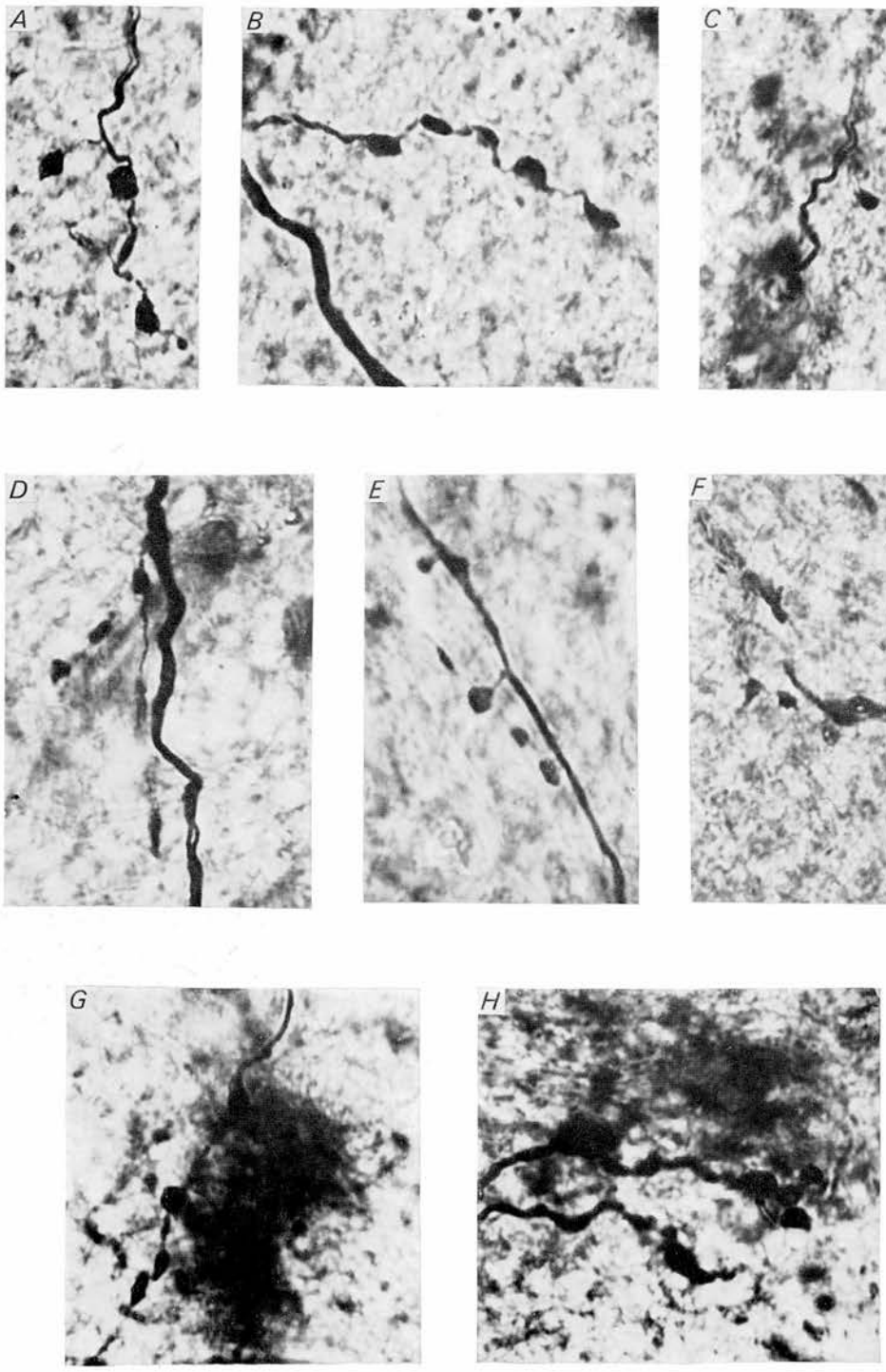

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EXPLANATION OF PLATES

All photomicrographs are of 100 μm thick transverse sections of the cord.

PLATE 1

Photomontage of a Ia afferent fibre collateral from medial gastrocnemius. This collateral is reconstructed in Text-fig. 2*A*.

PLATE 2

Terminal arborizations and synaptic boutons of Ia afferent fibre collaterals. *A–C*, in lamina VI, *D–F*, in lamina VII, *G*, *H*, in lamina IX. Sections *C*, *D*, *G*, *H*, were counterstained with cresyl violet.

In all photographs dorsal is at the top.

Synaptic contacts made by identified Ia afferent fibres upon motoneurones

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Injection of the enzyme horseradish peroxidase (HRP) into single Ia muscle afferent fibres has provided morphological information on the organization of their collaterals (Brown & Fyffe, 1978). In the present experiments HRP was injected into identified Ia afferents and motoneurones in cats anaesthetized with chloralose and paralysed with gallamine triethiodide.

Fig. 1. shows reconstructions of a single collateral from a Ia afferent in the posterior tibial nerve and a motoneurone antidromically activated from the same nerve. Five synaptic contacts were made, one single and one multiple contact, about 550 μm out on the dendritic tree of the motoneurone, on two different dendrites of the second and third order. No other contacts were seen between the Ia afferent fibre and this motoneurone.

In other experiments contacts between Ia afferents and motoneurones have been observed closer to the motoneuronal soma and also on more distal dendrites. Similar results have been obtained by R. E. Burke (personal communication). The technique

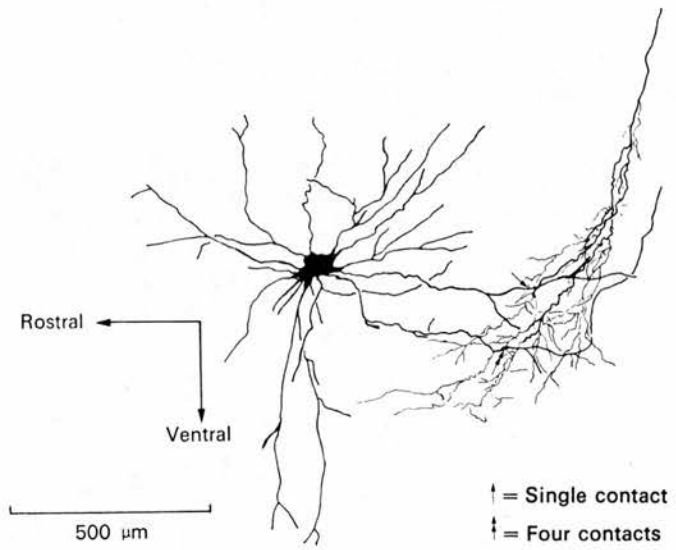

Fig. 1. Reconstruction, from sagittal sections, of the ventral part of a Ia afferent fibre collateral and a motoneurone with which it made five synaptic contacts (arrows). Only part of the motoneuronal dendritic tree is shown.

of HRP injection into pairs of neuronal elements monosynaptically linked will allow a direct estimate of the sites and numbers of synaptic contacts.

Supported by the M.R.C.

* M.R.C. Scholar.

REFERENCE

Brown, A. G. & Fyffe, R. E. W. (1978). *J. Physiol.* **274**, 111–128.

