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# Long-range afferents in the rat spinal cord. 1. Numbers, distances and conduction velocities

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## SUMMARY

The caudal extent of the penetration of primary afferent axons from the T12 and L1 dorsal roots and sural nerve has been investigated in adult decerebrate spinal rats. Microelectrode stimulation at the root entry zone (REZ) and at further caudal points in the spinal cord was used to generate antidromic action potentials in single fibres recorded in dorsal roots or peripheral nerves. A total of 209 units were recorded in T12 and L1 dorsal roots and 27% of these could be antidromically activated 10 mm caudal to the REZ. Fifteen percent of the units could be stimulated at the L4–5 border, 15 mm caudal to the T12 segment whereas 4.5% of the axons could be stimulated 25 mm caudally in the S4 segment, 11 segments caudal to the entry segment.

Similar recordings made from units in the sural nerve showed that of all the sural axons that penetrated to the L6 segment 50%, 18% and 2% of these reached the S1, S2 and S4 segments respectively. The conduction velocities of these units were clearly in the A-beta range when recorded in the nerve but decreased on entering the spinal cord and were reduced by 83% at their caudal end point.

The results show that substantial numbers of primary afferents have long-ranging caudal branches in areas beyond the regions of known postsynaptic effects. The functions of these caudal projections are unclear but they may represent a potential substrate for the development of functional connections under conditions of disease or denervation.


## 1. INTRODUCTION

A crucial factor that has permitted the investigation of sensory systems in the central nervous system has been the discovery of cells with receptive fields arranged in orderly maps. Each cell responds to a spatially limited fraction of the possible input and this fraction creates its receptive field. Neighbouring cells have neighbouring receptive fields that taken together create a complete map of the input. In the case of the spinal cord dorsal horn, which we examine here, the superficial laminae I–V contain an encoded representation of the complete body surface (excluding the face) (Wall 1960; Bryan *et al.* 1973; Brown & Fuchs 1975; Brown *et al.* 1980; Koerber 1980; Light & Durkovic 1984; Sorkin *et al.* 1986; Wilson *et al.* 1986; Woolf & Fitzgerald 1986). The cells in each segment respond mainly to the afferent sensory fibres which enter over that segment's dorsal root and which originate from that segment's dermatome on the skin. By using natural stimuli, 100% of cells recorded extracellularly in the medial two thirds of the rat L4 or L5 dorsal horn responded only to either toes or foot (Devor & Wall 1981). The same is seen in the cat (Brown & Fuchs 1975). The receptive fields of all these cells lie within the dermatome of their nearest root. In the lateral part of the dorsal horn, most cells also have their natural stimulus receptive fields restricted to the more proximal parts of the dermatome of that segments dorsal root (Brown & Fuchs 1975; Brown *et al.* 1980). However, there are a few cells in the extreme lateral part of the dorsal horn with very extensive receptive fields extending over many segments (Devor & Wall 1976). Mendell *et al.* (1978) found such cells constituted less than 1% of the dorsal horn cells. With electrical stimulation of roots or nerves, the synchronized afferent input provokes a much larger receptive field (Merrill & Wall 1972; Devor *et al.* 1977). In cat L7 dorsal horn, 33% of all cells responded to electrical stimuli applied 3–4 segments away (Mendell *et al.* 1978). Similar results have been shown after peripheral nerve stimulation in the rat (Markus *et al.* 1984). An even larger number, 48%, showed intracellular responses to such stimuli (Mendell *et al.* 1978) and this subliminal fringe has been shown by others (Brown & Fyffe 1981; Brown *et al.* 1987*b*; Woolf & King 1989; Woolf *et al.* 1989; Pubols *et al.* 1990). However, most of these long-range responses had a long latency and followed high frequency stimulation poorly and were therefore likely to be caused by polysynaptic input (Mendell *et al.* 1978). Most physiological evidence agrees that the monosynaptic inputs to dorsal horn cells originates from afferents entering over the nearest dorsal root or at most one or two segments rostral or caudal (Brown *et al.* 1973, 1987*a*; Devor & Wall 1976; Brown & Noble 1982). One would therefore predict that entering fibres

