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Long-Range Afferents in the Rat Spinal Cord. II. Arborizations that Penetrate Grey Matter

P. Shortland and P. D. Wall

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Long-range afferents in the rat spinal cord. II. Arborizations that penetrate grey matter

P. SHORTLAND AND P. D. WALL

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, U.K.

SUMMARY

1. The caudal extent of the collateral arborizations of entering sensory fibres in rat spinal cord was investigated by two methods: bulk labelling of peripheral nerves by injection of horseradish peroxidase conjugated to cholera toxin (B-HRP) and by antidromic stimulation using small currents from microelectrodes in the spinal cord while recording from single units in peripheral nerve or dorsal root.

2. The results show that injection of B-HRP into the sural or sciatic nerve labelled sural afferents in the grey matter three to four segments caudal to their root entry and sciatic nerve fibres were located in S4, the most caudal segment examined, four to six segments caudal to their root entry.

3. Detailed mapping with microelectrode stimulation showed that the parent descending fibres from filaments dissected from the L1 dorsal root coursed more than 20 mm, seven to eight segments caudal to the entry point in the dorsal columns and sent branches into the grey matter. Single units from the sural nerve were also followed caudally into the S2 and S3 spinal cord segments and also issued collateral branches into the grey matter.

4. The present results suggest that there is close agreement in the caudal penetration of long-ranging afferents by using complementary anatomical and electrophysiological methods.

1. INTRODUCTION

In a previous paper (Wall & Shortland 1991), we showed that substantial numbers of entering myelinated sensory afferents send descending branches caudally in the spinal cord over surprising distances. Of the caudal branches of dorsal root myelinated fibres, 8% were detected 20 mm (seven segments) caudal to their entry point. Here we re-examine the fine details of the terminals of these long-range afferents because they could be a pool of potential connections which might become effective in conditions of plasticity which are known to occur after peripheral nerve or root damage (Wall 1991). The existence of these long-range afferents is of interest because the anatomical distribution of the long-range afferents is considerably larger than the zone of cells which have been shown to respond monosynaptically to the entering afferents (Brown 1981; Shortland & Fitzgerald 1991). The mismatch between the large anatomical area covered by the afferents and the smaller zone of physiological responses could be explained if the extreme ends of these long-range afferents might be running only in white matter and failing to establish terminal branches in the grey matter. A further reason to determine the precise course of the long-range afferents is that evidence begins to appear that some of these fibres do not normally transmit orthodromic impulses, and that this impulse transmission blockade may be relieved by the degeneration of neighbouring dorsal roots (Wall & McMahon 1991). In this paper, we seek the existence of terminal arborizations of the long-range afferents in grey matter by using more recent transport methods of choleragenoid horseradish peroxidase (B-HRP) transport and by using higher resolution with electrophysiological methods.

One of the most widely used anatomical methods to study afferent terminations in the spinal cord has been the use of horseradish peroxidase (HRP) and its conjugates. There are three ways in which this method has been used. The first is the transganglionic transport of HRP or its conjugates wheat germ agglutinin (WGA-HRP) or B-HRP from peripheral skin region or from injections into single peripheral nerves (Molander & Grant 1985, 1986, 1987; Swett & Woolf 1985; Woolf & Fitzgerald 1986; Mense & Craig 1988; Molander *et al.* 1988; Brown *et al.* 1989; LaMotte *et al.* 1989, 1991; Florence *et al.* 1991; Maslany *et al.* 1991; McMahon & Kett-White 1991; Rivero-Melian & Grant 1991; Woolf *et al.* 1992). Such studies have revealed that each skin area or nerve projects to a precise and limited area of the dorsal horn and that the rostrocaudal extent of that area is dependent on the tracer used (tables 1 and 2). Under certain conditions of denervation, intact peripheral nerves or dorsal roots have been shown to have an increased rostrocaudal projection on the denervated side as compared with the control contralateral side (Fitzgerald 1985; Molander *et al.* 1988; LaMotte *et al.* 1989; McMahon & Kett-White 1991).

The second method is that of injection of HRP and

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its conjugates into individual dorsal root ganglia (Robertson & Grant 1985; Pfaller & Arvidsson 1988; Rivero-Melian & Grant 1988, 1990*a*,*b*; Neuhuber & Zenker 1989; Arvidsson & Pfaller 1990). Labelling of cervical dorsal root ganglia shows long caudal projections ending in lower thoracic segments (Pfaller & Arvidsson 1988; Neuhuber & Zenker 1989; Arvidsson & Pfaller 1990), whereas lumbar dorsal root ganglia send branches into sacral segments (Rivero-Melian & Grant 1990*a*).

The final method of application of HRP for labelling fibre terminals is that of intracellular or intra-axonal injecton into physiologically characterized afferents or dorsal root ganglion cells (Brown 1981; Semba *et al.* 1983, 1984, 1985; Meyers & Snow 1984; Hongo *et al.* 1987; Woolf 1987; Brown *et al.* 1991; Culberson *et al.* 1988; Kierstead & Rose 1988; Snow & Wilson 1989; Sugiura *et al.* 1988; Ritz *et al.* 1989, 1991; Shortland *et al.* 1989*a*, *b*; Hoheisel *et al.* 1989; Fitzgerald *et al.* 1990; Woolf *et al.* 1992). Unfortunately, although spectacular morphological displays of the arborizations are observed, the rostral and caudal range within which terminal arborizations occur is relatively short. The distance of axon and terminal fields observed was also dependent on the type of animal used, the class of afferent injected, and whether the injection occurred within an intact or denervated animal (Brown 1981; Sugiura *et al.* 1988; Snow & Wilson 1989; Fitzgerald *et al.* 1990; table 3).