J. Physiol. (1979), **296**, *pp.* 215–228
With 2 plates and 6 text-figures
Printed in Great Britain

THE MORPHOLOGY OF GROUP Ib AFFERENT FIBRE COLLATERALS IN THE SPINAL CORD OF THE CAT

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(*Received 27 March 1979*)

SUMMARY

1. The enzyme horseradish peroxidase (HRP) was injected into single Ib muscle afferent fibres in anaesthetized cats. Subsequently, histochemistry allowed the morphology of the axons and their collaterals in the lumbosacral spinal cord to be determined.

2. Eleven Ib axons were stained, seven from lateral gastrocnemius-soleus, one from medial gastrocnemius and three from muscles innervated by the posterior tibial nerve. Ten of the axons were traced into the dorsal roots and all but one (from the posterior tibial nerve) bifurcated upon entering the cord. Between 5·1 and 9·9 mm of each axon was stained and the fibres gave off eighty-four collaterals at intervals of 100–2300 μm, at an average spacing of about 900 μm. The spacing between collaterals on the (finer) descending axon branches was generally less than the intervals between collaterals on ascending branches.

3. All Ib collaterals had a characteristic morphology. The collaterals coursed cranially on a direct path through the dorsal horn to lamina IV or V before branching. They arborized widely in the intermediate region, mainly in lamina VI and in the dorsal part of lamina VII. Occasionally, less extensive arborizations were seen more dorsally in lamina IV and V. The rostro-caudal extent of individual collateral arborizations was limited to 200–400 μm and there was no overlap between adjacent collaterals. Each terminal arborization gave rise to 56–384 boutons, mainly of the 'en passant' type.

4. The results are discussed in relation to previous anatomical and electrophysiological studies.

INTRODUCTION

The central projections of group Ib afferent fibres from Golgi tendon organs have proved difficult to characterize. Classical anatomical methods are incapable of providing a definite identification of primary afferent fibre collateral arborizations in the spinal cord. Réthelyi & Szentágothai (1973) tentatively identify Ib afferent collaterals as the relatively large diameter collaterals that arborize widely in the intermediary region and do not reach the motor nuclei. The intermediary region corresponds with the area in which focal potentials greater than 20 % of maximum were evoked by electrical stimulation of Ib fibres (Eccles, Fatt, Landgren & Winsbury, 1954). Ib

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0022-3751/79/5820-0117 $01.50

afferents are also known to excite, monosynaptically, the cells of origin of the dorsal and ventral spinocerebellar tracts – the d.s.c.t. and v.s.c.t. respectively (Oscarsson, 1965, 1973; Lundberg, 1971; Lundberg & Weight, 1971).

The recent introduction of intra-axonal staining of electrophysiologically identified axons with horseradish peroxidase (HRP) allows the morphology of primary afferent collaterals to be determined (Brown, Rose & Snow, 1977, 1978; Brown & Fyffe 1978*a*). The present paper describes the morphology of collaterals of group I b muscle, afferent fibres. Some preliminary results were demonstrated to the Physiological Society at the Bristol meeting, January 1978 (Brown & Fyffe, 1978*b*). While we were completing this work a preliminary report by Hongo, Ishizuka, Mannen & Sasaki (1978) was published with findings essentially the same as ours.

METHODS

Full details of the methods have been published previously (Snow, Rose & Brown, 1976; Brown *et al.* 1977). The experiments were performed on eleven cats anaesthetized with chloralose (70 mg.kg^{-1}) and paralysed with gallamine triethiodide. For the present experiments on muscle afferents, the nerves to medial gastrocnemius, lateral gastrocnemius-soleus, and the posterior tibial nerve were exposed in both hind limbs and mounted on bipolar platinum or silver-silver chloride electrodes for stimulation in continuity. Ingoing volleys were monitored at the dorsal root entrance zone with a monopolar silver ball electrode. Conduction distances from peripheral nerve to the spinal cord were carefully measured at the end of each experiment. Glass microelectrodes filled with an 8% solution of HRP were used for recording and injecting axons near their entry into the spinal cord. Frozen, 100 μm thick transverse or longitudinal sections were cut and processed by the method recently developed by Hanker, Yates, Metz & Rustioni (1977). Some sections were counterstained with 0·1% methylene green.

RESULTS

Eleven I b afferent fibres with their collaterals were stained in the present experiments, seven from lateral gastrocnemius-soleus, one from medial gastrocnemius and three from muscles with axons in the posterior tibial nerve. For each axon the peripheral conduction velocity and the threshold to electrical stimulation of the nerve were measured. All the axons had peripheral conduction velocities between 80 and 98 m.sec^{-1}. None of the axons had ongoing activity when isolated and required noticeable stretch (manual extension or flexion of joints) to excite them. The differentiation of the group I muscle afferent fibres into I a and I b fibres on the basis of these characteristics was always supported by subsequent morphological data. Fibres identified as belonging to group I a had collaterals reaching the motor nuclei (Brown & Fyffe, 1978*a*). Group II fibres were excluded from our sample on the basis of conduction velocity. These fibres conduct at less than 70 m.sec^{-1} (Matthews, 1963) and have recently been shown to have collaterals with a characteristic morphology (Fyffe, 1979).

Successful staining of the I b afferent fibre collaterals, to the level of boutons in the intermediate region, required more than 150 nA.min of current to be passed. At the end of the injection period (up to 30 min) it was usually possible to continue recording the activity of the fibre and then to monitor a change in membrane potential when the electrode was removed.

Density and distribution of Ib collaterals

Fig. 1 shows, in diagrammatic form, the main features of the Ib axons and their collaterals. For each axon the following information is shown: the total rostro-caudal length of axon stained, whether or not it could be traced into the dorsal root, whether it bifurcated into ascending and descending branches upon entering the cord, and the number and spacing of the collaterals. Ten of the axons could be seen entering the cord through the dorsal roots and nine of these bifurcated into ascending and descending branches. The axon that did not appear to bifurcate ran in the posterior tibial nerve. In general the ascending branch was thicker than the descending branch of the axon. The total lengths of axon stained ranged from 5·1 to 9·9 mm ($7{\cdot}64 \pm 1{\cdot}8$ mm; mean ± S.D.). The ascending branches were stained for distances of 5·0–9·6 mm whilst the descending branches ranged from 0 to 3·8 mm. These values are similar to those for cutaneous and other muscle afferent fibres we have stained. None of the main branches in the present sample terminated in a collateral; presumably the axons were longer than the stained material indicated, and gave off further collaterals.