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Table 1. *Summary of caudal projections of myelinated afferents*

| method | animal | area studied | maximum distance caudal (no. segments or mm) | reference |
|---|---|---|---|---|
| golgi | cat | lumbrosacral cord | 2.5 segs | Szentagothai (1964) |
| nauta | cat | T9 | 4 segs | Liu & Chambers (1958) |
| | cat | C6–C8 | 4 segs | Sterling & Kuypers (1967) |
| | cat | lumbrosacral cord | 3 segs | Sprague & Ha (1964) |
| Fink–Heimer | cat | L5 | 5 segs | Culberson & Brown (1984) |
| | cat | Co3 | 4 segs | Ritz *et al.* (1985) |
| | cat | C1–C8 | 8 segs | Imai & Kusama (1969) |
| bulk label of DRGS | rat | C3 | 10 segs | Neuhuber & Zenker (1989) |
| | rat | L2–L6 | 4 segs | Rivero-Melian & Grant (1986) |
| | rat | C4–C8 | 17 segs | Arvidsson & Pfaller (1988) |
| | rat | C1–C3 | 14 segs | Pfaller & Arvidsson (1990) |
| intracellular injection of HRP | cat | HFA | 7.6 mm | Brown (1981) |
| | cat | Ia (muscle) | 5.5 mm | Brown (1981) |
| | rat | HFA/RA/SAI | 2.0 mm | Woolf (1987) |
| | rat | HFA | 2.0 mm | Shortland *et al.* (1989) |
| | rat | HFA | 5.0 mm | Fitzgerald *et al.* (1990) |
| electrophysiology: antidromic stimulation | cat | L2 | 6 segs (73 mm) | Wall & Werman (1976) |

Table 2. *Caudal projections of C fibres*

| method | animal | area studied | maximum distance caudal (no. segments or mm) | reference |
|---|---|---|---|---|
| golgi | cat | lumbrosacral cord | 1 seg. | Szentagothai (1964) |
| intracellular injection | guinea pig | L6 (somatic) | 1 seg. | Sugiura *et al.* (1988) |
| | | T13 (visceral) | 3 segs | |
| antidromic stimulation | rat | sural/gastroc. nerves | 4 mm | McMahon & Wall (1985) |
| other immuno histochemistry | rat | L4 | 5 segs | Traub *et al.* (1989) |

would terminate within one or two segments of their entry point. This paper examines that prediction and finds that many fibres range far beyond the predicted area.

As afferent fibres enter the spinal cord they form a 'T' junction with rostral and caudal running branches (Cajal 1909; Rethelyi & Szentagothai 1973). The rostrally directed branch may run towards distant targets such as Clarke's column and the dorsal column nuclei, we have not examined this branch as we would not have been able to differentiate axons destined to end in spinal cord from those which would terminate in distant structures. We concentrate here only on the caudal branch that can only terminate in the spinal cord. The extent of this branch has been studied by many authors and their results are summarized in tables 1 and 2. Six different methods have been used, each with its advantages and limitations. One must warn that some authors were not attempting to establish the full extent of the caudal range and reported penetrations only as far as they looked. Four findings stand out. First, the startling long runs of 8–17

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segments are all reported as originating in cervical afferents which could mean no more than that these afferents have a much greater possible range of caudal penetration than lumbar afferents. Secondly, unmyelinated fibres appear to run shorter distances than myelinated fibres. Thirdly, the beautiful single-fibre horseradish peroxidase (HRP) transport methods show very limited extensions in contrast to other methods. A possible explanation for this marked disparity is that the transport method particularly emphasizes the true terminal arborizations with boutons termineaux whereas other methods may reveal in addition long-running stem fibres. Lastly, it is quite clear that many methods reveal that substantial numbers of incoming fibres extend beyond the region predicted by physiological studies. There is a marked mismatch between the physiological explorations and the anatomical results.

We are therefore left with the question of the extent and numbers of stem axons that penetrate long distances with or without terminal arbors. The answer to this question is important because it would affect the embryological question of the precision of growth of entering nerve fibres toward their adult functional target. If long-ranging afferent fibres existed beyond an area where they exert even subthreshold excitatory effects in the adult, they could represent an exhuberent overgrowth in the embryo which had not been withdrawn during maturation. If such fibres existed, even inactive, they could be the substrate for the subsequent development of functional connections under conditions of disease or denervation. For these reasons we search here for afferent fibres whose axons extend beyond the region of known postsynaptic effects.

