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AFFERENT FIBRES IN CAT VENTRAL ROOTS: ELECTROPHYSIOLOGICAL AND HISTOLOGICAL EVIDENCE

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SUMMARY

1. Electrophysiological experiments using averaging techniques, as well as anatomical experiments using horseradish peroxidase staining, have provided further evidence of afferent axons in lumbosacral ventral roots of cats.

2. Recording from dorsal root filaments in L7, S1 or S2, following stimulation of the companion ventral root close to the dura, often shows action potentials of slow conduction velocity belonging to the Aδ or C group. Stimulation applied to the proximal part of the ventral root failed to evoke such responses.

3. Recording from multiple sites along a centrally cut ventral root filament shows responses of two types: (a) action potentials of long latency to peripheral nerve stimulation which are seen at all recording locations and which are not seen following dorsal root stimulation. These appear to be afferent fibres which enter the cord via the ventral root; (b) action potentials which follow dorsal root stimulation and which are usually seen only at the most distal ventral root recording site. Some of these were also activated by stimulation of some skin or muscle nerves. At appropriate intervals collision of impulses from dorsal root or peripheral nerve can be demonstrated. Such axons appear to have a recurrent course in the ventral root.

4. Section of the spinal nerve at points progressively closer to the dorsal root ganglion abolishes the dorsal to ventral root continuity of most recurrent type axons at 2 mm distal to the ganglion.

5. Following application of horseradish peroxidase to crushed ends of distal stumps of cut dorsal roots, thin fibres marked by the enzyme are observed in the distal part of companion ventral roots. U-turns of axons have been observed in the distal part of ventral roots and in the spinal nerve near the pole of the ganglion.

INTRODUCTION

It has been known for many years (Magendie, 1822; Bernard, 1858) that stimulation of the distal stump of a cut ventral root elicits painful reactions, provided the companion dorsal root is intact. The 'recurrent sensitivity' of ventral roots, as

Magendie termed this phenomenon, has often been ascribed to the stimulation of afferent fibres which, before entering the spinal cord through dorsal roots, follow a recurrent course in ventral roots (see Cranefield, 1974; Coggeshall, 1980; Laporte, 1983). Several recent observations strongly suggest the existence of such fibres. Risling & Hildebrand (1982) reported that the number of non-myelinated axons in cat lumbar ventral roots is much larger in distal portions of the roots than in proximal ones. Risling, Dalsgaard, Cukierman & Cuello (1984) found no non-myelinated axons at some junctions of ventral roots with the spinal cord and observed U-turns of axons in the ventral roots. Dorsal horn cells were found to be activated both by electrical stimulation of distal stumps of ventral roots and by mechanical skin stimulation (Chung, Lee, Kim & Coggeshall, 1985). Kim & Chung (1985) have recorded action potentials of Aδ and C fibres in dorsal root filaments after stimulation of the companion ventral root and, conversely, action potentials in ventral root filaments after stimulation of the distal stump of the companion dorsal root.

The present study provides additional evidence, based on averaging techniques, for the existence of afferent axons, both Aδ and C, in ventral roots. Many such axons take a recurrent course in the distal portion of a ventral root and then travel via a dorsal root towards the spinal cord. Others course centrally in the ventral roots. The peripheral origin of such axons has been demonstrated. Further, after application of horseradish peroxidase to the distal stump of dorsal roots, thin axons marked by the enzyme were visualized in the distal part of companion ventral roots and some axons were seen to make a recurrent loop.

METHODS

Electrophysiological studies

Results were obtained from adult cats of either sex, anaesthetized with sodium pentobarbitone (Nembutal) 50 mg/kg intraperitoneally, supplemented as required for the maintenance of full anaesthesia with subsequent intravenous doses. Laminectomy was performed, exposing the lumbosacral cord and removing bone so as to expose a maximal length of spinal nerves L7–S2. A bath of warm paraffin oil over the dissected region was contained by skin flaps elevated around it. The dorsal and ventral roots of these segments were cut close to the cord and the cord removed after being ligated and transected at about L5. In some experiments the nerve to posterior biceps semitendinosus and the posterior femoral cutaneous nerve were exposed and cut distally, also being covered by a paraffin oil pool. Body temperature was maintained at 37 °C by an electric heating pad under the animal and the paraffin oil was kept at a similar temperature by immersion heaters.