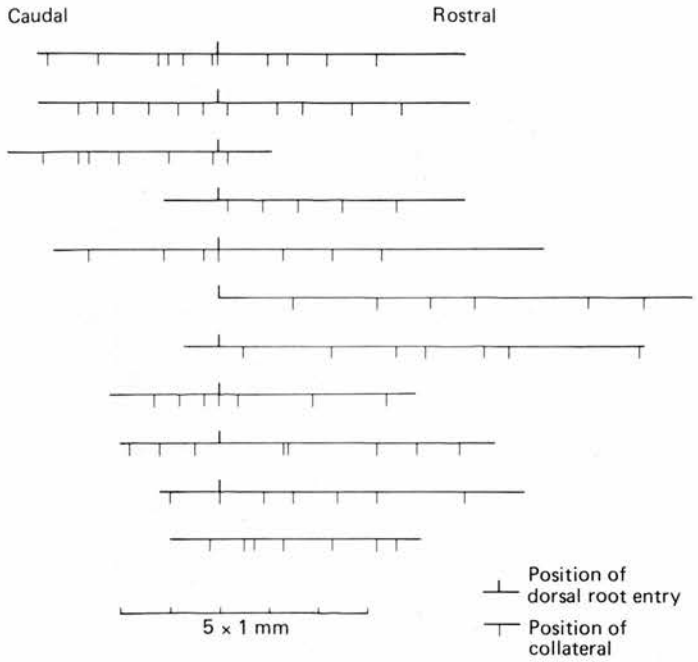

Fig. 1. Diagrammatic representation of the branching pattern of Ia afferent fibres in the spinal cord. The total stained length of each axon is shown, together with (where possible) the position of its entry to the cord through the dorsal root and the origin of stained collaterals. All but one of the axons that could be traced into the dorsal root can be seen to bifurcate upon entering the cord. For further description see the text.

We have no evidence that the HRP fails to stain collaterals between the most rostral and caudal ones, and therefore we obtain some useful data on the spacing of collaterals. Typically a few collaterals close to the injection site were well stained and

demonstrated extensive branching and filling of fine terminal branches and boutons. Collaterals arising further away were more lightly stained; few if any terminal branches or boutons could be observed.

The main rostral and caudal branches moved medially from their entry into the cord and bifurcation, to assume wavy courses in the dorsal column. A total of eighty-four collaterals arose from the eleven Ib axons, with a range of five to eleven from each axon ($7{\cdot}4 \pm 2{\cdot}0$; mean $\pm$ S.D.). Fifty collaterals arose from the ascending branches, twenty-seven from the descending branches and seven from the axon which could not be traced into a dorsal root. The latter axon was probably part of the ascending branch of the Ib afferent – as it was traced rostrally its course assumed a deeper position in the dorsal columns.

The distance between the most rostral and most caudal collaterals of a stained axon ranged from 3·4 to 8·0 mm, and the collaterals were spaced at intervals of 100–2600 μm (890 ± 506 μm; mean $\pm$ S.D.). There is no significant difference between this value and that obtained for Ia afferents, which have a mean spacing between collaterals of about 1 mm (Brown & Fyffe, 1978*a*). However in the present sample of Ib axons it was apparent that collaterals arising from the descending branches of the axons were more closely spaced than collaterals arising from the ascending branches. Intervals on descending branches ranged from 200 to 1500 μm (690 ± 331 μm; mean $\pm$ S.D.) whilst spaces between collaterals on ascending branches ranged from 100 to 2600 μm (1080 ± 524 μm; mean $\pm$ S.D.). The difference between collateral spacing on the ascending and descending branches is highly significant ($P < 0{\cdot}001$). There was no significant increase in the spacing of collaterals arising further from the root entrance of the parent axon. This was so even on ascending branches stained for up to 9·6 mm from the entrance.

Morphology of Ib collaterals

The main morphological features of a variety of Ib afferent fibre collaterals from triceps surae and muscles innervated by the posterior tibial nerve are shown in Figs. 2–5 and Pl. 1. The characteristic branching patterns are strikingly similar for all Ib collaterals stained in this sample.

Collaterals usually entered the dorsal horn at the dorsomedial or dorsal edge and ran ventrally in a direct course to lamina IV or V before branching. As can be seen most clearly from the sagittal reconstructions of Fig. 4, collaterals ran slightly rostrally and approximately in parallel with each other for this initial part of their course through the dorsal horn. Branching, which started in lamina V, gave rise to an extensive fan shaped arborization, 400–800 μm wide in the transverse plane, in which the terminal collateral branches were mainly located in the medial and central parts of lamina VI and in the dorsal part of lamina VII.

In contrast to the wide transverse distribution of Ib terminal arborizations in the intermediate region, the rostro-caudal extent of any collateral was usually restricted to 200–400 μm. There was no overlap between adjacent collaterals so that in effect each collateral from a Ib axon occupied a discrete volume of the spinal cord. Even when the origins of adjacent collaterals were as close as 100 μm, the courses taken by the collaterals and the restricted rostro-caudal extent of the aborizations, resulted in the aborizations being separated by as much as 300–400 μm (see Fig. 4).

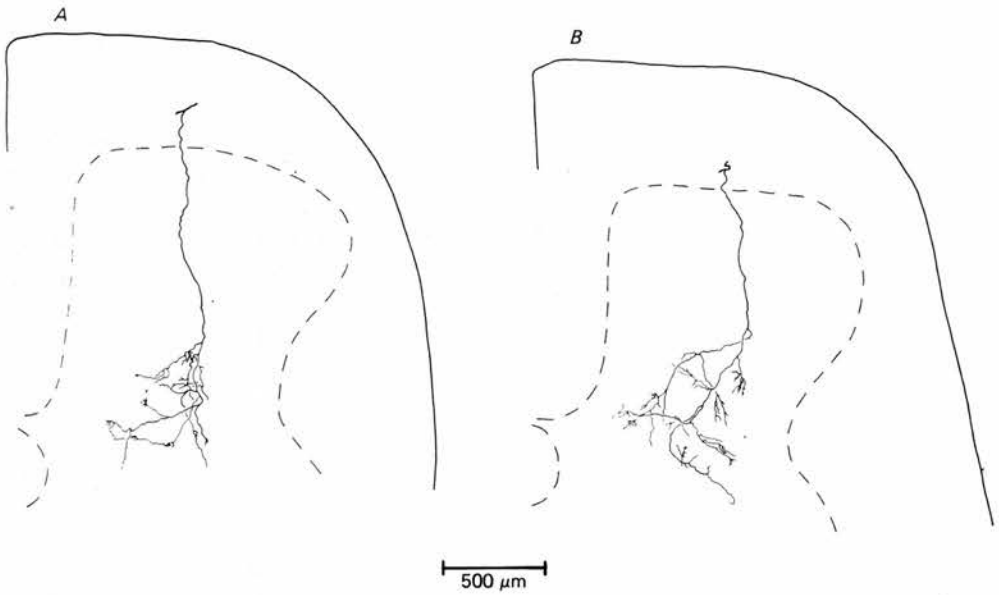

Fig. 2. Reconstructions, in the transverse plane, of two adjacent collaterals from the descending branch of a medial gastrocnemius Ib afferent. Collateral *A* was caudal to collateral *B*. The collaterals enter the dorsal horn at its dorsal edge and run ventrally to lamina V before branching into the characteristic fan shaped arborizations in lamina VI and the dorsal part of lamina VII. The outline of the grey matter is indicated by the dashed line.

