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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(*Received 27 June 1977*)

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).

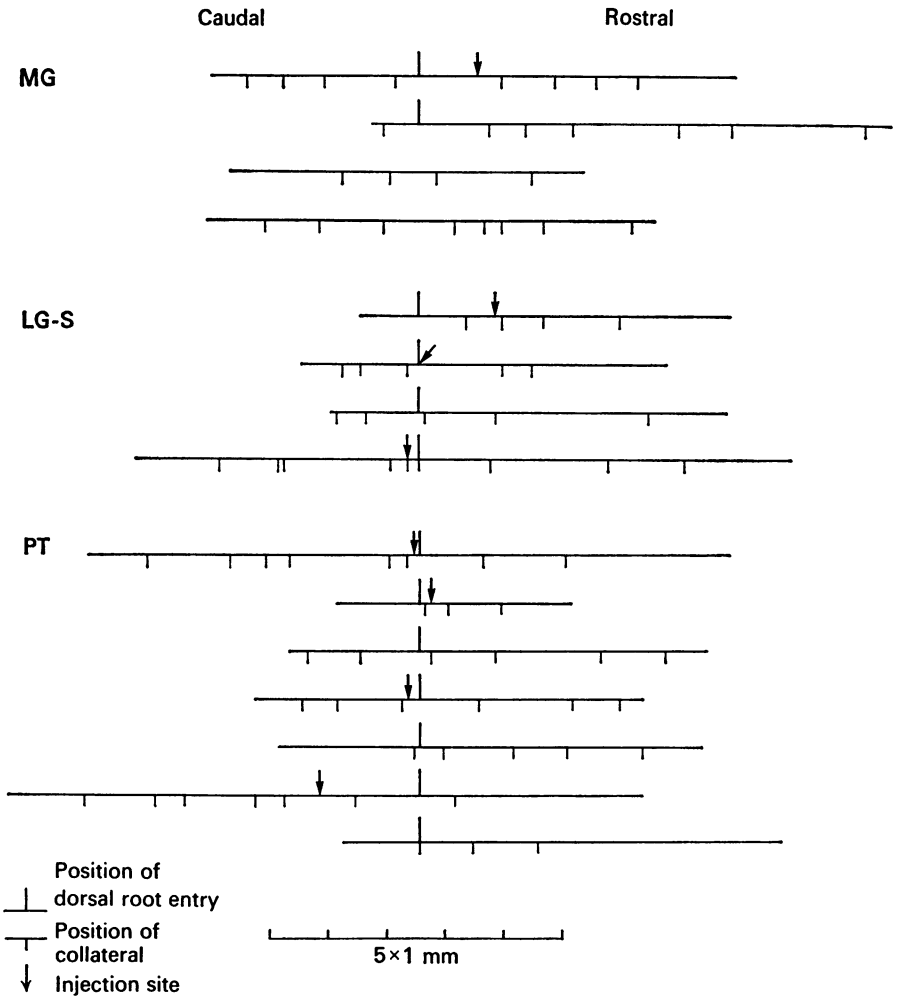
As in our other primary afferent material the most intensely stained collaterals were near the site of HRP injection and only these showed terminal boutons in the motor nuclei and lamina