An alternative and independent method for mapping the location and distribution of fine branches of single axons is provided by microelectrode mapping (Wall & Werman 1976; McMahon & Wall 1985*a*,*b*). It is possible to stimulate unmyelinated axons in the central nervous system (CNS) by passing current through a nearby microelectrode. The antidromic action potential provoked by this stimulus can be recorded on a strand of peripheral nerve or root containing a single active fibre. By moving the stimulating microelectrode and seeking the point of lowest threshold to evoke the antidromic action potential, the location of the central axon can be determined within 25 μm (McMahon & Wall 1985*b*).

The previous evidence suggested there was some mismatch between the extent of long-range afferents as shown by anatomical against physiological methods. In the present study, we have addressed the problem of how far caudal terminals from the sural or sciatic nerve can be located in the spinal grey matter using anatomical tracing techniques. We compare this with results obtained using electrophysiological methods from filaments innervating the same dorsal root ganglion or peripheral nerve.

2. METHODS

(*a*) *HRP transport*

Male Sprague-Dawley rats (200–260 g) were anaesthetized with fentanyl (0.3 mg per kilogram, intramuscular) and diazepam (5 mg per kilogram, intraperitoneal). Under sterile conditions, the sural or sciatic nerve was exposed for intraneural injection of 0.1–0.5 μl of 1.5% dissolved in distilled water B-HRP (List laboratories) using fine glass microelectrodes. A little hole was made in the nerve sheath, and the electrode (external tip diameter 30–60 μm) was inserted. The B-HRP was delivered via pressure injection. The microelectrode was withdrawn and the injection site ligated with 5-0 Mersilk to prevent leakage out of the nerve and distal to the injection site. The musculature and skin were then sutured, and the animal was allowed to recover. After a period of 72 h, the animal as again anaesthetized and perfused with a standard HRP perfusion medium (Hanker *et al.* 1977).

After the perfusion of nerve-labelled animals, segments L4–S4 were identified and pinned. Segments L4 and L5 were sectioned transversely at 60 μm saving every other section, and segments L6–S4 were serially sectioned transversely at 60 μm. All sections were stained by using the TMB reaction procedure (Mesulam *et al.* 1978) for visualization of HRP reaction product. Sections were viewed under dark field and the distribution of the HRP reaction product drawn with the aid of a camera lucida. Laminae borders were defined according to Molander *et al.* (1984). Photographs of the resolution we achieved have been published (Woolf *et al.* 1992).

(*b*) *Microelectrode mapping*

All experiments were done on male Sprague Dawley rats ranging from 200 g to 300 g in mass and deeply anaesthetized with intraperitoneal urethane (1.5 g per kilogram). The carotid artery and trachea were cannulated. Temperature, ECG and expired CO_2 were monitored throughout the experiment which continued only while these and the observed circulation on the surface of the exposed cord were in the normal range. Fine strands were dissected either from dorsal roots, L1 or T13, or from the sural nerve in the popliteal fossa and placed on a recording fine silver hook. Recordings were made with respect to a pin in nearby muscle through conventional amplifying circuits with their filters set to admit frequencies from 1 kHz to 15 kHz. When an antidromic spike was recorded from an axon following stimulation in the spinal cord, its shape was retained on a memory trace so that it could be compared throughout the remainder of the stimulus search with the evoked spike to be certain that the same unit was observed. The presence of a single unit was confirmed by observing the all-or-none characteristic of the spike shape as the stimulus intensity was raised. The existence of a continuous axon between the stimulus and recording site was confirmed by showing that the refractory period for two stimuli at twice threshold was always below 1.5 ms.

The spinal cord was transected at T10 to prevent the exposed cord being flooded with descending cerebro-spinal fluid (CSF) and to isolate the cord from descending effects. An extensive laminectomy exposed the cord from the lower thoracic to the caudal sacral segments, and this area was covered with warm paraffin oil. The stimulating microelectrodes were glass-covered tungsten with 25 μm exposed tip of the