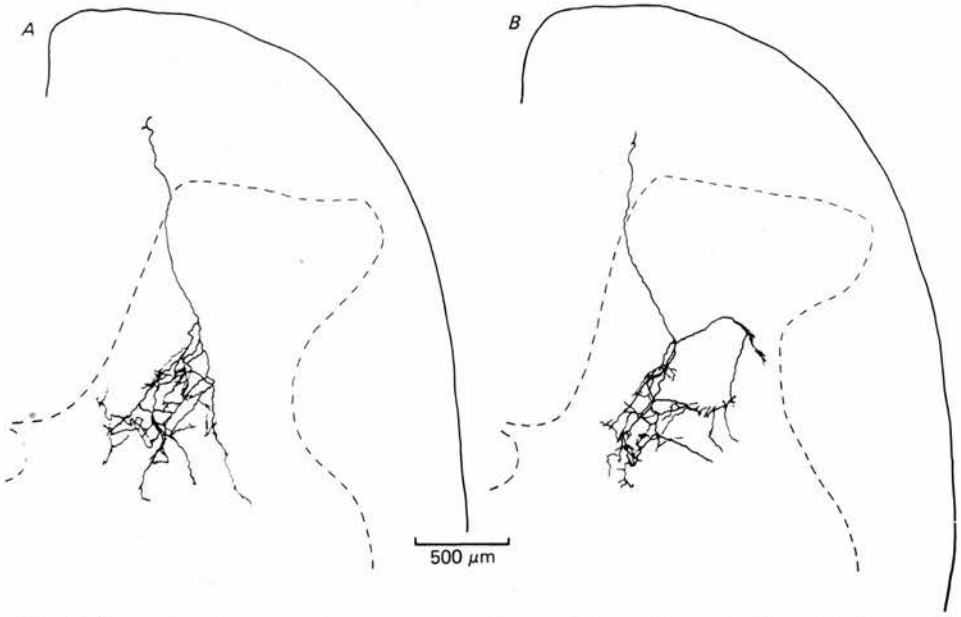

Fig. 3. Reconstructions, in the transverse plane, of two adjacent collaterals from the ascending branch of a lateral gastrocnemius-soleus Ib afferent. The main arborizations in medial and central lamina VI are similar to those in Fig. 2. The more rostral collateral (*B*) also projects to the lateral parts of laminae V and VI.

Although the branching pattern of the Ib collaterals usually generated a single extensive arborization in the medial half of lamina VI some Ib collaterals projected to other regions. One lateral gastrocnemius Ib afferent injected close to the dorsal root entrance had three well stained collaterals with minor branches given off more dorsally, in lamina IV. These gave rise to small arborizations in the centre of lamina IV. All of these collaterals arose from the descending branch of the Ib axon, about 2–3 mm caudal to the dorsal root entrance. The most caudal of these collaterals is shown in Fig. 5*A*. Apart from this minor arborization the general pattern of this collateral was similar to collaterals from other Ib axons; further branching produced the main fan-shaped terminal arborization in lamina VI.

The other region to which some of the present sample of Ib collaterals projected was the lateral part of laminae V and VI. Projections to this area were observed more commonly than the projections dorsally in lamina IV, especially on collaterals located

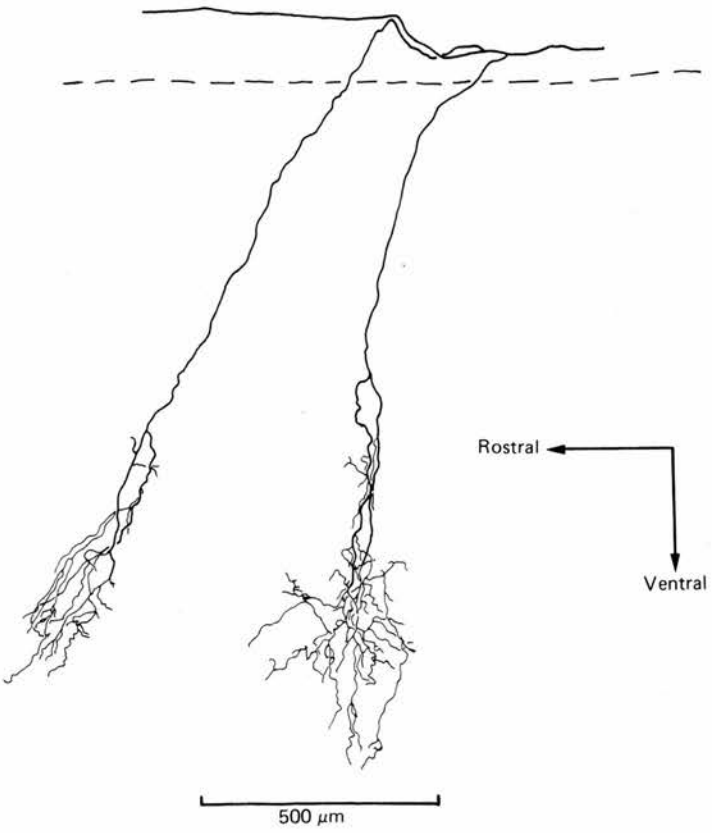

Fig. 4. Reconstructions, in the sagittal plane, of two adjacent collaterals from the ascending branch of a lateral gastrocnemius-soleus Ib afferent. This view shows the cranial trajectory of the collaterals towards their terminal arborizations. Although the collaterals arise close to each other on the parent axon there is no overlap between the two arborizations. The rostro-caudal extent of each arborization is restricted to less than 400 μm in contrast to the wide transverse spread seen in Figs 2, 3 and 5. The dashed line indicates the dorsal border of the dorsal horn.

in the upper L7 segment. Two examples, from different Ib axons are shown in Figs. 3*B* and 5*B*. The collaterals shown in these reconstructions were located 6–8 mm rostral to the entry of their respective parent axons into the spinal cord. In both cases the ascending branch of the Ib axon had been injected 3–4 mm rostral to the dorsal root entry. In Fig. 3 the collaterals are from the same Ib axon. Both arborize characteristically in medial and central lamina VI but additionally the more rostral collateral (3*B*) sends branches to terminate within 100 μm of the lateral border of lamina V and also in the lateral part of lamina VI. Fig. 5*B* is a reconstruction of a rostral collateral from a different Ib axon. This collateral branched soon after entering the medial part of the dorsal horn and sent branches ventrolaterally to terminate in lateral lamina VI and in dorso-lateral lamina VII. Other branches of the collateral arborized extensively in the medial half of laminae V and VI.

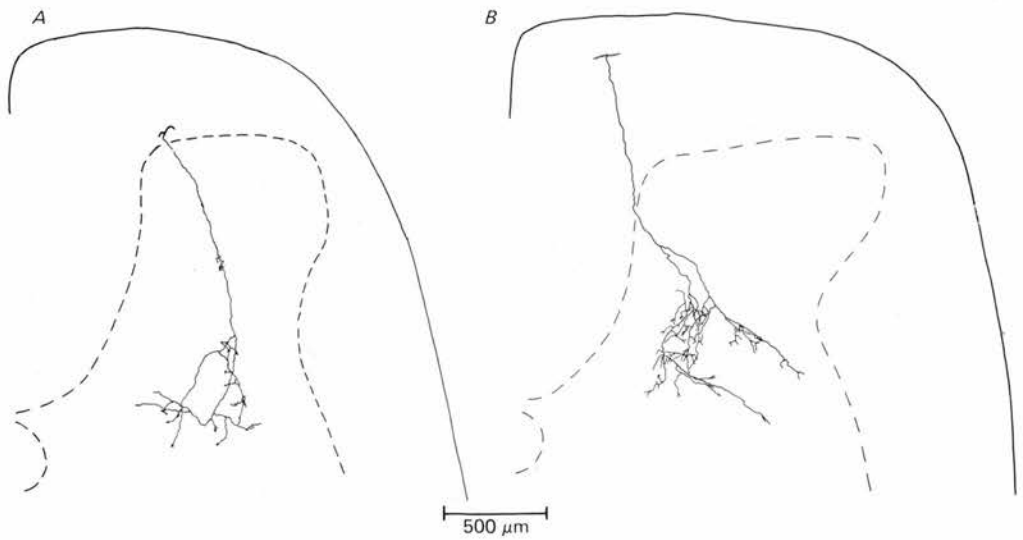

Fig. 5. Reconstructions, in the transverse plane, of two collaterals from different Ib axons. Collateral *A* is from the descending branch of a lateral gastrocnemius-soleus afferent and has a small arborization in lamina IV as well as the major zone of termination in central lamina VI and the dorsal part of lamina VII. Collateral *B* is from a lateral gastrocnemius-soleus afferent ascending the cord. The collateral arises about 7 mm from the dorsal root entry. The main arborization is in the medial half of laminae V and VI. There are also prominent projections to the lateral part of lamina VI, and to dorso-lateral lamina VII.