VII. Collaterals further away from the injection site showed the main branching pattern and boutons in lamina VI and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

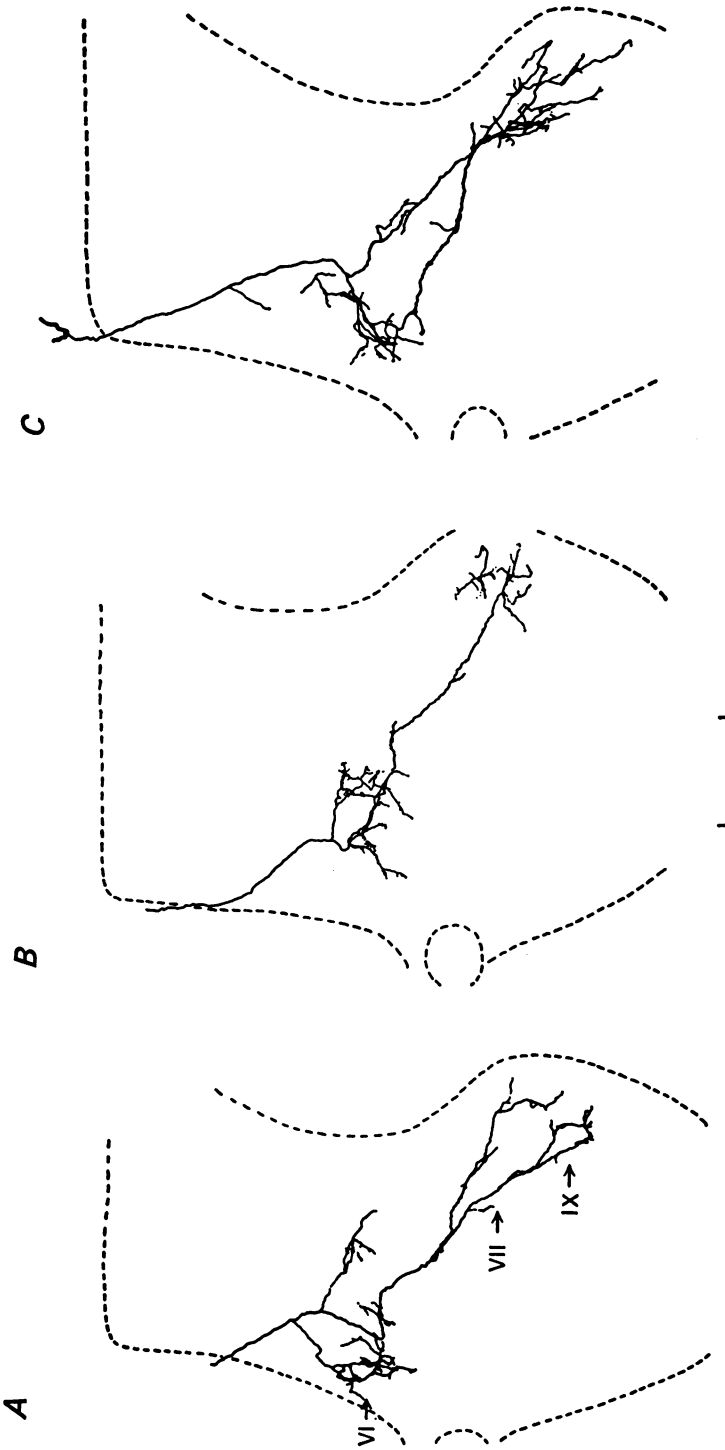
(1040 ± 513 μm; mean ± S.D. n = 73). Therefore, on the average, a collateral arose about every mm along the axon. Examination of Text-fig. 1 reveals that there was no obvious tendency for Ia collaterals to be