Roots or nerves were stimulated by 1 ms rectangular pulses of variable amplitude (up to 18 V) delivered through isolation units. Electrodes were made of platinum iridium wire.

Potentials were recorded by Grass model P5 a.c. amplifiers whose filters were usually set at 1 Hz for the low frequency and 3 kHz for the high-frequency filter (3 dB attenuation). Signals were averaged, usually over 50–100 trials, using a PDP 11/23 Plus computer. The program permitted sampling for a specified duration from three channels simultaneously, with a minimal sampling interval of 350 μs. More frequent sampling from one channel, at an interval of 120 μs, showed no significant difference in the responses. The interval between stimuli was usually set at 2·13 s, avoiding a multiple of the mains frequency and permitting adequate time for recovery of C axons between stimuli. Between stimuli and subsequent sampling periods, the cumulative responses could be observed on an oscilloscope. On completion of a series, the averaged digitized responses were stored on a disk (RL02) and were subsequently plotted using a Tektronix 4662 plotter.

Anatomical methods

Results were obtained from twelve cats of either sex, weighing 2·5–4·3 kg. They were anaesthetized with sodium pentobarbitone (Nembutal, 50 mg/kg, I.V.) and allowed to recover after surgery from the effects of anaesthesia to permit transport times of 5–8 days. Core temperature was maintained at 38 °C by a feed-back-controlled electrical blanket during the surgical procedure. After surgery, they received antibiotic treatment (sodium benzylpenicillin, 250000 i.u. (bipenicillin Specia)) mixed with 100 mg chloramphenicol (solnicol) for 4 days. Recovery and post-operative survival were uneventful.

Horseradish peroxidase (HRP, Sigma type VI) was dissolved in a 2% DMSO (dimethyl sulphoxide) solution until a semi-solid paste was formed. After drying at room temperature, a solid HRP crystal resulted which was fragmented into small pieces. A 'slow release gel' was prepared by adding 30% HRP in distilled water to a solution of polyacrylamide gel that was allowed to polymerize (Griffin, Atkins & Mayer, 1979). Pieces of wet gel were then used for implants.

A laminectomy was performed on four vertebral lumbar segments. The dura was reflected and the identified dorsal root (either L7 or S1) was cut near its entrance to the cord under the warmed paraffin oil. The severed root was then drawn by suction into a 25 mm length of silicone tubing whose inside diameter just accommodated the root. HRP crystals were then placed on the crushed end of the dorsal root, followed by small pieces of gel. The central end of the tubing was then hermetically sealed with a surgical silver clip in order to avoid leakage. It was then sutured to the inner wall of the wound in order to avoid any displacement. The dorsal root, the cord and the surrounding tissues were flushed repeatedly with saline and the exposed area closed with muscle, aponeurosis and cutaneous sutures.

After a survival time of 5–8 days, each cat was anaesthetized and then perfused through the left ventricle with 500 ml of heparinized saline followed by 2·5 l of 1% paraformaldehyde–1·25% glutaraldehyde in 0·1 M-phosphate buffer at pH 7·4 for 30 min. This was followed immediately by a 30 min flush with 2 l of cold (4 °C) 0·1 M-phosphate buffer containing 10% sucrose (Rosene & Mesulam, 1978).

The following material was excised: the lumbar cord with the pair of roots whose dorsal root was exposed to HRP, the two adjacent rostral and the two adjacent caudal pairs, the root contralateral to the treated dorsal root and the spinal ganglia with adjoining segments of spinal nerves. This tissue was placed in a solution of 0·1 M-phosphate buffer containing 30% sucrose overnight and then frozen with dry ice and serially sectioned at 40 μm on a sliding microtome. The spinal cord and the ganglia were sectioned either in horizontal or in sagittal planes, and the roots were longitudinally sectioned.

All sectioned material was reacted immediately for HRP activity according to the tetramethylbenzidine (TMB) technique of Mesulam (1978), and mounted on gelatin-coated slides. All slides were examined under dark-field microscopy for the presence of HRP reaction product and some of them were photographed (Pl. 1).

RESULTS

Electrophysiological experiments

The present experiments were designed to detect the presence of afferent axons in lumbosacral ventral roots and to determine their paths, their conduction velocities and, in some cases, their peripheral origins. Action potentials were recorded in filaments of dorsal and ventral roots and averaged in order to detect responses in axons of small diameter. During the course of this study, Kim & Chung (1985) published the results of electrophysiological experiments which showed that certain axons in the C and Aδ group are in continuity between dorsal and ventral roots. Our findings confirm their results and provided additional information on afferent axons in ventral roots.