Terminal arborizations and synaptic boutons of Ib collaterals

Successive branching of the pre terminal axons gave rise to fine terminal branches usually located in lamina VI and in dorsal lamina VII. Most of the terminal branches were oriented more or less in the dorso-ventral or medio-lateral directions, with very few instances of terminal axons running longitudinally up or down the cord. This fine branching pattern expressed itself in the gross morphology described in the previous section, as wide transverse arborizations with restricted rostro-caudal spread. Branch-

ing in and around lamina VI did not always occur at simple bifurcations; often an axonal branch would divide into three daughter branches as illustrated in Pl. 2*A*.

Fig. 6 and Pl. 2 illustrate some of the terminal patterns and bouton arrange-

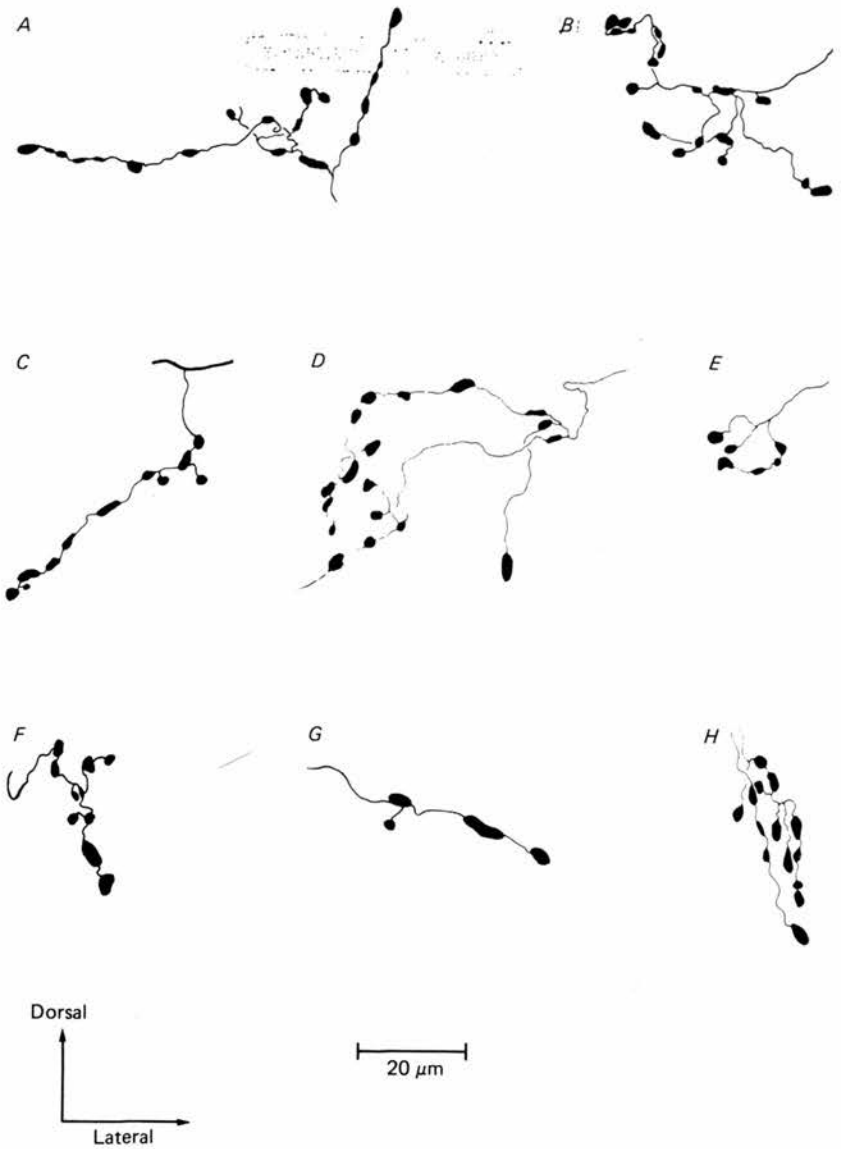

Fig. 6. Reconstructions, in the transverse plane, of some terminal patterns from HRP-labelled Ib afferents. The terminals in *A–E* were located in the medial and central parts of lamina VI and illustrate examples of boutons 'en passant' along the fine terminal branches. More complex branching and clustering of boutons is also evident. Single boutons are often offset from the terminal branch on short fine stalks. In *F–H* are reconstructed some terminals in lateral parts of lamina VI. Boutons were fewer in this region, but even close to the lateral border of the neck of the dorsal horn (e.g. in *H*) complex arrangements could be seen.

ments observed on the Ib collaterals. The predominance of boutons 'en passant' along the terminal branches was characteristic of the Ib collateral arborizations. In transverse sections the boutons 'en passant' lay in the plane of the section. In lamina VI, where most of the Ib boutons were observed, another feature was that single boutons were often offset from terminal branches on short, fine stalks (e.g. Fig. 6 *C*). Some terminal branches divided several times in their final 20–30 μm to give rise to quite complex clustering of boutons 'en passant' and 'terminaux' (e.g. Fig. 6 *B*, *D*). Branches which terminated in dorsal lamina VII generally had rather simpler arrangements, consisting of only a few boutons 'en passant' along the terminal branches. Boutons were also observed on those collateral branches projecting more laterally in laminae V, VI and dorso-lateral VII. Again, the terminal branches carried a few 'en passant' boutons with single boutons offset from the main terminal branch. The few collaterals from the Ib axon which projected to lamina IV, as well as lamina VI, generated quite complex arborizations in the centre of lamina IV. Pl. 2 *B* illustrates the arrangement of one of these minor arborizations.

Boutons in the main terminal arborization in lamina VI were sometimes seen in close association with cell body profiles in sections counterstained with 0·1 % methylene green. The most commonly observed pattern was where a fine branch divided to deliver two to six boutons, on two to three terminal branches, to the stained cell body. Pl. 2 *E* shows four boutons in relation with a medium sized lamina VI neurone. Pl. 2 *D* shows an example of 'en passant' boutons near or on a cell in lamina VI – this arrangement was quite rarely seen. In lateral lamina VI apparent contacts were also observed on some cell bodies (Pl. 2 *F*). In fact only a small proportion of the boutons from the Ib collaterals seemed to be involved in possible contacts on cell bodies. The predominant boutons 'en passant' along the final 20–50 μm of the terminal branches may then be expected to be involved in forming 'climbing' contacts along the dendrites of interneurones in this area.