## 2. METHODS

All experiments were done on adult Sprague Dawley rats of either sex weighing 200–300 g. The animals were deeply anaesthetized with urethane (50 mg $kg^{-1}$ intraperitoneally (IP). After anaesthesia, one carotid artery and the trachea were cannulated. The urethane-anaesthetized animals were decerebrated, paralysed with gallamine and artificially ventilated. Rectal temperature was measured and maintained at 36 °C. Expired $CO_2$, heart rate and ECG shape were continuously monitored. Blood loss was replaced with degraded gelatine (Haemacell, Behring). The spinal cord was sectioned at T10–T11 to isolate the lower segments and to prevent CSF flooding the root under study. A laminectomy then exposed the segments needed for stimulation from T12 to the coccygeal segments. The dura was then opened and the cord covered with warm paraffin oil. In some experiments the sural nerve was exposed in the popliteal fossa and covered with oil.

Stimulation of the entire L1 dorsal root was achieved by placing the dissected dorsal root on a pair of silver–silver chloride hooks as close as possible to the root entry zone (REZ). Stimuli were square waves 0.02 ms, 1 Hz, up to a maximum of 100 μA. Stimulation in the spinal cord was through glass-covered tungsten microelectrodes (Merrill & Ainsworth 1972) with a 25 μm exposed tip. They were placed in the appropriate place by a three-dimensional micromanipulator under microscopic observation. The stimulus parameters were 0.02 ms, 1 Hz, up to 100 μA or 0.2 ms, 1 Hz up to 10 μA with the microelectrode tip negative with respect to a distant electrode.

Recordings of single units were made on fine strands dissected with pairs of sharpened jeweller's forceps either from dorsal roots or from the sural nerve. The strand was mounted on a hook electrode that led to a conventional amplification system with the filters set to a wide-band high frequency setting and with the low frequency cut-off at 0.1 Hz to limit the stimulus artifact recording. The strand was recorded with respect to a nearby electrode touching the muscle (the indifferent electrode). At the end of the experiments all roots and segments were identified by extensive dissection of the foraminae.

## 3. RESULTS

### (*a*) *The proportion of primary afferents that penetrate 10 mm caudal to their entry point in the spinal cord*

For this experiment, recording strands were dissected from the L1 dorsal root and stimulating electrodes were placed proximal to them on the whole L1 root and along the length of the cord as shown in figure 1. In this and all other subsequent experiments it is important to emphasize that we are intentionally dealing only with large myelinated axons. The reason is that it was necessary to limit the stimulus intensity to minimize the artefact size and to limit the stimulus spread so that accurate localization of the fibres could be obtained. The first step in this set of experiments was to determine the number of conducting units in the dissected strand on the recording electrode some 4 mm from the REZ. The entire root was stimulated through

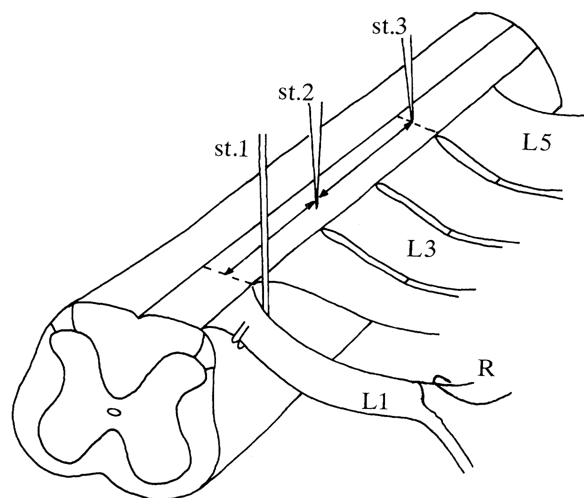


Figure 1. Diagram of the recording position on a strand dissected for the L1 dorsal root, R, and the three stimulus positions: st. 1 on the entry region of the root; st. 2, 5 mm caudal to the stimulated root and st. 3, 10 mm caudal to the stimulated root as indicated by the arrows.