spaced closer together near the dorsal root entrance of the axon. This is in contrast to the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

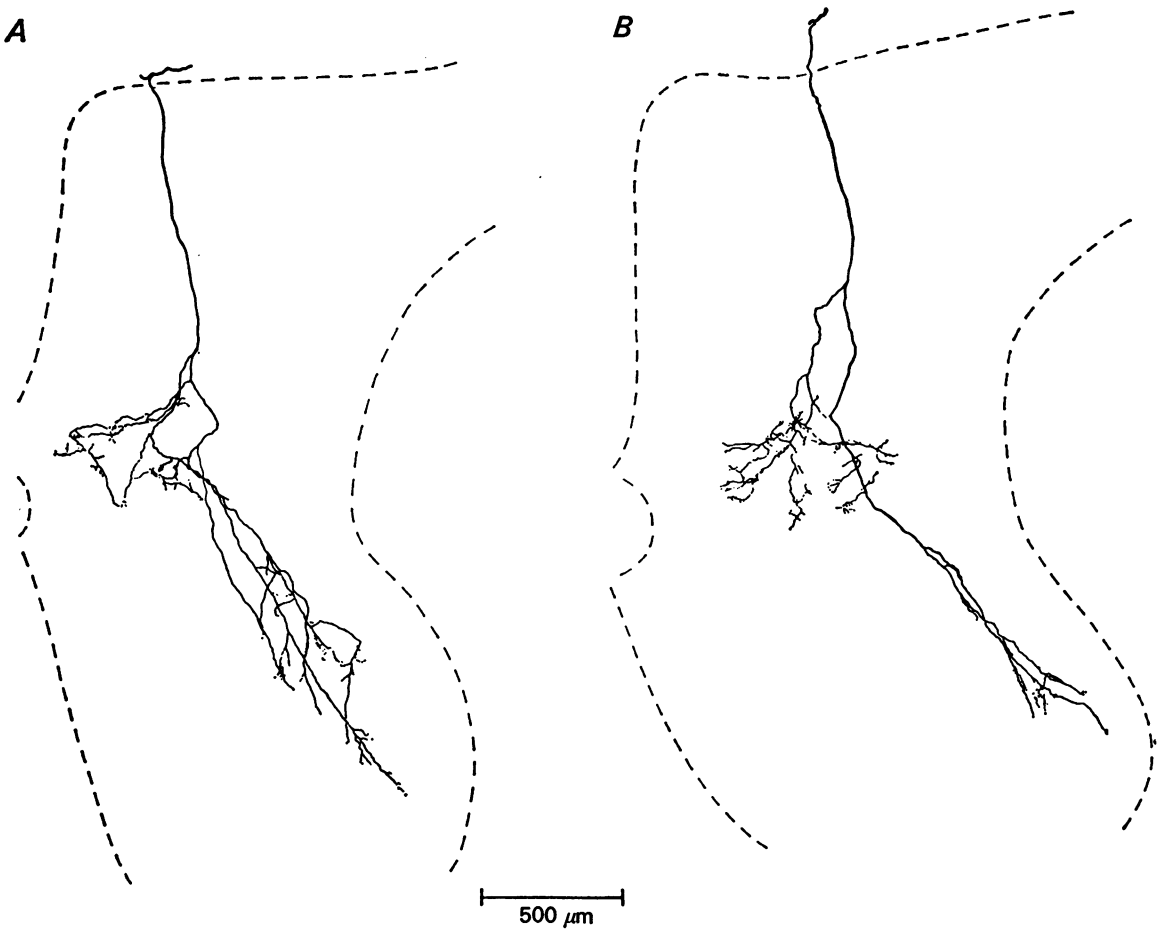
Morphology of Ia collaterals

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*.

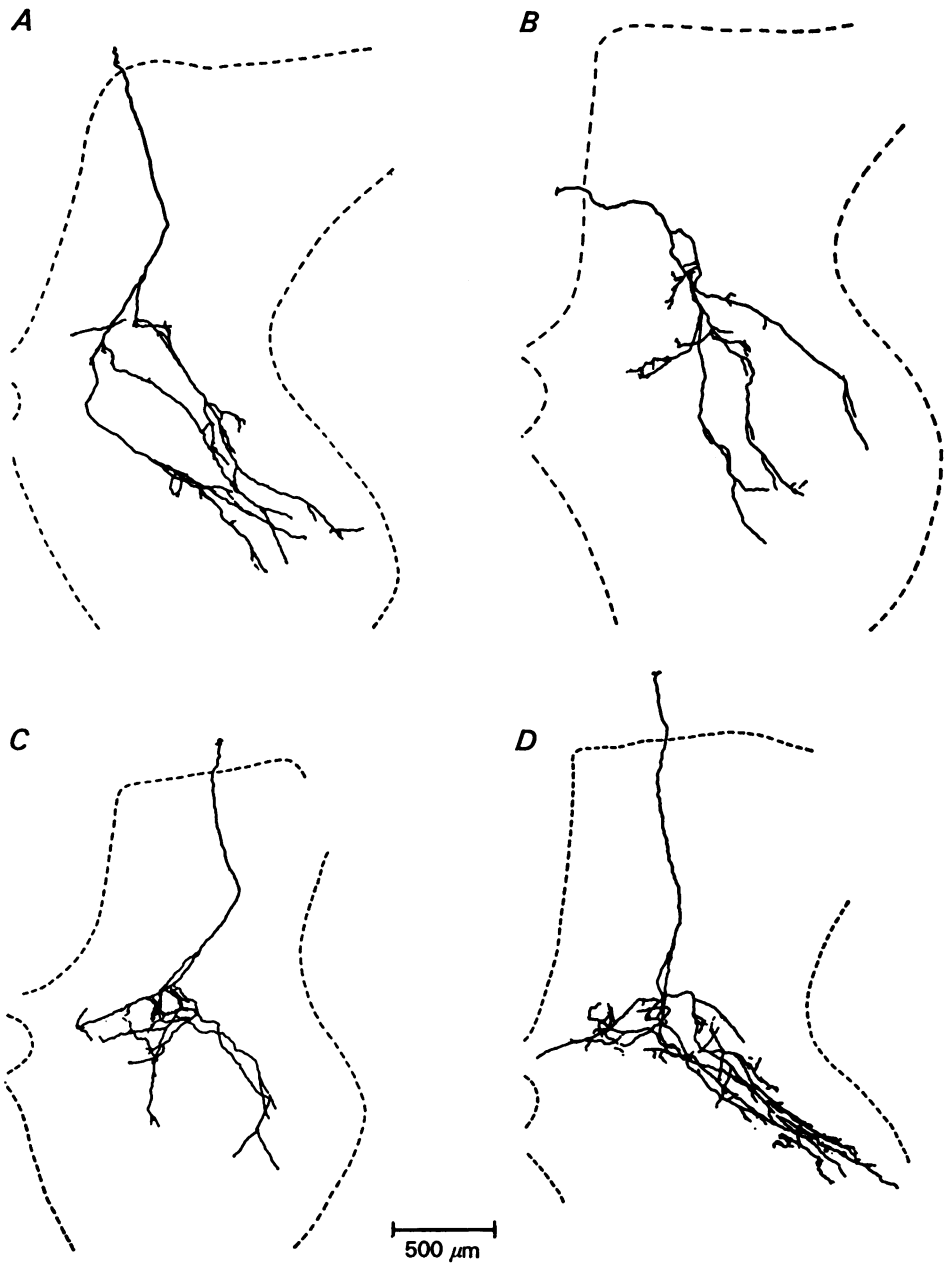
same muscle in different cats. Indeed the similarity extended to collaterals from Ia fibres of muscles