The total number of boutons from a single Ib collateral ranged from 56 to 384 (179 ± 114; mean $\pm$ S.D.). This number is much larger than the number of boutons in the lamina VI arborization of Ia collaterals (up to ninety-seven, A. G. Brown & R. E. W. Fyffe, unpublished) and reflects the more extensive arborization of Ib collaterals and predominance of boutons 'en passant'. The size of Ib boutons ranged from $1{\cdot}0 \times 1{\cdot}0$ μm to $5{\cdot}0 \times 3{\cdot}0$ μm ($3{\cdot}1 \pm 0{\cdot}97 \times 1{\cdot}7 \pm 0{\cdot}53$ μm; mean $\pm$ S.D., $n = 170$).

DISCUSSION

The technique of intra-axonal staining of electro-physiological identified axons has recently been applied to demonstrate the morphology and central connexions of primary afferent fibres in several systems including the monosynaptic Ia pathway to motoneurones (Brown & Fyffe, 1978*a*, *c*), hair follicle afferent fibre collaterals (Brown, 1977; Brown *et al.* 1977), collaterals from afferent fibres of slowly adapting type I units (Brown *et al.* 1978) and the collaterals of other types of cutaneous and muscle afferent fibres (A. G. Brown, R. E. W. Fyffe, P. K. Rose & P. J. Snow, unpublished results). The present paper extends the sample of stained, identified primary afferent fibres to include the group Ib afferents from Golgi tendon organs of the cat.

The identification of muscle afferent fibres into groups Ia, Ib and II (see Matthews,

1972) was based during each experiment on the conduction velocity of the peripheral nerve fibre and a subjective assessment of its response to passive stretch of the muscle. The group II muscle afferents from muscle spindle secondary endings conduct impulses at less than 70 m.sec^{-1} and are excluded from the present sample of axons which all had conduction velocities greater than 80 m.sec^{-1}. This is important since some of the group II muscle afferents have recently been shown to have monosynaptic excitatory connexions to motoneurones (Kirkwood & Sears, 1974; Stauffer, Watt, Taylor, Reinking & Stuart, 1976) and also to project to the intermediate region and the dorsal horn (Fu & Schomburg, 1974; Fu, Santini & Schomburg, 1974). These findings have been supported by intra-axonal injection of HRP into identified group II fibres which show a characteristic branching pattern vastly different from the Ia and Ib collaterals (Fyffe, 1979). The tentative classification of the group I fibres into Ia and Ib carried out during recording was always correlated with the subsequent demonstration of a collateral morphology characteristic of receptor type. The Ia fibres had a regular ongoing discharge when isolated and could be excited by weak stretch or tapping the muscle. All the Ia fibres projected to the appropriate motor nuclei (Brown & Fyffe, 1978*a*). The Ib fibres were silent when isolated and required noticeable stretch of the muscle to excite them. Ib collaterals only projected as far ventrally as dorsal lamina VII.

Successful staining of Ib axons and their collaterals required more than 150 nA.min of current to be passed. The extent of labelling observed was similar to that from previous injections of primary afferent fibres. All but one of the stained Ib fibres was seen to bifurcate on entering the cord and to ascend or descend the cord in the dorsal columns. Generally the rostral branch of the stem axon was thicker than the caudal branch.

Assuming that all the collaterals between the most rostral and most caudal ones were stained, the average distance between adjacent collaterals was about 890 μm. This is similar to the value obtained for the inter-collateral distances on Ia fibres (Brown & Fyffe, 1978*a*) and considerably longer than values obtained from Golgi studies of primary afferent fibres in young animals (Scheibel & Scheibel, 1969). However it was apparent that collaterals arising from the descending branch were more closely spaced, with a mean distance between adjacent collaterals of 690 μm, than collaterals on the ascending branch of the axon, which had average intervals of 1080 μm. Although the ascending branches were always stained for greater distanees than the descending branches, there was no significant increase in the collateral spacing further away from the dorsal root entry. In the 3 mm either side of the dorsal root entry collaterals were, as stated, more closely spaced on the descending branches. In a recent preliminary report, Hongo *et al.* (1978) also observed longer inter-collateral distances on the ascending branches of a small pooled sample of stained Ia and Ib axons. The significance, if any, of this observation is obscure.

Ib collaterals had a characteristic gross morphology which agreed with expectations from many previous anatomical and electrophysiological experiments. In the transverse plane the general pattern is strikingly similar to the collaterals shown in Fig. 5*A* of Réthelyi & Szentágothai (1973). The collaterals run directly through the dorsal horn to lamina V before branching extensively and arborizing in laminae V, VI and dorsal lamina VII, mainly in the medial half of lamina VI. None of the Ib collaterals

in the present sample projected more ventrally than dorsal lamina VII. Only one Ib axon had (three) collaterals which projected dorsal to lamina V. These collaterals, which projected to the centre of lamina IV, all arose from the descending branch of that axon.

Ib collaterals pursue a cranial trajectory towards their terminal arborizations. This is similar to the path taken by Ia collaterals and this feature has not been observed in anatomical investigations of primary afferent fibre projections using Golgi staining methods in young animals (see for example Fig. 9 in Scheibel & Scheibel, 1969). Presumably the cranial trajectory of the collaterals develops as the kitten grows.

The wide fan-shaped arborization seen in lamina VI and dorsal VII in transverse sections contrasts with the sagittal view of the Ib arborizations. Each collateral has a restricted rostro-caudal spread, with most of the terminal branches oriented transversely or in the dorso-ventral direction. There was never any overlap between adjacent collaterals; each arborization occupied a discrete zone of the spinal cord. In the previous study of Ia fibres, some collaterals gave rise to an almost uninterrupted sagittal column of terminals in the intermediate region, although the lamina VI arborizations of Ia collaterals are much less complex in transverse spread and bouton density than the Ib arborizations.

The zone of termination of Ib collaterals corresponds very closely with the localization of focal synaptic potentials greater than 20 % of maximum evoked by electrical stimulation of Ib afferents from hind limb muscles (Eccles, Fatt, Landgren & Winsbury, 1954). The effects of tendon organ activation must be mediated by interneurones located in, or with dendritic arborizations in, this part of the intermediate region. The mechanism of autogenetic inhibition of homonymous and synergic muscles has been intensively studied. Laporte & Lloyd (1952) associated this inhibition, and the reflex facilitation of antagonistic motoneurones, with activity in tendon organ afferents. They also postulated a disynaptic linkage mediating these effects. Since these early experiments a wealth of data on the segmental reflex response patterns to the Ib impulses has been provided by intracellular recording from interneurones and motoneurones (see, for example, Eccles, Eccles & Lundberg, 1957; Eccles & Lundberg, 1959; Jankowska & Lindström, 1972). Recently Lucas & Willis (1974), using adequate stimulation of tendon organs, demonstrated monosynaptic excitation by Ib afferents of interneurones in the medial parts of laminae VI and VII.