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Merrill-Ainsworth type (1972) held in a three-dimensional micromanipulator. The stimulus was tip negative with respect to a pin in nearby muscle. The current strength used was up to 100 μA, 200 μs duration delivered at 5 Hz or 50 Hz. The routine for localization of the central course of the axon used the following programme. The presence of a long-range descending branch of an entering axon was initially detected by stimulating on the surface of dorsal columns with a 0.5 mm silver ball negative electrode. Then the exact location of the axon at that start point was determined by stimulating with a microelectrode in a series of mediolateral tracks separated by 100 μm. At 50 μm intervals in the dorsoventral direction of each track, the threshold for stimulating the fibre was determined. The depth of dorsoventral penetration was 2 mm. The minimal threshold points, always less than 10 μA, were taken as the axon's location. Having located the axon in one cross section of the cord, the procedure was repeated with the next transverse grid 100 μm caudally in the fine search and 250 μm or 500 μm caudally in the course search. The co-ordinates of each stimulus point were measured with respect to the surface, the midline and the root entry. Key grids were marked with a pin on the contralateral side. At the end of the experiment, the cord was fixed in formol saline and transverse sections cut at 50 μm thickness to allow an outline of the cord and grey matter to be drawn for each stimulated transverse grid. Once the parent axon had been located in the initial transverse map, the subsequent more caudal maps could be done much more rapidly. It was only necessary to search for the lowest threshold point in the limited area which was known to contain the axon in the more rostral search plane. Branch points were obvious because, instead of detecting a single point of lowest threshold, two low-threshold points became apparent. When this was observed, mapping with high resolution in three dimensions was done to record the location of clearly separate branches to be certain that the serpentine coure of a single axon was not the cause of the appearance of branches.

3. RESULTS

(*a*) *Injection of B-HRP into peripheral nerves*

We first examined spinal cord dorsal horns for signs of spread of the injection from the sural nerve territory to the sciatic nerve territory. Those signs were the labelling of large numbers of motorneurons if the sciatic was involved (LaMotte *et al.* 1991), as against the small numbers if the injection was restricted to the sural (Swett *et al.* 1986), and a wide mediolateral spread of label across the dorsal horn if the sciatic was injected.

In two animals where only the sural nerve was labelled, label extended from L4 to S1 with a sparser projection to S2 (figure 1). The details of the laminar arrangement of sural afferent terminals has been described elsewhere (Woolf *et al.* 1992). The description here will be confined to the more caudal regions. In rostral L6 terminal staining in the dorsal horn is

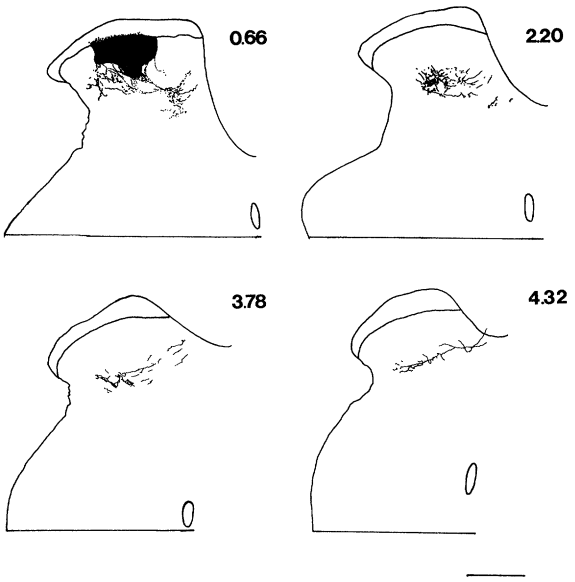

Figure 1. Transverse sections of spinal cord showing the dorsal horn termination sites of sural nerve afferents retrogradely labelled with B-HRP. Numbers indicate the distance in millimetres of label caudal to the L5–6 segmental boundary. Solid line in the dorsal horn indicates the laminae II–III border. Scale bar 250 μm. The upper sections are in L6, the lower left is in S1 and the lower right is in S2.

seen in laminae IIi–V, similar to L5. More caudally terminal staining was restricted to deeper laminae, being present only in laminae III-IV and restricted to laminae IV in S1. The terminals are located in the reticulated region of the dorsal horn. In these caudal transverse sections, stem axons could not be seen travelling in the dorsal columns. In S2, penetrating collaterals became sparser, adjacent collaterals being separated by distances of 200–400 μm. Very few collaterals reached S3. In the two animals studied, penetrating collaterals into the grey matter were observed 4.32 mm and 4.98 mm caudal to the L5–6 segmental boundary. The extreme caudal arborizations had a relatively simple branching pattern (figure 1) but showed terminal-like swellings which is taken at the light microscopic level as evidence for boutons terminaux (Cruz *et al.* 1987, 1991). There are two possible reasons for the more ventral location of the terminals in more caudal segments. One could be that they are displaced ventrally by afferents arriving over roots which supply the caudal segments (Ritz *et al.* 1989). The other is that the simple restricted arborizations which we observed caudally do not extend dorsally in the manner of the more complex arbors which are observed in segments close to the dorsal root over which the fibres enter (Woolf *et al.* 1992).

When the sciatic nerve was labelled with B-HRP in two animals, descending collateral fibres were seen as far caudal as S4, the most caudal segment examined, and a dense network of stem axons could be clearly seen in the dorsal columns in these far caudal segments. The laminar location of the more caudal regions of the sciatic nerve territory will be described here because the more rostral terminations in the

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lumbar enlargement have been described elsewhere (LaMotte *et al.* 1991; Woolf *et al.* 1992). In rostral L6, label was found in laminae I, IIi-V and the motorneuron pool, but by caudal L6 label was restricted to laminae III-IV in the dorsal horn. The occasional contralateral projection was seen at this level but they became more numerous in sacral segments. In S1 and S2 the distribution of terminals moved medially towards the midline region and was confined to laminae IV–V. Motorneurons were present as far caudal as rostral S1. The number of penetrating collaterals decreased caudally so that in S3 and S4 individual collaterals could be identified descending from the stem axon in the dorsal column. In these regions collaterals terminated near the midline region dorsal to the central canal. For the two animals studied, penetrating collaterals (and descending parent axons) were observed for distances of 6.90 µm (figure 2) and 9.12 mm caudal to the L5–6 boundary and probably extend well beyond this region into more caudal regions.