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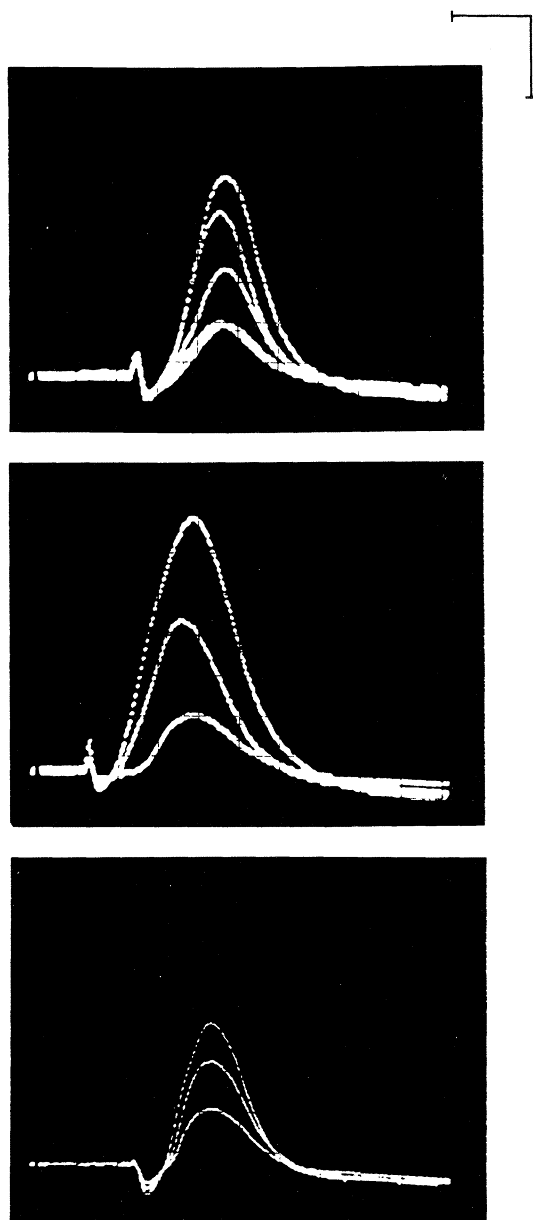

Figure 2. Recording of single units on strands dissected from the L1 dorsal root after stimulation of the root close to the dorsal root entry zone (REZ). The stimulus and recording positions were at st. 1 and R as shown on the diagram in figure 1. The stimulus strength was gradually increased until the smallest unit was recorded. On further increase of the stimulus strength, the recording jumped to the second, third and fourth positions as shown in the upper trace. In the lower two recordings only three heights were recorded. It is proposed that the largest potential in the upper recording is a compound action potential made up of four units and that the lower two examples contain three units each. Time bar: 0.2 ms. Amplitude bar: 0.5 mV.

hook electrodes placed at the REZ. The stimulus intensity was gradually raised until an all or none unit appeared on the recording sweep (figure 2). With a further increase of stimulus intensity there was no change until there was a sudden jump in spike height to a new level. This was taken to be recruitment of a second unit. This procedure was continued step by step counting the number of quantal jumps which were taken to represent the number of axons contributing to

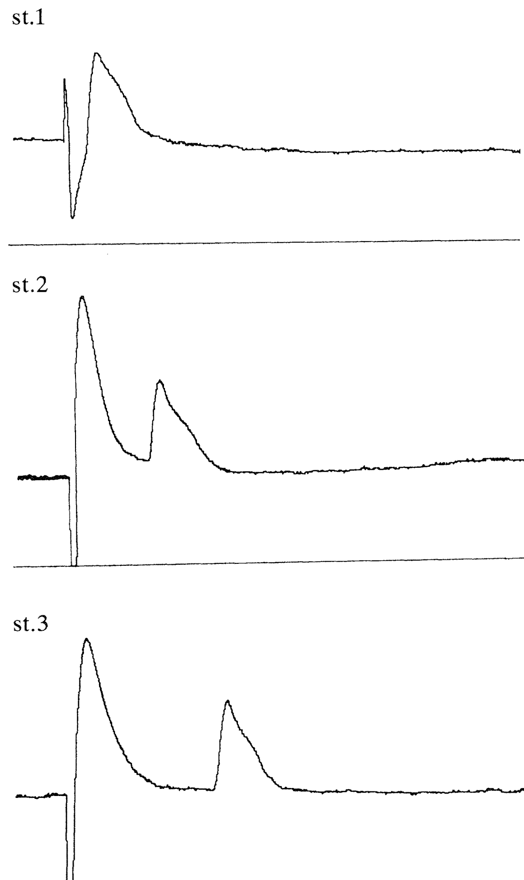


Figure 3. Recording from the same single unit in a strand from the L1 dorsal root after stimulation on the REZ of the dorsal root, st. 1 and st. 2, 5 mm caudal to the root entry and st. 3, 10 mm caudal to the root. The stimulus positions are shown in the diagram in figure 1. The amplitude of the action potential was 1.2 mV. The delay between the start of the trace and the beginning of the stimulus artifact is 0.5 ms.