In the present experiments the course of afferent axons in ventral roots has been studied in two ways: (a) ventral roots have been stimulated, in some cases at varying

distances from the dura, while recording from dorsal root filaments. By recording at two points along the dorsal root filament conduction velocities of the axons could be determined. In these experiments the spinal nerve was sectioned at 8–10 mm from the distal pole of the spinal ganglion; (b) a dorsal root or peripheral nerve was stimulated while recording from multiple loci along a ventral root filament at varying distances from the dura. Both methods provided evidence consistent with the view that many afferent fibres course for a short distance centrally in a ventral root and then turn retracing this course and joining the dorsal root to travel to the spinal cord.

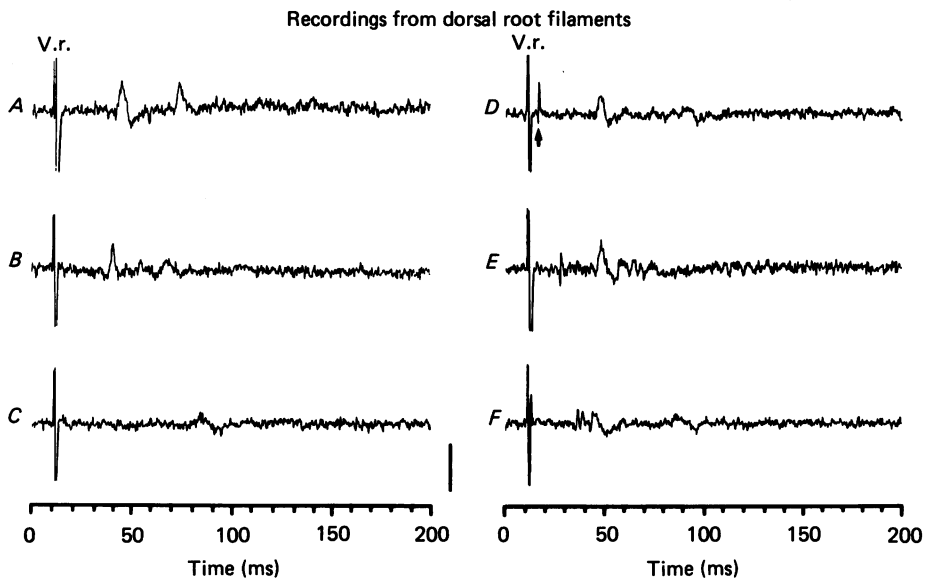

Fig. 1. Average action potentials recorded from six S1 dorsal root filaments in the same experiment (*A–F*) after stimulating the corresponding ventral root (v.r.). Spinal nerve sectioned 8 mm from the distal pole of the spinal ganglion. Stimulating electrodes 4 and 8 mm from the dura; recording electrodes 7–9 mm from the dura. Calibration bar: 1 mV for fifty trials, i.e. 20 μV per trial.

Ventral root stimulation and recording from dorsal root filaments. In these experiments the spinal nerve was sectioned 8–10 mm distal to the ganglion in order to eliminate any peripheral effects produced by motor axons. In most cases we recorded from filaments in which action potentials from several units were present although responses from individual axons could be recognized. In a few cases these were further subdivided so as to record from single units but, in general, dissection was minimized so as to avoid damage to the axons along the length of the filament. A total of thirty out of forty dorsal root filaments that were tested contained afferent axons which coursed in a ventral root. Representative responses are illustrated in Figs. 1–3.

In the records of Fig. 1, responses in six different dorsal root filaments are shown to stimulation of the same ventral root, with the cathode placed 4 mm from the dura. The portion of the ventral root distal to the cathode was grounded to prevent current spread and the stimulus strength was sufficient to excite all C fibres in a dorsal root

of similar size (not shown). In all six filaments (*A–F*) action potentials from slowly conducting axons could be seen, most of which conducted at velocities characteristic of C fibres. In *D* (arrow) a more rapidly conducting axon was recorded, probably from an Aδ axon.