in the functional group triceps surae. These similarities are shown in Text-figs. 2–4. Text-fig. 2 consists of reconstructions of three adjacent collaterals from a medial gastrocnemius Ia axon, Text-fig. 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.

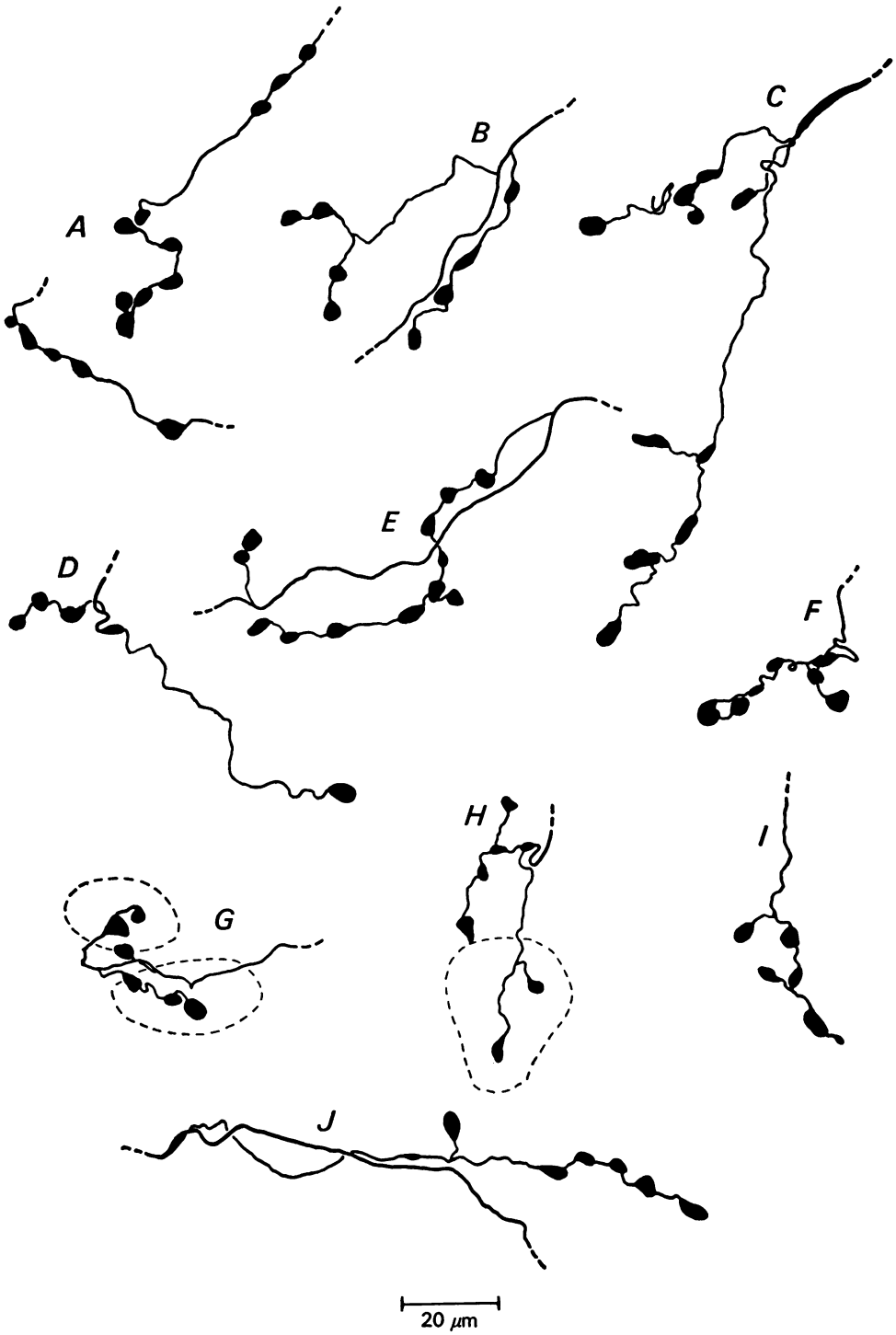
Terminal arborizations and synaptic boutons of Ia collaterals

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.