Studies using either electrical or adequate stimulation of Ib afferents (Eecles, 1965; Jankowska & Lindström, 1972; Lucas & Willis, 1974) have indicated that most of the interneurones in the intermediate region activated by group I afferents belong to the Ib pathway from Golgi tendon organs. The projection of Ib afferent fibre collaterals described in this paper provides an anatomical basis for such excitation of interneurones in the intermediate region. Where counterstained sections were examined, two to six boutons could occasionally be seen in close apposition to stained cell bodies. Furthermore, Ib collateral terminal branches in and around the medial half of lamina VI commonly carried five to ten boutons 'en passant'. Such arrangements may indicate 'climbing' bouton connexion along dendrites, and provide a powerful synaptic input.

Detailed electrophysiological work has also been carried out on the distribution of Ib effects to pathways ascending from the spinal cord to the cerebellum, especially

through the d.s.c.t. and v.s.c.t. (for review see Oscarsson, 1973). The d.s.c.t. takes its origin in the cells of the nucleus dorsalis or column of Clarke (Clarke, 1859; see Rexed, 1954) an anatomically well defined column of cells extending from thoracic segments down into the fourth lumbar segment. Thus primary afferents entering the cord at lower lumbar levels have to ascend for at least two segments before giving collaterals to these cells. It would clearly be of interest to stain Ib collaterals at the level of Clarke's column in the upper lumbar segments. None of the present sample of Ib axons was stained for that distance. However, the most rostrally located collaterals described in this paper had indeed marked arborizations in the medial parts of the intermediate region and if this pattern is also characteristic of Ib collaterals in upper lumbar segments then it would provide an anatomical basis for Ib excitation of d.s.c.t. cells in these locations.

Ib input to the v.s.c.t. has also been studied by various workers (e.g. Oscarsson, 1957; Burke, Lundberg & Weight, 1971). Hubbard & Oscarsson (1962) described cells of origin of the v.s.c.t. in a widespread region dorsomedial to the ventral horn. In later studies, Lundberg and coworkers (Burke *et al.* 1971; Lundberg & Weight, 1971; Lundberg, 1971) described the spinal border cells of Cooper & Sherrington (1940) as being the major source of the v.s.c.t. Many of the dorsomedial cells receive monosynaptic Ib input (Eccles, Hubbard & Oscarsson, 1961; Hubbard & Oscarsson, 1962) whilst the spinal border cells receive predominantly monosynaptic Ia input. However since some of these also receive monosynaptic Ib input, the two sources of the v.s.c.t. may complement each other. Spinal border cells giving rise to the v.s.c.t. have been found as far caudally as the sixth lumbar segment. It is interesting to note that the most rostrally stained collaterals in our present sample project towards the lateral border of lamina VI and the dorso-lateral part of lamina VII.

Thus the present report describes projections of single Ib afferent fibres in the lower lumbar segments of the spinal cord. The extensive collateral arborizations in lamina VI and the dorsal part of lamina VII correlate with previous electrophysiological studies of group Ib projections.

We wish to thank Mr R. B. Hume for continued excellent technical assistance. Animals were held in the Wellcome Animal Research Laboratories, Faculty of Veterinary Medicine. This work was supported by a grant from the Medical Research Council.

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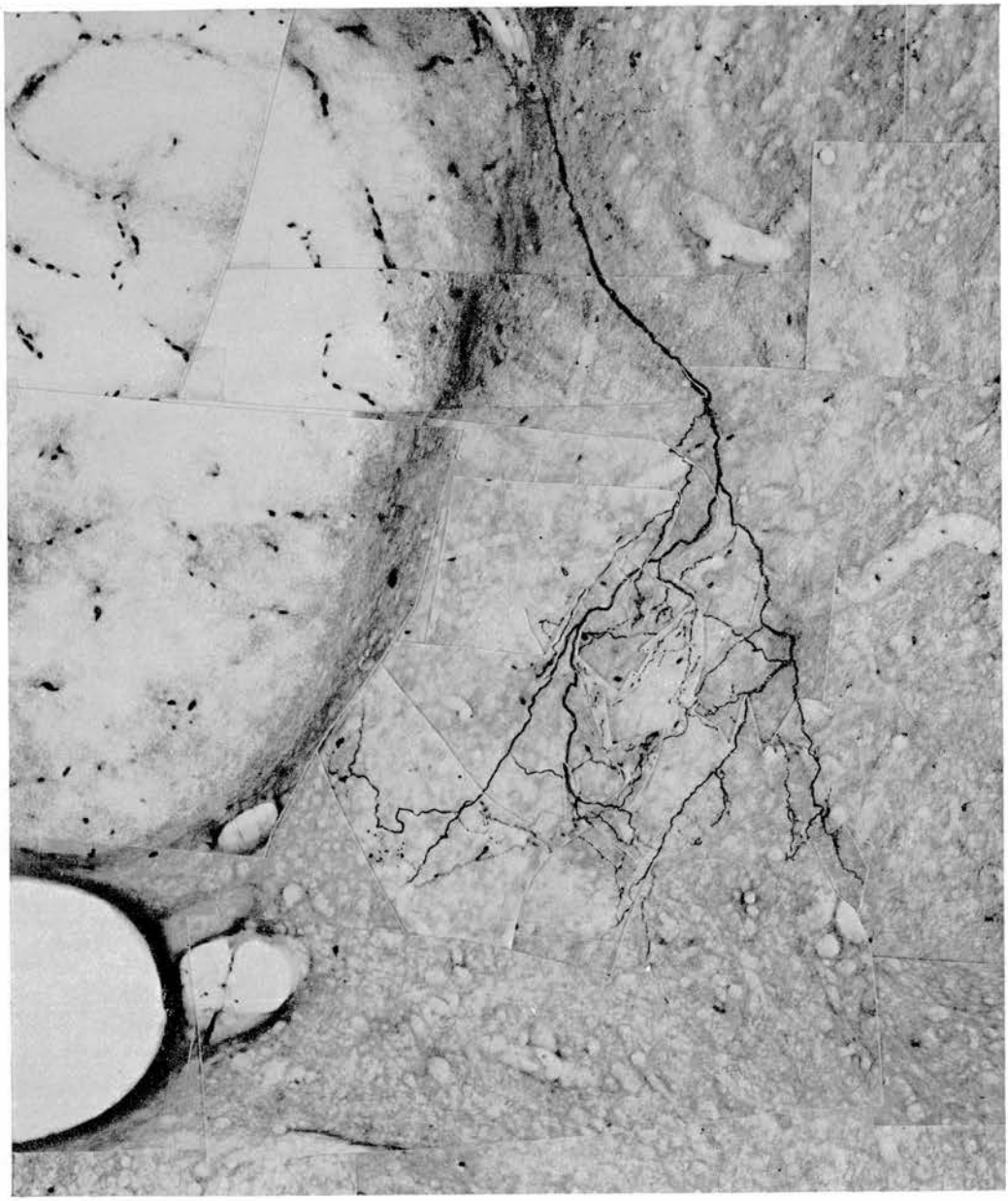