Slowly conducting action potentials in dorsal root filaments were seen only when stimulation was applied to the distal portion of the ventral root. In the experiment illustrated in Fig. 2, three positions of a pair of stimulating electrodes were used successively on a ventral root and action potentials were simultaneously led from two

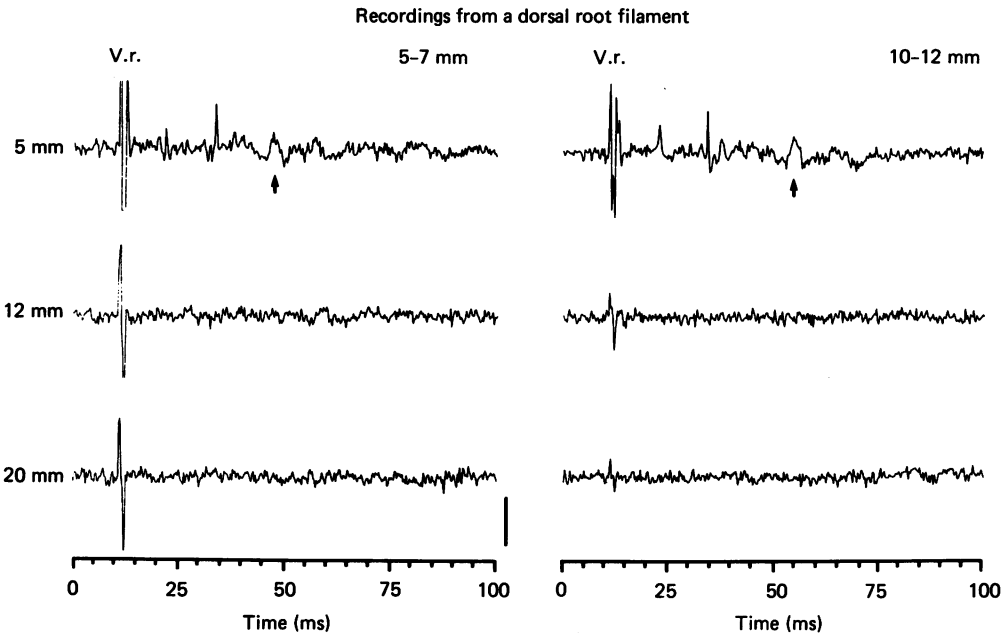

Fig. 2. Average action potentials recorded from an S2 dorsal root filament with two pairs of recording electrodes placed respectively at 5 and 7 mm (left column), and 10 and 12 mm (right column) from the dura. The upper, middle and lower traces were obtained with the stimulating cathode on the S2 ventral root (v.r.) respectively at 5, 12 and 20 mm from the dura. Stimulating electrodes with an interelectrode distance of 4–5 mm. Calibration same as in Fig. 1.

pairs of recording electrodes, placed on a dorsal root filament respectively at 5 and 7 mm (left column) and 10 and 12 mm (right column) from the dura. It can be seen that only when the cathode was placed 5 mm from the dura were detectable action potentials recorded in the dorsal root filament. Spread of stimulating current beyond the cathode to the ganglion or the dorsal root fibres themselves was carefully avoided by laying the most distal few millimetres on the bottom of the preparation or, in some cases, by placing a ground electrode distal to the cathode. We observed, in control experiments, that crushing the ventral root where it penetrates the dura abolished the action potentials.

The conduction velocity in a dorsal root of axons that are activated by stimulation of the distal part of the corresponding ventral root was measured by recording from two pairs of recording electrodes at different distances along the dorsal root filament.

The two pairs of recording electrodes were kept relatively close because non-myelinated axons might not remain undamaged over a long stretch in dissected thin filaments. Comparing the latencies of the potentials marked by arrows in the upper traces of Fig. 2 shows that the conduction velocity in this axon was about 0·6 m/s, consistent with its being a C axon. Action potentials with much shorter latencies may be

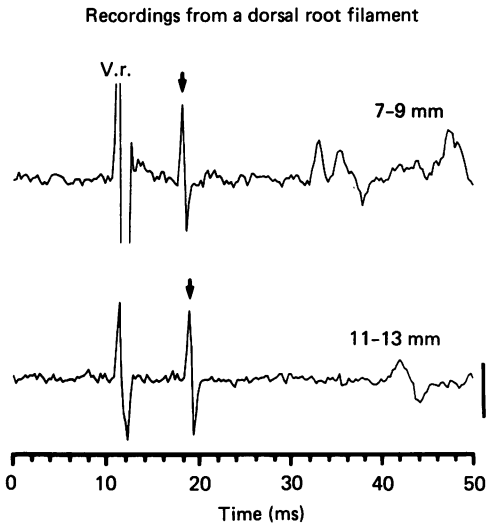

Fig. 3. Average action potentials of an axon with a conduction velocity in the Aδ range (see arrows). An S1 ventral root (v.r.) was stimulated 7 mm from the dura (interelectrode distance 4 mm) and the action potentials were led from a dorsal root filament with two pairs of recording electrodes, respectively situated at 7 and 9, 11 and 13 mm from the dura. Calibration same as in Fig. 1.