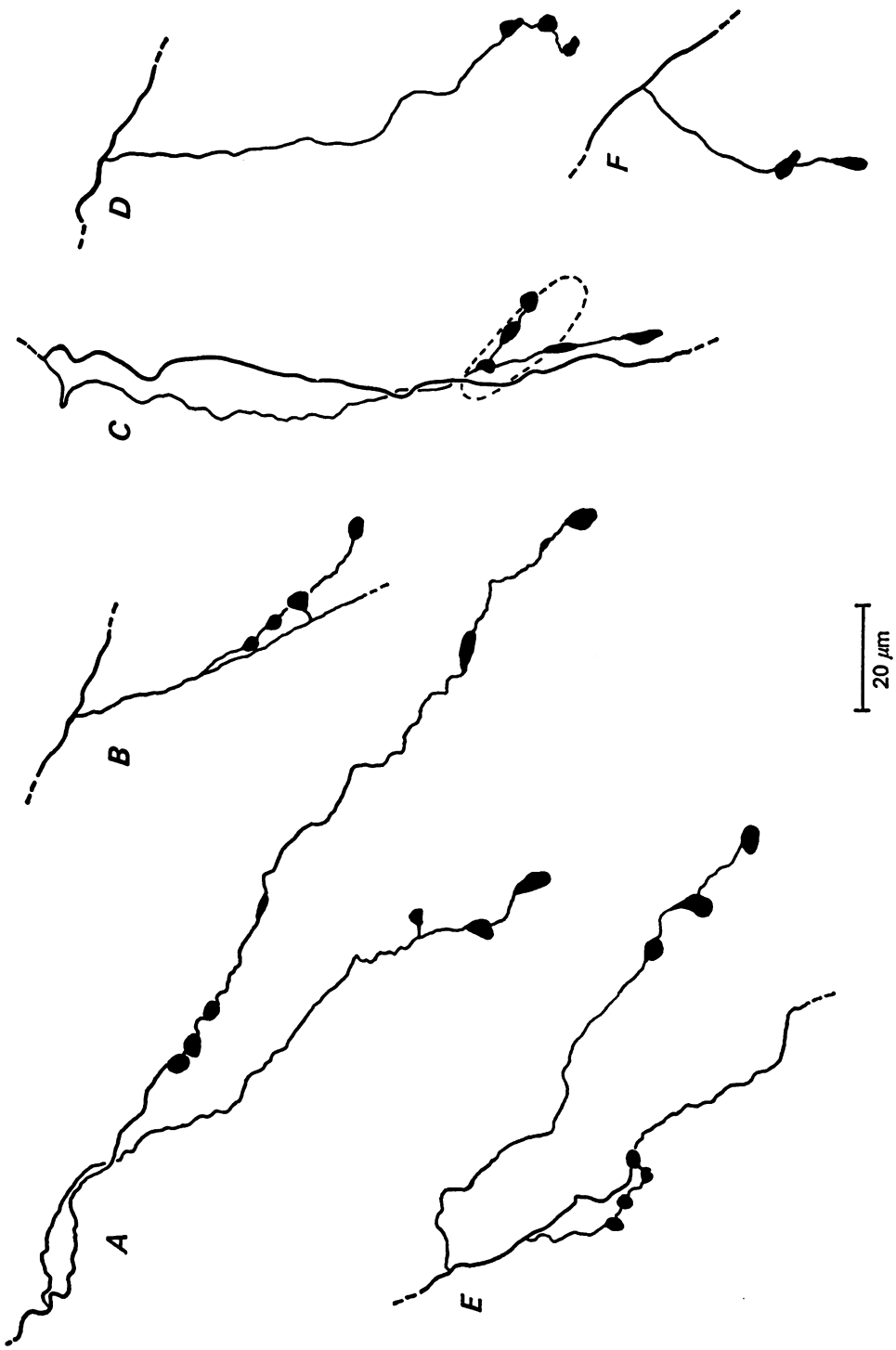
Lamina VI. The terminal arborizations were more complex and the density of synaptic boutons was higher in the intermediate region than in either lamina VII or IX (Text-figs. 5–7, Pl. 2). For axons in which adjacent collaterals were well-stained (e.g. those of Text-figs. 2 and 3) the terminal arborizations 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.