(*b*) *Electrophysiological mapping of the location of descending axons and their terminal branches*

In figure 3, four examples are shown of the location of branches of axons from the sural nerve which had entered the cord in the rostral part of the L5 dorsal root and which descended into the sacral segments. The percentage of fibres which descend is shown in Wall & Shortland (1991). Single axons were first recorded in filaments of the sural nerve which penetrated as far as the L6–S1 border, and then their subsequent caudal course was followed in detail. These four are representative examples of fibres whose descending path was mapped in detail in 20 animals. Transverse grids of stimulus points were made at 20 µm or 500 µm caudal intervals. The fibre was located as the point of lowest threshold which was always well below 10 µA for a 200 µs duration stimulus. The same single unit was recorded in the sural nerve throughout the mapping as it had an all-or-none shape which remained the same during the session where each antidromic spike matched the spike which had been recorded at the beginning and which was held on a memory trace. All fibres had a conduction velocity characteristic of myelinated fibres in the peripheral nerve (Wall & Shortland 1991). All fibres were uninterrupted descending branches of the peripheral axon as there was no variation of the conduction time, and the refractory period from any one stimulus point for two stimuli at two times threshold was less than 1.5 ms.

The mapping of each descending branch began in the L6 segment where they were found to lie superficially in the dorsal columns. At successive caudal transverse maps, the descending main branch remained in the dorsal columns and moved medially. It was frequently not possible to follow the extreme caudal reach of these fibres because they moved medially to a position ventral to the midline dorsal vein where it was not possible to probe with the penetrating stimulating microelectrode. At intervals

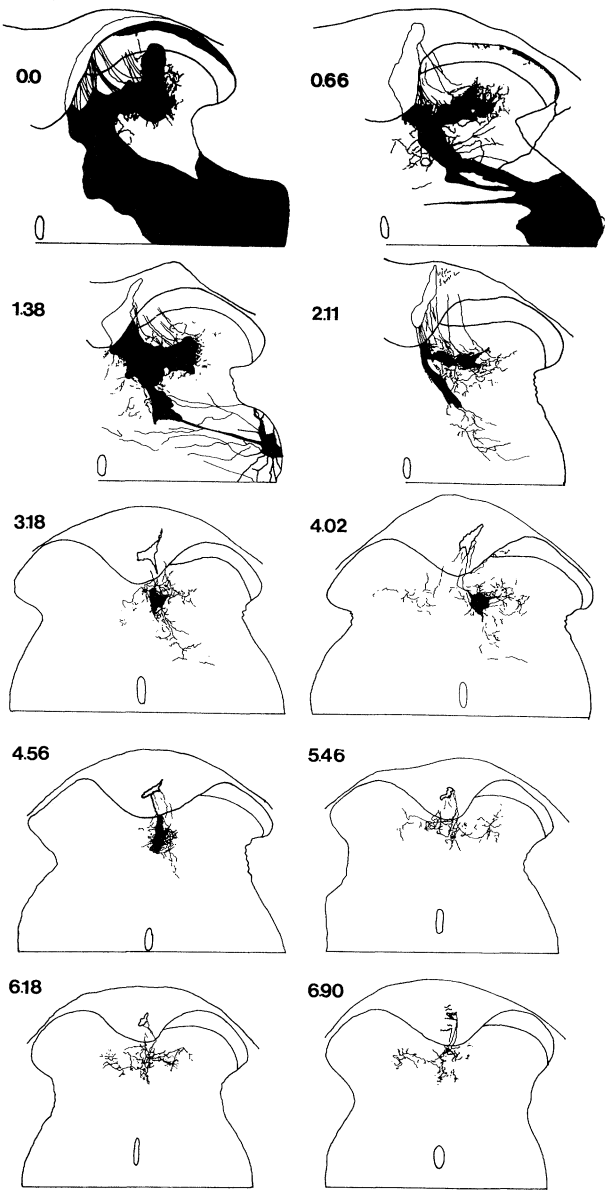

Figure 2. Transverse sections of spinal cord showing the grey matter termination sites of sciatic nerve afferents retrogradely labelled with B-HRP. Numbers indicate the distance in millimetres of label caudal to the L5–6 border. Only regions dorsl to the central canal are shown. The solid line in the dorsal horn represents the laminae II–III border. Solid shading in the grey matter represents areas of dense label, and open shading in the dorsal columns represents caudally travelling stem axons. Scale bar 250 µm. The upper pair of sections are in L6 and the successive pairs of sections are in S1, S2, S3 and S4.

along the course of the main descending axon in the dorsal columns, branches were detected which penetrated ventrally for short distances into the grey matter. As transverse maps were made at 250 µm or 500 µm intervals, it is likely that some of these penetrating branches of the descending parent axon were missed.