the compound action potential. This technique has been developed extensively by Devor in the peripheral nerve (Devor & Govrin-Lippmann 1979). Only those strands which contained five or less units were used for further investigation. Thicker strands with more units produced too complex a compound action potential for us to identify single units and these strands were placed aside. Dorsal root reflexes were identified and excluded because the impulses have a variable latency and fail to follow high frequency stimulation.

The next step was to determine unit by unit if the fibre penetrated either 5 or 10 mm caudal to the entry point. An example of how this was done is shown in figure 3. The top sweep, st. 1, shows the action potential of a single unit recorded on the strand following stimulation of the L1 dorsal root. The middle sweep, st. 2, shows the same unit activated by stimulation with a microelectrode in the dorsal columns 5 mm caudal to L1 in the L3 segment. The lower sweep, st. 3, again shows the same unit this time activated by a stimulus through the microelectrode 10 mm caudal to the fibres entry over the L1 root where it could be stimulated at the boundary of segments L4 and L5. To ensure that the action potential observed after root stimulation is the same as that evoked by cord stimulation, collision tests were done on all spikes. Stimuli to st. 1 and st. 2

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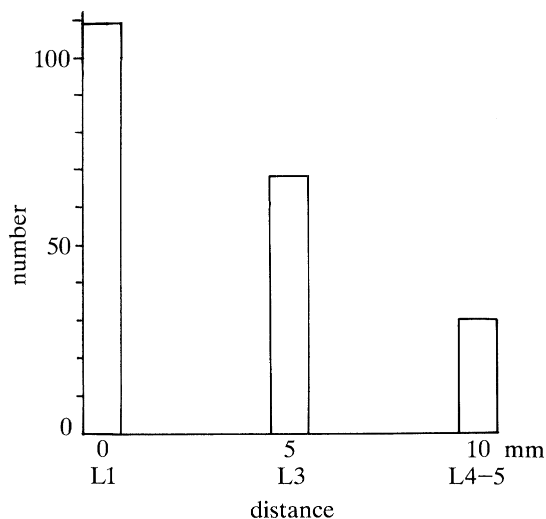


Figure 4. The diagram illustrates the penetration of the 109 fibres recorded as entering over the L1 root of which 68 or 32 % were penetrating for 5 mm to the L3 segment and that 30 or 27 % were penetrating for 10 mm to the junction of the L4 and L5 segments.

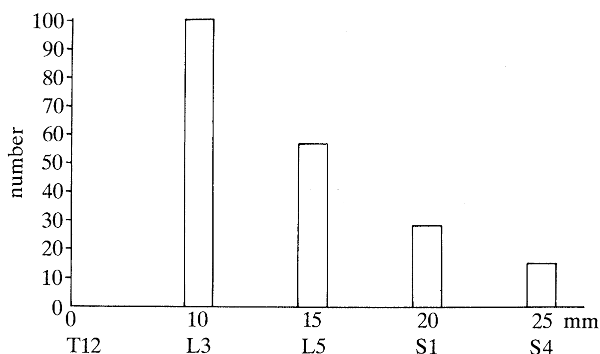


Figure 5. One hundred units were recorded on the T12 dorsal root which had penetrated for 10 mm in a caudal direction as far as the L3 segment. The diagram shows the number of these that penetrate for 15, 20 and 25 mm as far as the border of the L4–5 segments and to the S1 and S4 segments.

were given simultaneously to test if the response from st. 1 collided with that from st. 2 and abolished it so that only the st. 1 response remained.

We isolated 109 units in L1 filaments which responded to L1 stimulation. Of these, 68 (62 %) could be stimulated from the dorsal columns 5 mm caudal to the root entry and 30 (27 %) could be stimulated from 10 mm caudal (figure 4). The location of each axon was found to lie within the dorsal columns by moving the stimulating electrode mediolaterally to identify the lowest threshold point. This was done to ensure that there was no distant stimulus spread.