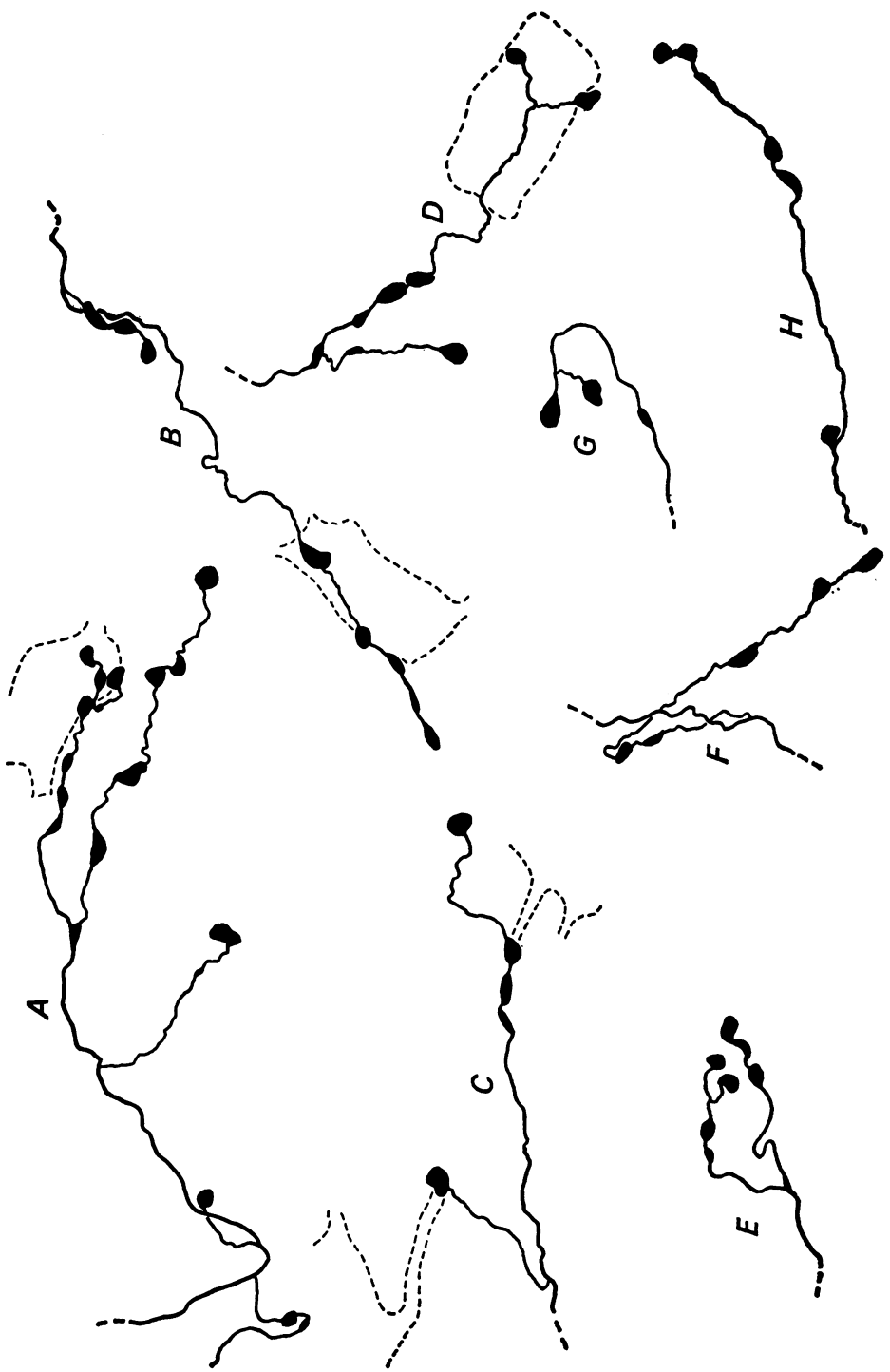
In sections counterstained with cresyl violet these clusters of boutons could be seen to be arranged in relation to the somata and proximal dendrites of lamina VI neurones and were, presumably, making synaptic contact onto these neurones. The outlines of some lamina VI neurones are indicated in Text-fig.