To determine the location of these penetrating branches in more detail, the experiment was modified in two ways for ten fibres. The recording site was

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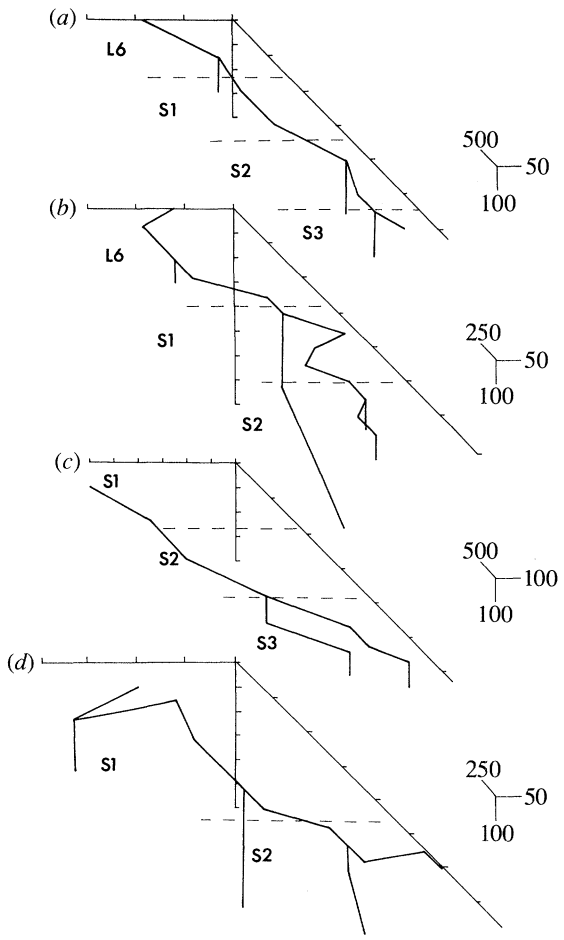

Figure 3. The course of descending branches of four myelinated sensory fibres originating in the sural nerve. The single unit was recorded on a strand dissected from the sural nerve. The recorded axons entered the cord over the rostral part of the L5 dorsal root. The location of the descending branch of the axon was determined beginning in the L6 segment by making a transverse grid of stimulus points with a tungsten microelectrode. The grid was made with tracks separated by 100 μm mediolaterally with the stimulus delivered every 50 μm in a dorsal ventral direction. The axon was located as the lowest threshold point in the grid which was always less than 10 μA at 200 μs duration. The search grids were repeated at 50 μm or 100 μm intervals. The coordinate scale is shown in three dimensions. It should be noted that the scale varies for each of the fibres. The slanting line was the midline of the spinal cord. Each axon is shown to descend in the dorsal columns and to send intermittant branches which penetrate the grey matter.

moved from the sural nerve to single myelinated fibres in the L1 dorsal root in ten animals. This allowed us to study the location of terminal branches further caudally to their entry point than was possible for the sural axons which enter over L4 and the L5 dorsal roots. The location of these fibres was first measured 20 mm caudal to their entry point. These fibres from the L1 dorsal root were examined in the S1 and S2 segments 20–25 mm caudal to their entry point. To achieve a finer detail of localization, the search grid interval was reduced to 50 μm in the dorsoventral, mediolateral and rostrocaudal directions. This finer grade search is illustrated for three such fibres in figure 4.

In figure 4*a*, the descending branch in the dorsal columns ended in segment S2 because a more caudal search failed to reveal any sign of this axon. However, immediately rostral to this point two branches were detected which penetrated grey matter, one entering lamina VI and the other lamina X. Similar penetrating branches were seen in all ten fibres, and two examples are shown in figure 4*b*,*c*. There is no reason to believe that this technique revealed the final terminals of these penetrating branches as they were tapering as judged by the rapid decrease of their conduction velocity (Wall & Werman 1976), and therefore their threshold was likely to rise beyond the maximal limit (10 μA) we had set for stimulus intensity.

4. DISCUSSION

Our first aim was to compare the caudal extent of long-range descending branches of sensory afferents entering the spinal cord as revealed by various techniques. Transport methods will necessarily be limited by four factors: the amount injected, the time available for transport, the efficiency of transport, and the sensitivity of the detection method. In the case of HRP injection of single afferent axons introduced by Snow & Brown (1976), which reveals such beautiful detail of nearby central terminals, there will be only a small amount injected into the single axons and the transport time is limited. It is therefore not too surprising that this technique reveals only a limited caudal spread (table 3). It should also be noted that a small proportion of primary afferents do not have caudal projecting branches (Brown 1981; Woolf 1987). Comparison of injection of different markers (HRP, WGA-HRP and B-HRP) into peripheral nerves or dorsal root ganglion shows that the rostrocaudal spread depends on the technique used (LaMotte *et al.*, 1991; Maslany *et al.*, 1991; table 1). These various markers are transported by different classes of fibres; WGA-HRP is preferentially transported in small-diameter fibres, and B-HRP is transported by large myelinated fibres (Robertson & Grant 1985; LaMotte *et al.* 1991) with little overlap between the two populations (Robertson *et al.* 1991), whereas free HRP is transported by both classes. Also, WGA-HRP and B-HRP bind to receptors on the afferent membranes and can be internalized at a greater rate (Gonatas *et al.* 1980) and are transported at different rates to the central terminals. It is also acknowledged that in most PNS neurons B-HRP appears to be a more sensitive retrograde, anterograde and transganglionic transported substance than free HRP (Trojanowski 1982; Wan *et al.* 1982) and this appears to be true for labelling the central terminals of cutaneous afferents in the spinal cord (LaMotte *et al.*, 1991; Maslany *et al.* 1991). Whereas it is inevitable that the sensitivity of the enzyme detection method will limit the ability to detect low levels of transported material in distant tapering axons, it is equally clear that choice of transport marker and hence class of fibre labelled is also important. It is therefore not surprising that some