observed; their amplitudes are usually larger and their durations shorter than those of C axons. An example of such potentials is shown in Fig. 3; the comparatively large action potentials recorded at the two sites (arrows) are due to an axon conducting at about 5 m/s belonging to the Aδ group. The conduction velocity of axons along their course between ventral and dorsal root cannot be accurately measured since their exact course is not known (see Fig. 8).

The majority of recurrent axons appeared to be C fibres, as evidenced by latency of their potentials and by measurements of conduction velocity between two sites in dorsal root filaments. A small number of slow Aδ axons were also seen (see for example the short-latency potential in record *D* of Fig. 1 and in the upper record of Fig. 8).

Simultaneous recording from the distal, medial and proximal regions of ventral root filaments following stimulation of peripheral nerves or dorsal roots. Whenever possible, naturally occurring filaments were used to avoid damage of axons along their course. A total of forty-four out of ninety-six ventral root filaments that were examined showed responses to dorsal root stimulation. The ventral roots were sectioned at their entry to the spinal cord. The electrode pairs, with nearly equal interelectrode distances, were placed such that the distal pair was near the dura, the proximal pair near the central end of the root and the medial pair approximately midway between.

Two types of response have been observed: in the first, following peripheral nerve stimulation a slowly conducting action potential was observed at all three recording sites with a progressive increase in latency as the impulse was conducted toward the spinal cord; dorsal root stimulation failed to excite the axon. This type response

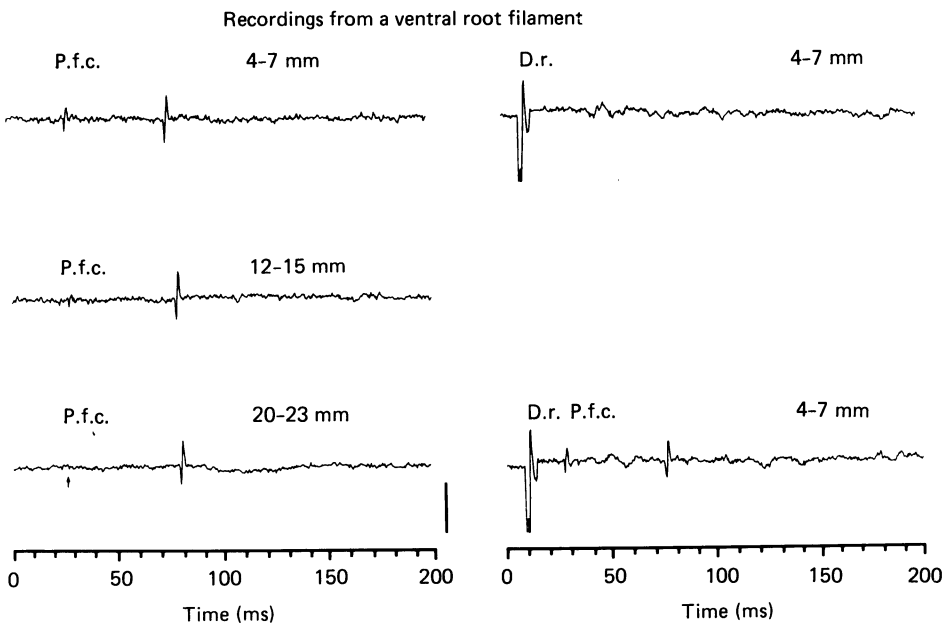

Fig. 4. Average action potentials of an axon of the posterior femoral cutaneous nerve (p.f.c.) led from a ventral root filament. Conduction distance between the stimulating cathode on the p.f.c. and the dura: 58 mm. Left column, potentials recorded with three pairs of electrodes from an S1 filament situated respectively at 4 and 7, 12 and 15, and 20 and 23 mm from the dura following p.f.c. stimulation. Note that the stimulation of S1 dorsal root (d.r.) does not activate this axon in the ventral root (upper trace, right column) nor interfere with the response to the stimulation of p.f.c. when it is applied before this stimulation (lower trace, right column). Calibration same as in Fig. 1.

apparently occurred in a non-recurrent ventral root afferent fibre that probably entered the cord by that route. The second type response was seen at the most distal ventral root recording site only and could be excited by both dorsal root and peripheral nerve stimulation. This response is apparently due to recurrent afferent axons which course for a limited distance in the ventral root and then turn back entering the cord by the dorsal root. The second type is by far the more common in our experience since we observed only once the first type of response which is illustrated in Fig. 4.