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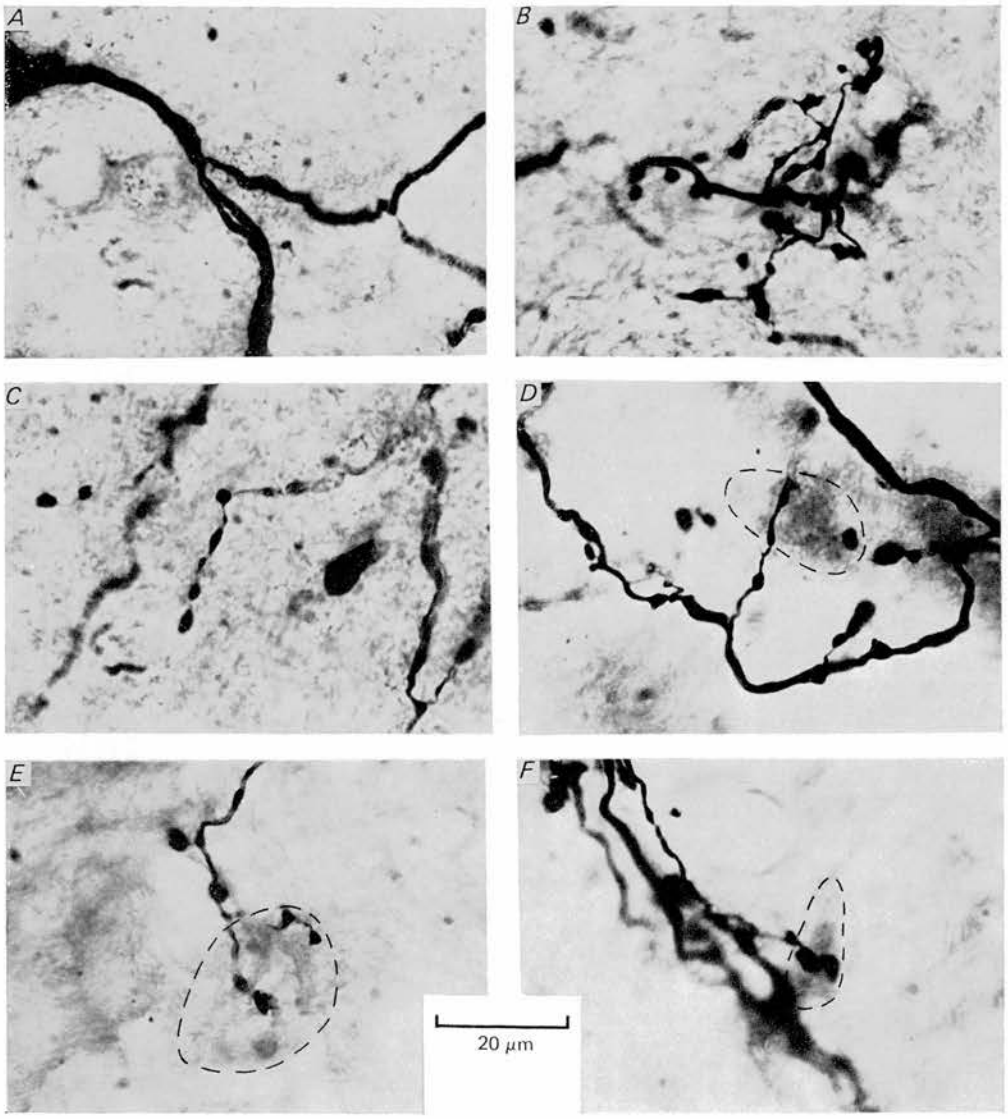
(*Facing p.* 228)


A
B
C
D
E
F
20 μm

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EXPLANATION OF PLATES

All photomicrographs are from 100 μm thick transverse sections of spinal cord.

PLATE 1

Photomontage showing part of the extensive branching and terminations in lamina VI of a Ib afferent fibre collateral. This collateral is reconstructed in Fig. 3*A*.

PLATE 2

Photomicrographs showing details of Ib terminal arborizations.

A, example of a fibre dividing into three daughter branches in lamina VI.

B, complex clustering of boutons observed in the central part of lamina IV, dorsal to the main arborization.

C–F, boutons in lamina VI. *D–F* are from counterstained sections. Soma profiles are outlined. *C*, branch carrying several 'en passant' boutons. *D*, 2 boutons 'en passant' contacting a stained cell body. *E*, four contacts on a medium sized lamina VI neurone. *F*, two to three contacts on a cell near the lateral border of lamina VI.

[*From the Proceedings of the Physiological Society*, 13–14 *July* 1979
Journal of Physiology, **296**, 39–40*P*]

The morphology of Group II muscle afferent fibre collaterals

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Horseradish peroxidase was injected ionophoretically into single Group II axons in the lumbosacral cord of cats anaesthetized with chloralose (70 mg kg^{-1}) and paralysed with gallamine triethiodide. All axons innervated triceps surae muscle and had peripheral conduction velocities of 54–61 m sec^{-1}.

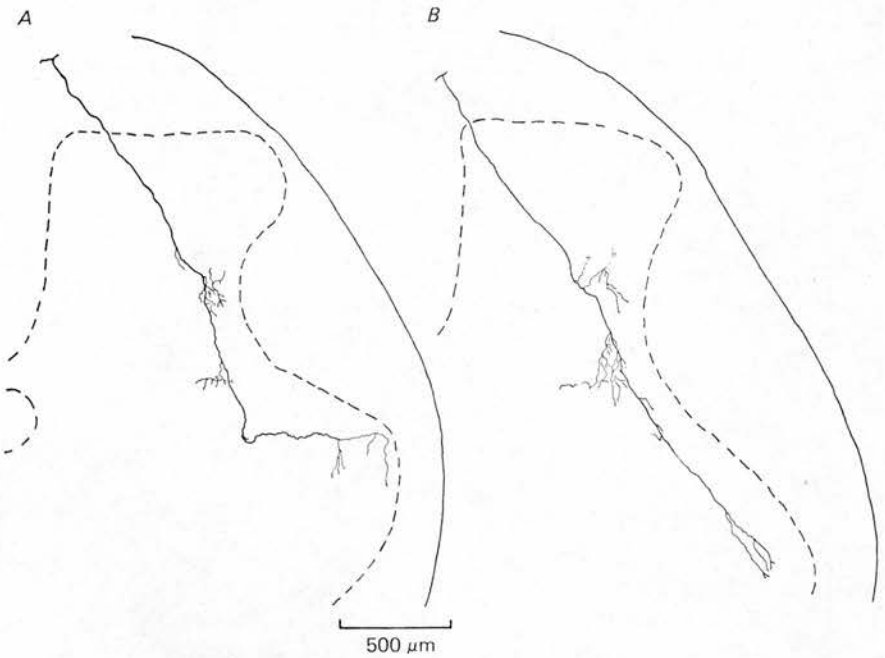

Fig. 1. Reconstructions, from transverse sections of the spinal cord, of two adjacent collaterals from a Group II afferent fibre innervating lateral gastrocnemius-soleus muscle. The grey-white border and surface of the cord are indicated by dashed and solid lines respectively.

Group II muscle afferent collaterals in the spinal cord (see Fig. 1) had terminations in the dorsal horn, intermediate region and in the ventral horn. The anatomy of Group II collaterals differed from the Ia and Ib afferent fibre collaterals (Brown & Fyffe, 1978, 1979). The location of the Group II collateral arborizations was similar to the sites of Group II focal synaptic potentials described by Fu, Santini & Schomburg (1974) and provides morphological support for the recent evidence (Kirkwood & Sears, 1974; Stauffer, Watt, Taylor, Reinking & Stuart, 1976) that Group II muscle spindle afferents make monosynaptic excitatory connexions with motoneurones.

Supported by grants from the M.R.C.

* M.R.C. Research Student.

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