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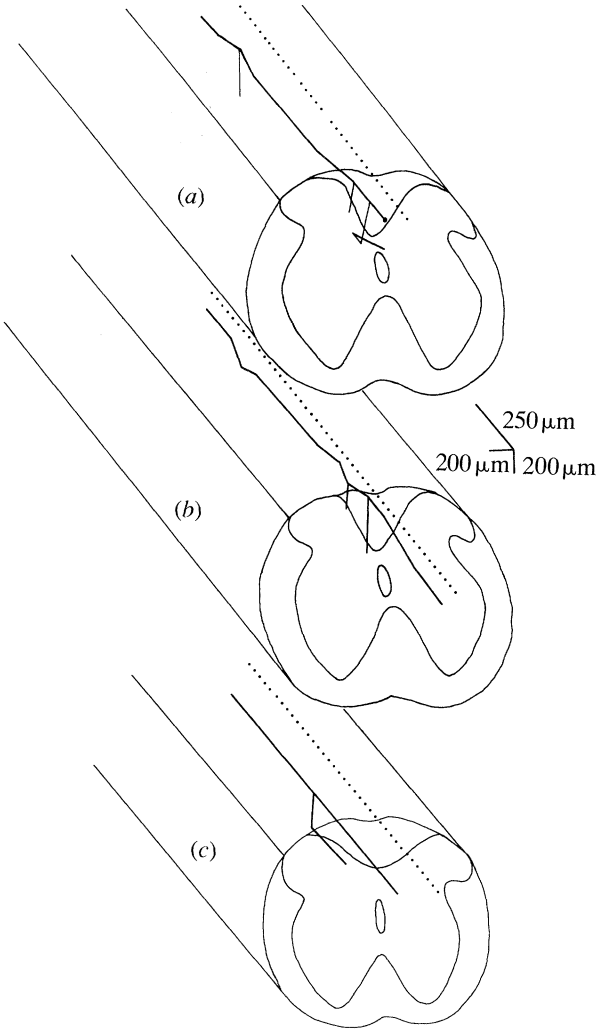

Figure 4. The course of three descending axons originating from myelinated axons in the L1 dorsal roots. The location of these fibres was detected by the method used in figure 3, but the search grids were on a finer scale in which the stimulus points were separated by 50 μm in all three dimensions. Each axon is shown more than 20 mm caudal to its entry point in segments S1 and S2. The parent descending axon lies in the dorsal columns and sends penetrating side branches into the grey matter. (*a*) The round point on the end of the parent axon in the dorsal columns marks the termination of this fibre as it could not be stimulated in more caudal search grids. (*b*) and (*c*) The fibres continued more caudally in the dorsal columns beyond the area which was searched in detail.

transport methods do not necessarily reveal the full extent of terminal aborizations.

Bulk labelling of peripheral nerves with HRP and its conjugates has shown that each nerve has a somatotopically precise termination area, the rostrocaudal extent of which depends on the tracer. For example, when the sural nerve is labelled with WGA-HRP, labelling is seen as far caudal as caudal L5 (Swett & Woolf 1985) and rostral L6 when free HRP is used (Woolf & Fitzgerald 1986; table 1). The present results reveal penetrating axons and terminals as far as S2 so that, at least in the case of peripheral nerve projections, the anatomy matches the antidromic mapping when the appropriate tracer is used. The nature of the receptive class of these caudal penetrating afferents remains to be determined. They may be cutaneous, muscle or subcutaneous in nature. The sural nerve in the rat is mainly cutaneous but it does contain some muscle afferents (Swett *et al.* 1986) which project to the deeper laminae (IV–VI) of the dorsal horn (Shortland & Woolf, personal observation). The rostrocaudal extent of fibres from deep tissue is greater than that of cutaneous fibres (Brown 1981; Mense & Craig 1988). Skin injections which label only a few cutaneous afferents show a more limited rostrocaudal projection (Molander & Grant 1985; P. Shortland, unpublished observations) than those observed after whole-nerve injection. It is therefore possible that the long-range afferents are of deep origin but we await their physiological identification.