In this experiment the posterior femoral cutaneous nerve (p.f.c.) was stimulated at a strength sufficient to excite C fibres and the potential changes recorded by three pairs of electrodes located at 4 and 7, 12 and 15, 20 and 23 mm from the dura (records on left). The action potential, which has essentially the same form in all three records, conducted at a velocity of approximately 2·5 m/s. The axon apparently did not make a recurrent loop since, if there were such a loop, the action potential of a distally

conducted impulse should have been seen at the distal electrode pair. Stimulation of the dorsal root at C strength failed to excite the axon indicating that the axon did not course in the dorsal root; it was most likely a very small myelinated axon.

An example of the second type of response is illustrated in Fig. 5. Stimulation of the posterior biceps semitendinosus (p.b.s.t.) nerve evoked a long latency response in a ventral root filament recorded at 4–7 mm from the dura and a much smaller response at 12–15 mm (Fig. 5, left).

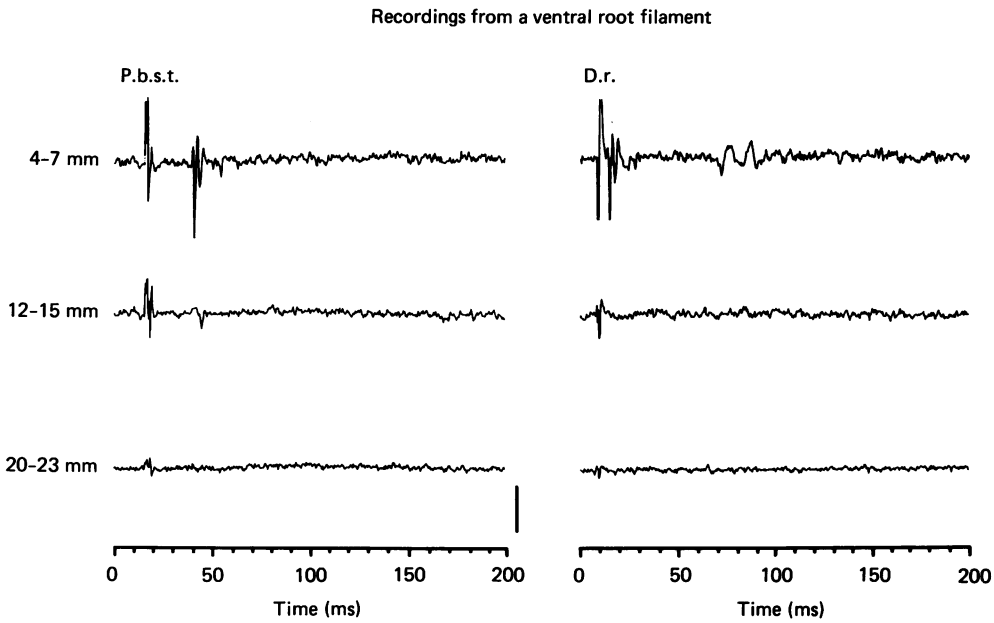

Fig. 5. Average action potentials of an axon of p.b.s.t. led from a ventral root filament. Conduction distance between the stimulating cathode on p.b.s.t. and the dura: 55 mm. Note that in this filament there were no α or γ action potentials. Left column, responses recorded from an S1 filament with three pairs of electrodes respectively situated at 4 and 7, 12 and 15, and 20 and 23 mm from the dura, following p.b.s.t. stimulation. Right column, responses recorded from the same positions but after stimulating the S1 dorsal root (d.r.) (cathode 5 mm from the dura, interelectrode distance 8 mm). Calibration same as in Fig. 1.