### (*b*) *The number of primary afferents that penetrate 10–25 mm caudal to the entry point in the spinal cord*

To examine this length of penetration it was necessary for us to move more rostrally from L1 to T12 for recording. This root was too short to allow us to count the total numbers of fibres in the filament because the artifact overrode the recording. Therefore we chose 100 fibres running in this root which penetrated at least 10 mm and then checked each fibre to see if it penetrated 15, 20 and 25 mm caudally (figure 5). The answer is that of the original 100 fibres, 57 penetrated as far as 15 mm, 28 to 20 mm and 15 to 25 mm. These last fibres were located 11 segments caudal to their entry point.

Assuming the same proportion of T12 and L1 fibres penetrate for 5 and 10 mm, the results from experiments 1 and 2 can be added together. Figure 6 shows the results of combining the total of 209 units. The percentage of fibres entering over T12 which would penetrate caudal distances up to 25 mm as far as S4 is shown. We believe this to be an underestimate as some very caudal fibres moved below the central vein into regions we could not reach with the stimulating microelectrode.

### (*c*) *The caudal penetration of sural nerve afferent fibres*

Recording single units on the sural nerve and examining their caudal penetration had two advantages. The conduction velocity of the fibre from the dorsal root to the peripheral recording site could be accurately measured and as the sural nerve supplies the skin and because the conduction velocities were mainly in the A-beta range it is certain that the majority of these fibres were low threshold mechanoreceptors (Lynn & Carpenter 1982). The disadvantages are that the sural nerve fibres arrive in the cord over the L3–L5 dorsal roots, so that it was necessary to locate the precise point of entry for some measures. Secondly, the far caudal penetrations reached into caudal sacral segments where the cord is narrow and the cord surface veins proportionately large so that the extreme ends of the fibres may have disappeared into the inaccessible region underneath the central vein.

Sixty single units in the sural nerve were located by the same means as those used for root recording. Each unit penetrated as far caudally as the L6 segment. All such units were therefore stimulated 1–3 segments caudal to their entry point. The further caudal extent was then traced for each unit by searching the cord at 1 mm steps caudal to the initial stimulus point. The results are shown in figure 7. It will be seen that half the units penetrated at least a further 2.5 mm, equivalent to one further segment.

### (*d*) *Conduction velocities of the caudal extensions of sural units*

The conduction velocities of the parent fibres in the sural nerve was measured by locating with the stimulating microelectrode the rootlet containing the fibre and then measuring the latency to recording the spike on the sural strand. The conduction velocity of the fibre from the entry point to the L6 segment was calculated by the difference in latencies from these two stimulus points. The conduction velocity from the L6 segment to the farthest detectable point of penetration was obtained from the peripherally recorded latencies from these two stimulus points. The results are shown

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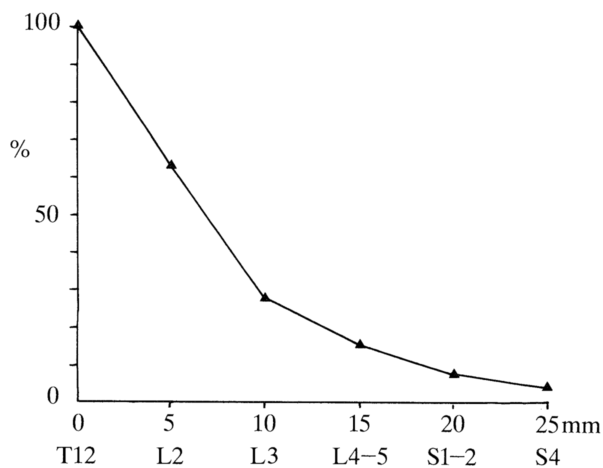


Figure 6. The estimated percentages of those fibres entering the T12 dorsal root which reach various segments. This figure is obtained by combining the results shown in figures 5 and 6 as explained in the text.