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

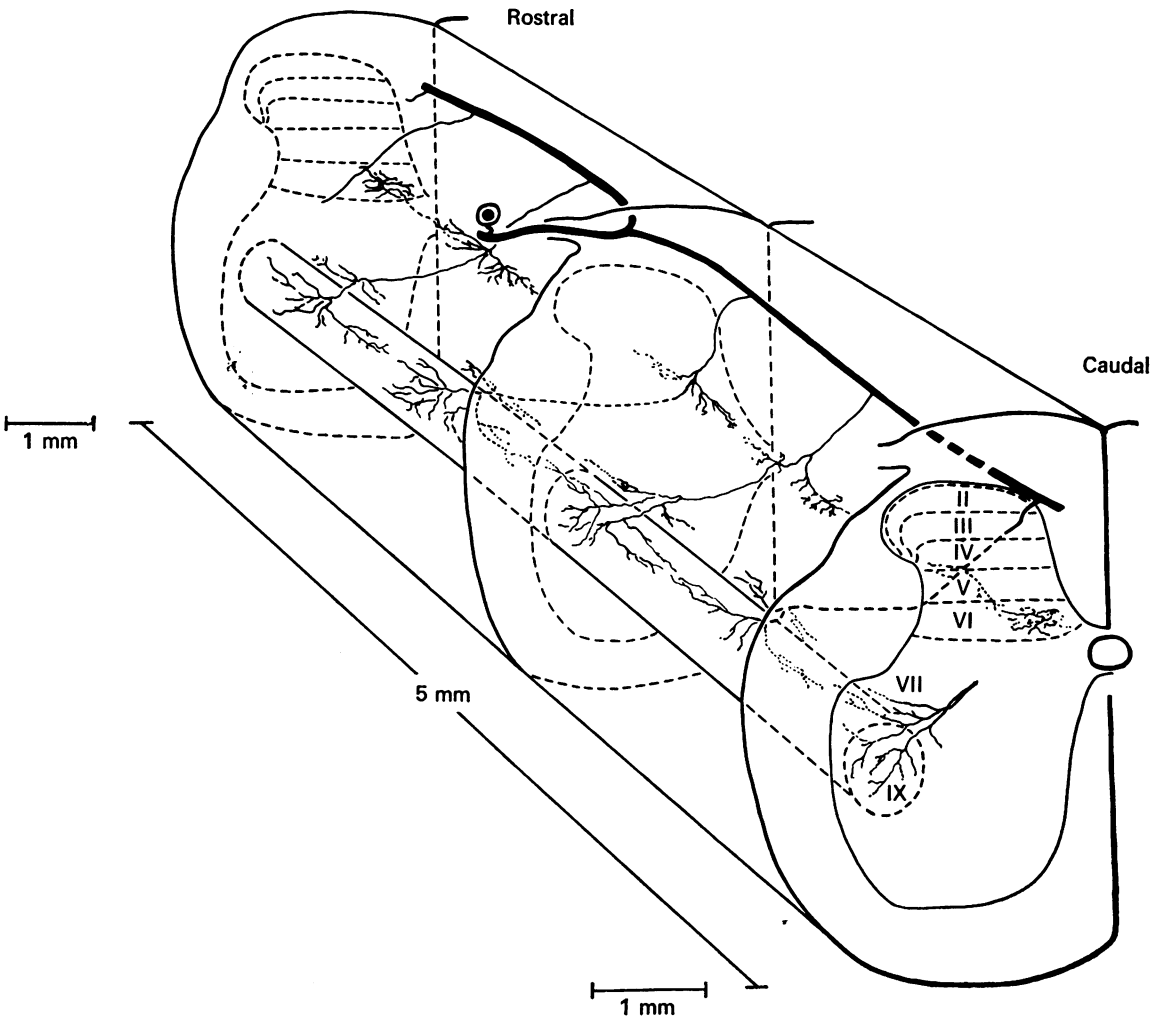
Lamina IX (motor nuclei). Terminal arborizations in the motor nuclei were intermediate in complexity to those in laminae VI and VII. In adjacent well stained collaterals they also formed a relatively uninterrupted column of