Similarly, our results, and those of LaMotte *et al.* (1991), find that B-HRP shows much more caudal penetration of sciatic afferents than other tracers (table 1). We observed grey matter penetrations in the dorsal horn in S4 with clear indications that they proceed even further caudally. These caudal collaterals are unlikely to be low-threshold cutaneous mechanoreceptors as their morphology does not resemble that reported for cutaneous primary afferents (Woolf 1987; Shortland *et al.* 1989) and as judged by the large number of contralateral projections. Cutaneous and muscle afferents from the tail region are known to terminate in this region (Ritz & Bailey 1989; Ritz *et al.* 1991) as do pudendal nerves (McLachan & Janig 1983). This again raises the possibility that these caudal fibres are deep, subcutaneous or articular fibres rather than cutaneous afferents, assuming B-HRP labels these populations equally. Alternatively, it is possible that as caudal collaterals terminate in a region that is not normally their main central target area they assume the appropriate morphology for collaterals of that region. This is known to occur when sprouting hair follicle afferents enter denervated regions of spinal cord that would normally contain glabrous skin terminals and vice versa (Fitzgerald *et al.* 1990). The terminals of these long-range afferents appear to accumulate tracer in end knob-like swellings and it is therefore highly unlikely that we were observing transynaptic spread of the tracer and this is supported by the negative evidence that B-HRP moves across synapses (Robertson & Grant 1985). No signs of label were apparent in cell bodies.

Bulk labelling of the L1 dorsal root ganglion (DRG) with B-HRP shows a dense projection to the L1 and L2 dorsal horn with a sparser projection to laminae III–IV of segments L3–L5 (Rivero-Melian & Grant 1990*a*), a distance of greater than 12 mm caudal to the root entry zone. The L1 DRG contains axons from the lateral and anterior femoral cutaneous (LFC and AFC) nerves and possibly the saphenous nerve (Rivero-Melian & Grant 1991). The saphenous nerve is known to have a split terminal field to the L2–3 and caudal L5 dorsal horn (Swett & Woolf 1985; Woolf & Fitzgerald 1986; Rivero-Melian & Grant 1991; LaMotte *et al.* 1991), and the LFC projects as far caudal as L6 (Rivero-Melian & Grant 1991) as

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Table 1. *Caudal projections of primary afferents from sural and sciatic nerves into rat spinal cord following the injection of various markers*

		lumbar		sacral				
nerve	marker	5	6	1	2	3	4	reference
sural	WGA[a]	→						Swett & Woolf (1985)
	WGA[a]	→						Molander & Grant (1986)
	F[b]	→	→ → →					Woolf & Fitzgerald (1986)
	B[c]	→	→ → → → → → → → → →					this paper
sciatic	WGA[a]	→	→					LaMotte *et al.* (1991)
	B[c]	→	→ → → → → → → → → →					LaMotte *et al.* (1991)
	B[c]	→	→ → → → → → → → → → → → → → → → → →					this paper
	F[b]	→	→ → → → → →					Woolf & Fitzgerald (1986)
	WGA[a]	→	→					Swett & Woolf (1985)

[a] WGA, wheatgerm agglutinin conjugated to HRP.
[b] F, free unconjugated HRP.
[c] B, choleragenoid conjugated to HRP.

Table 2. *Summary of rostrocaudal projections of primary afferents using transganglionic HRP labelling methods in the spinal cora*

animal	area studied	rostrocaudal extent	marker	reference
rat	saphenous neuron	T12–L5	B-HRP	Rivero-Melian & Grant (1991)
	anterior femoral cutaneous neuron	T11–L6		
	lateral femoral cutaneous neuron	T11–L6		
rat	saphenous neuron	L2–L5	WGA-HRP	Swett & Woolf (1985)
	sural neuron	L3–L5		
	tibial neuron	L3–L5		
	superficial peroneal neuron	L4–L5		
	posterior cutaneous neuron	L4–L6		
rat	saphenous neuron	L2–L4	Free HRP	Woolf & Fitzgerald (1986)
	sural neuron	L3–L6		
	tibial neuron	L2–L4		
rat	tibial neuron	L3–L6	WGA-HRP	Molander & Grant (1986)
	medial Plantar neuron	L4–L5		
	lateral plantar neuron	L4–L5		
	saphenous neuron	L2–L4		
	superficial peroneal neuron	L4–L6		
	sural neuron	L4–L6		
	lateral femoral cutaneous neuron	L2–L4		
	obturator	L2–L5		
rat	gastrocnemius neuron	T10–L5	Free HRP/	Molander & Grant (1987)
	deep peroneal neuron	T11–L5	WGA-HRP	
	femoral neuron	T11–S2		
	hamstring n.	T13–L5		
rat	sciatic n.	T8–S2	B-HRP	LaMotte *et al.* (1991)
		L2–L5	WGA-HRP	
	saphenous	L2–L5	B-HRP	
		L2–L3	WGA-HRP	
	median	C1–T6	B-HRP	
		C4–T3	WGA-HRP	
	ulnar	C1–T7	B-HRP	
		C5–T2	WGA-HRP	
cat	gastrocnemius n.	L4–S3	Free HRP	Mense & Craig (1988)