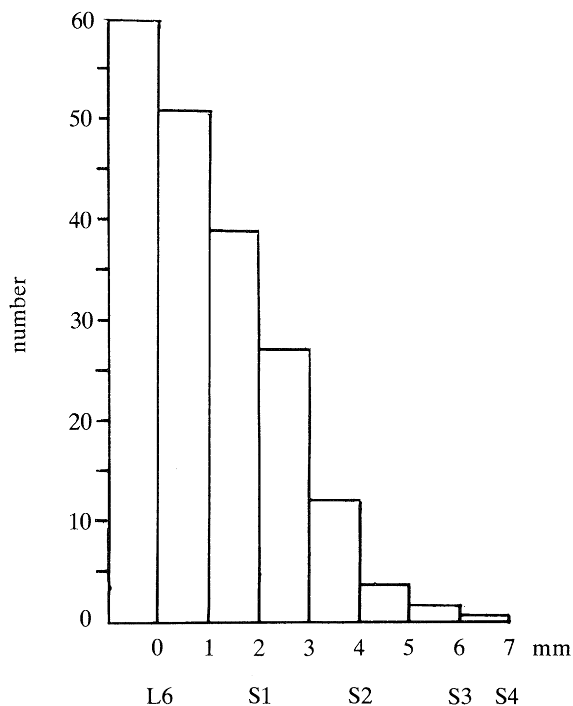


Figure 7. Sixty single units recorded in the periphery on the sural nerve were found to have penetrated as far caudally as the L6 segment. The diagram shows the number of these fibres that could be followed more caudally for up to 7 mm as far as the S4 segment.

Table 3. *Conduction velocities of sural afferents*

| | | conduction velocity/($ms^{-1}$) | | |
|---|---|---|---|---|
| | no. units | mean ± s.d. | fastest | slowest |
| root to sural | 54 | 24.1 ± 5.8 | 42 | 13 |
| L6 to root | 16 | 10.0 ± 4.6 | 19 | 5 |
| end point to L6 | 30 | 4.1 ± 2.2 | 10 | 1 |

in table 3. They show that there is a two stage decrease in the conduction velocity (cv) after entering the spinal cord. The cv is slowed by 58 % by the time it reaches the L6 segment and by a further 25 % at the end point, an 83 % slowing of cv compared with that recorded in the sural nerve. Whereas the cv is clearly in the A-beta range in the peripheral nerve, the cv at the distant end is only just above that found for C fibres.

## 4. DISCUSSION

The results suggest that substantial numbers of afferents have long-range branches that penetrate the spinal cord in a caudal direction. These fibres extend considerably beyond the area where monosynaptic postsynaptic responses have been observed and beyond the zone where most transport studies have shown the existence of afferent terminal arbors (Brown *et al.* 1981; Swett & Woolf 1985; Molander & Grant 1986; Woolf & Fitzgerald 1986).

As suggested in the introduction, the choice of technique and segmental level both markedly influence the observed length of caudal projections seen (tables 1 and 2). For large fibres, Fink–Heimer degeneration, some bulk labelling transport and single fibre antidromic recording provides evidence for very long-range penetration. However, single fibre HRP transport in large myelinated afferents shows only small lengths of caudal penetration. The reason for this difference might be that the transported HRP fails to penetrate the farther reaches of the terminal arbor. There is some evidence for this (Brown 1981; Meyers & Snow 1984; Woolf 1987; Shortland *et al.* 1989). However, in most cases where afferents have been intracellularly stained the stem axon proceeds beyond the last observable caudal collateral (Woolf 1987; Shortland *et al.* 1989), although Brown (1981) saw one axon that was presumably part of a caudal branch of an afferent which ran for 7.6 mm and terminated as a collateral arbor. Technical factors such as the physiological condition of the animal, HRP transport rate, HRP injection amount and post injection survival time will also influence the distance of axon recovered. Another possibility related to bulk labelling methods is that the main rostrocaudally running stem axon proceeds much further than the area in which terminal arborizations and synaptic contacts are elaborated. It could be that the HRP accumulates selectively in the high density region of boutons and is present in much smaller amounts in long-running axons so that it is not readily apparent and may be missed particularly when the axons are cut in cross section but are observed in longitudinal section (Pfaller & Arvidsson 1988; Arvidsson & Pfaller 1990). Many bulk labelling studies have used WGA–HRP as the transported substance and this is known to be preferentially located in the central terminals of small diameter fibres (Robertson & Grant 1985). Because it is generally agreed that C fibres have shorter caudal projections (tables 1 and 2) the true caudal extent of the larger fibres may not have been observed. However, studies using different HRP conjugates reveals no differences in the central projections from a given nerve territory (Woolf & Fitzgerald 1986). In contrast, the degeneration techniques and the antidromic stimulation methods could well favour the discovery of long-running parent axons.