terminals; each collateral had terminals spread over at least 300–500 μm in the 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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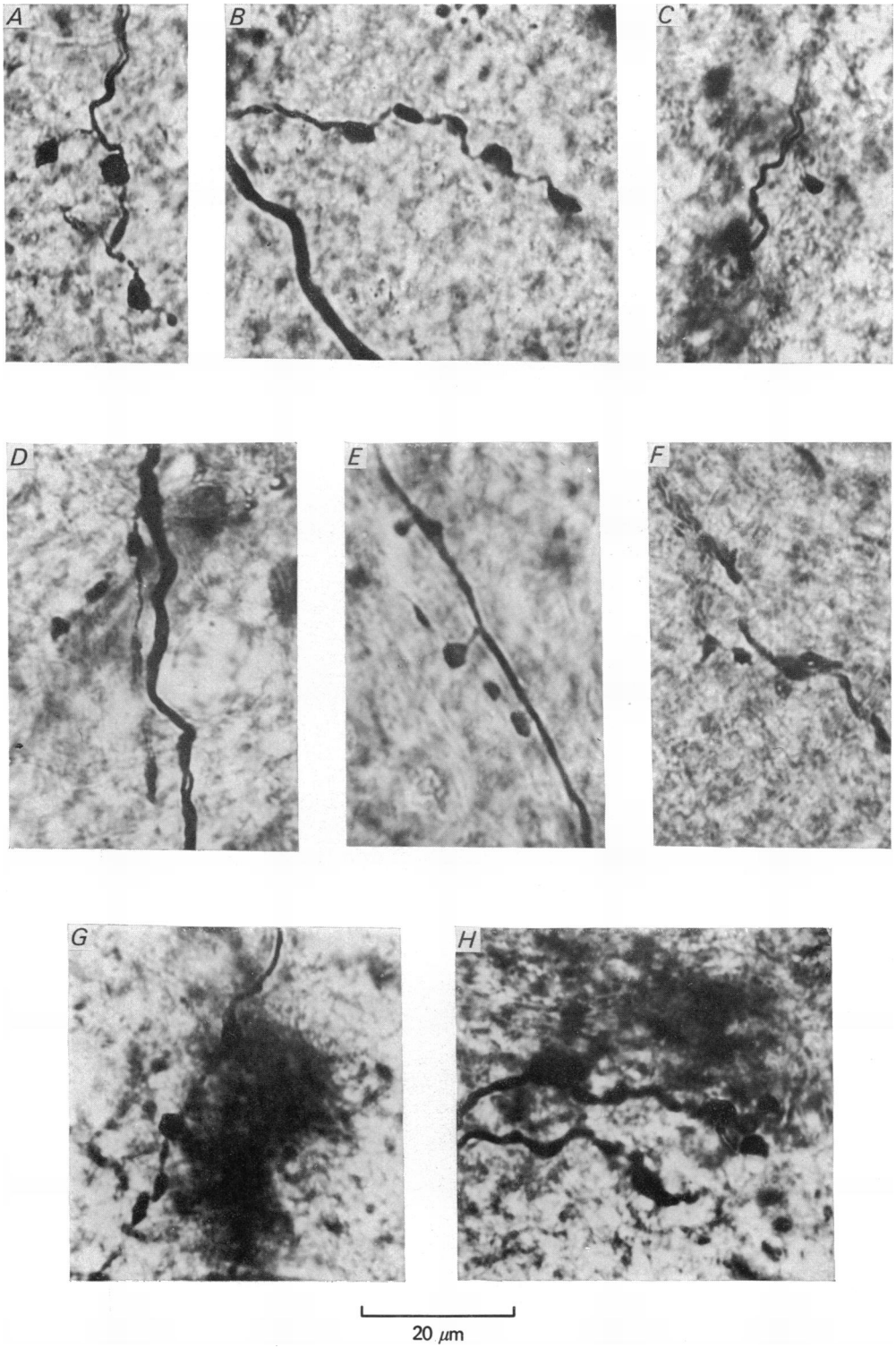
500 μm



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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.