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Table 3. *Summary of rostrocaudal projections of primary afferents using intraaxonal injection of HRP in the spinal cord*

animal	number	type	maximum rostrocaudal extent (mm)	reference
cat	31	sensory	< 1 segment	Brown *et al.* (1991)
cat	43	SAI[a]	6.89	Ritz *et al.* (1989)
		SAII[b]	5.62	
		HFA[c]	8.40	
cat	51	muscle	7.80	Ritz *et al.* (1991)
cat	7	RA[d]	4.0	Semba *et al.* (1983)
	12	PC[e]	7.0	Semba *et al.* (1984)
	28	SAI[a]	6.0	Semba *et al.* (1985)
cat	24	muscle	18.0	Hongo *et al.* (1987)
cat	42	muscle	8.1	Hoheisel *et al.* (1988)
cat	196	tibial	6.2	Koerber *et al.* (1989)
cat	6	HFA[c]	10.0	Snow & Wilson (1989)
cat		sensory	10.5	Brown (1981)
		muscle	10.5	
guinea-pig	27	visceral	13.2	Sugiura *et al.* (1988)
	12	somatic	5.0	
rat	24	HFA[c]	5.9	Shortland *et al.* (1989*a*)
rat	28	RA[d]	6.0	Shortland *et al.* (1989)
rat	16	sensory	5.7	Woolf (1987)
rat	18	HFA[c]	6.5	Fitzgerald *et al.* (1990)

[a] SAI, slowly adapting type 1.
[b] SAII, slowly adapting type 2.
[c] HFA, hair follicle afferent.
[d] RA, rapidly adapting afferent.
[e] PC, Pacinian corpuscle.

revealed by using bulk labelling methods. The present electrophysiological recordings may be identifying axons from the nerves innervating the mid-axial line of the limb (Swett & Woolf 1985) or from the LFC nerve. The DRG injections are unable to distinguish axons from different nerves. Previous bulk-labelling studies of the L1 DRG have only looked at caudal segments in transverse sections (Rivero-Melian & Grant 1990*a*,*b*) and may have missed the very caudal ends of these axons as these are sparse and not easily seen in transverse sections; they are better visualized in horizontal sections (Pfaller & Arvidsson 1988). The resolution achieved by the present electrophysiological methods are only sufficient to show that the parent axon courses caudally in the dorsal columns and that some collateral branches penetrate the grey matter of distant segments. Thus for DRG afferents the fine detail of the caudal penetration of terminal arborizations is not completely revealed by electrophysiological or anatomical methods.

The complementary evidence of transport and of electrophysiology agrees that there are remarkably long-range caudal extensions of entering afferent fibres. The sural fibres with long-range penetrations originate from large-diameter myelinated fibres in the periphery (Wall & Shortland 1991), and because the sural is mainly a cutaneous nerve and as large diameter cutaneous afferents are mainly low-threshold mechanoreceptors (Campbell *et al.* 1989; Shortland *et al.* 1989) we can propose that these fibres should be excited by low-level mechanical stimuli in the periphery. This raises a considerable functional paradox as there is no evidence that the postsynaptic cells in the region of the long-range afferents respond to stimulation of the peripheral receptive field of the afferent fibres. For example, detailed mapping of extracellular cell responses to natural stimuli of the sural nerve peripheral skin region fails to find responding cells caudal to the L5–6 border (Shortland & Fitzgerald 1991), and yet we find afferents in S2. Intracellular recording reveals a somewhat wider peripheral receptive field from which postsynaptic excitatory potentials can be evoked (Woolf & King 1989; Pubols 1990) but the expansion is minor. Electrical stimulation of the sural nerve reveals excitatory receptive fields in 9% of dorsal horn cells outside the normal low-threshold innervation territory of the sural nerve by strengthening of ineffective synapses (Pubols *et al.* 1986). It appears that the remarkably precise somatotopic postsynaptic map (Brown 1981; Shortland & Fitzgerald 1991) is supplied by more diffusely mapped afferents, some of which end in areas inappropriate for the postsynaptic map.

There are four possible explanations. One would be that there are too few of the long-range afferents to generate clear postsynaptic responses. However, 30% of entering myelinated afferents send axons at least three segments caudal to their entry point (Wall &

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Shortland 1991), which takes them into a very inappropriate area of the somatotopic map. Second, it could be that the distant terminal branches are blind ending and do not contain vesicles or a synaptic apparatus as suggested by Meyers & Snow (1984), and are unable to excite dorsal horn cells. Some somatotopically inappropriate collaterals are invaded by action potentials (Meyers & Snow 1984*b*). However, in papers by Traub *et al.* (1989, 1990), A-delta afferents were shown to terminate many segments from their root entry segment, and electron microscopy showed that the distant terminals contained vesicles and synaptic specializations. It has been suggested (Shortland *et al.* 1989) that transganglionic transport of HRP accumulates in regions of high synaptic density and does not occur in blind-ending collaterals, although Lamotte *et al.* (1991) report that these blind collaterals reside in the appropriate nerve territory as demonstrated by labelling with B-HRP. Although a clear answer to this would require electronmicroscopy, the terminals we have seen here end as swellings in grey matter which are believed to be associated with synapses (Cruz *et al.* 1987, 1991). Third, it is the muscle and deep fibres rather than cutaneous afferents which show the greatest caudal extent. This may explain some of the discrepancies in the receptive field mapping studies because stimuli to deep tissue were not used (Shortland & Fitzgerald 1991).

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