The segmental level also influences the distance that

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axons can be traced caudally. Caudal projections of between 4–17 segments have been seen from the bulk labelling or degeneration of cervical dorsal roots and ganglia (table 1) whereas more modest projections of three segments have been recorded for thoracic afferents (Imai & Kusama 1969; Ritz *et al.* 1985). For lumbar cord the values range between 2–5 segments, depending on the root and up to 4 segments for more caudal (coccygeal) roots (table 1). It has been suggested that the very long caudal projections seen in cervical cord represents a specialized projection aimed at Clarke's Column (Arvidsson & Pfaller 1990). The present study shows that 4% of T12–L1 afferents can project as far as 11 segments caudally, up to S4, further than any previous reports for studies in the rat.

The units recorded in the present study fulfilled the standard criteria of single units with fixed all-or-none amplitudes and latencies and they were blocked by collision. The spikes reported here appeared to be generated in continuous axons. They were certainly not dorsal root reflexes which were frequently observed with long and variable latencies and an inability to follow high frequency stimulation. Refractory periods to double pulses applied to the central axons were always less than 2 ms. In invertebrates, some long-running axons are in fact multiple cells coupled by tight junctions (Pappas & Bennett 1966). No such junctions have been reported in adult vertebrates. Even if these axons reported here were of this type, we would expect that, on passing beyond the tight junction, another cell with branches would be revealed. This was never seen. We observed an apparent abrupt end to the axon.

From the sural studies, we describe here only fibres with a relatively fast peripheral conduction velocity. Fibres in this group are mainly cutaneous low threshold mechanoreceptors (Lynn & Carpenter 1982). There are three possible reasons for this limitation. First, the short relatively thick strands we used for recording are not suitable for thin fibre recording. Secondly, it is possible that there are no long range branches from fine afferents as most methods agree that such fibres have a much more limited area of central endings than large diameter fibres (but see Traub *et al.* 1989). Thirdly and most likely, because we limited the amplitude of the stimulus to avoid spread and to decrease the stimulus artifact we might not have stimulated small afferents.

On entering the cord there is a marked decrease of conduction velocity in the afferents. This has been reported repeatedly for ascending fibres since the first measurements by Lloyd & McIntyre (1950) and more recently for A-delta and C fibres (Traub & Mendell 1988; Waddell *et al.* 1989). It evidently applies equally to the descending branch and the most likely explanation is that it is due to the change in extracellular space and from tapering associated with the emission of collaterals. There is anatomical and electrophysiological evidence that dorsal root axon diameters are smaller than those of peripheral axons (Suh *et al.* 1984; Lee *et al.* 1986). These thinner caudal axons may have a thinner myelin sheath that may result in a loss of insulating ability and so slow the conduction velocity. The final stretch of the axon was, however, still conducting at speeds higher than that of unmyelinated afferents (Lynn & Carpenter 1982). We know from previous studies (McMahon & Wall 1985) that the technique is capable of stimulating fine, central, unmyelinated fibres. We therefore searched in detail with high stimulus strengths the area beyond the apparent end of the descending fibres and assured ourselves that we had not missed an even further extension of the axon as a thin unmyelinated branch or continuation.

We are left then with evidence that some afferents extend in the cord far beyond the region where they have been shown to have a monosynaptic postsynaptic effect. It is possible that the long-running axons emit no terminal arborizations or have branches with no boutons (Meyers & Snow 1984; Woolf 1987; Shortland *et al.* 1989). If these long-range axons turn out to be blind-ending, it would set an interesting embryological question. There are many examples in embryological development of early exuberant growth of connections followed by a phase of competition and reorganization of appropriate terminations associated with the withdrawal of inappropriate endings (pruning) (Purves & Lichtman 1985; Innocenti 1988). If the long-range afferents described here are blind-ended in adults, it would mean that pruning is only effective on the extreme terminals, leaving intact the axons from which the final arborization grew. It is not known at present whether blind-ending collaterals exist in the neonatal spinal cord. Alternatively, they may simply be long running caudal axons with no collaterals at all, representing aberrant growth in the spinal cord. Finally, it is possible that the long-range fibres have synaptic contacts with cells but the contacts are so scattered and so disadvantageously placed on dendrites that no obvious postsynaptic effect occurs. To prove the existence of such ineffective contacts might push present intracellular averaged techniques beyond their limits. It would seem more reasonable to search for minor contacts under conditions of disinhibition and partial deafferentation where we know that cells become explosively excitable and where minor inputs would be exaggerated and amplified.

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