At 20–23 mm no action potentials appeared. Dorsal root stimulation at C fibre strength evoked a nearly identical potential complex at 4–7 mm as followed p.b.s.t. stimulation but at shorter latency and no response was seen at the two more proximal electrode pairs. The dorsal root stimulus elicited other action potentials with longer latency at the most distal electrode pair which were not seen following p.b.s.t. stimulation, either because their latency was beyond the sampling period or because their axons had a different peripheral distribution.

This type of response to muscle or skin nerve stimulation was observed in twenty-nine instances. The observation that stimulation of either a peripheral nerve or a dorsal root could elicit a nearly similar action potential in the distal portion of the ventral root filament suggests the presence of a dorsal root fibre which follows a recurrent course within the ventral root. Further evidence is provided by the

collision of such impulses following stimulation of dorsal root and peripheral nerve at appropriate intervals.

Fig. 6 presents the evidence for such a collision. Records *A* and *B* show responses to stimulation of p.b.s.t. and of the dorsal root respectively (same experiment as Fig. 5). When the dorsal root stimulation preceded the p.b.s.t. stimulation by 10 or 30 ms, the response to p.b.s.t. stimulation was abolished (*C*, *D*) showing that the

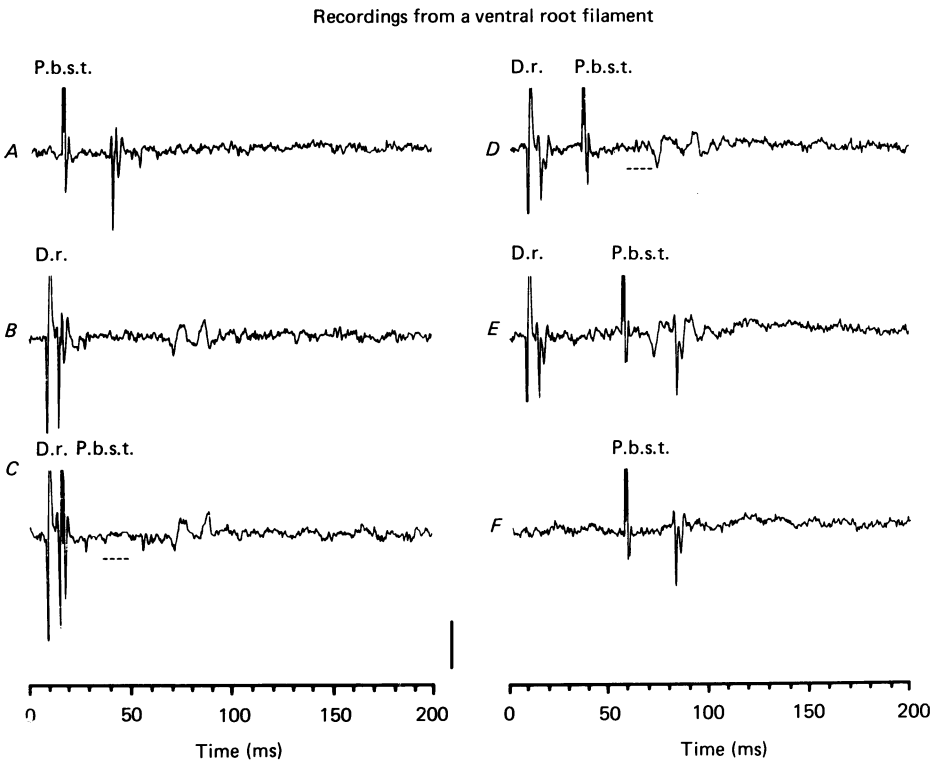

Fig. 6. Occlusion of the response in a ventral root filament to p.b.s.t. stimulation by prior stimulation of the dorsal root (d.r.) (same experiment as in Fig. 5). *A*, stimulation of p.b.s.t. alone; *B*, stimulation of the dorsal root alone; *C*, dorsal root stimulation precedes p.b.s.t. stimulation by 10 ms; note that the response to dorsal root stimulation is distorted by the shock artifact of the p.b.s.t. stimulation and that the response to p.b.s.t. stimulation is abolished; the dashed line under the trace indicates the place where that response should have appeared in the absence of dorsal root stimulation. *D*, dorsal root stimulation precedes p.b.s.t. stimulation by 30 ms; no response to p.b.s.t. stimulation. *E*, when the time interval between the two stimulations is 50 ms, the response to p.b.s.t. stimulation is observed again. *F*, control stimulation of p.b.s.t. alone. Calibration same as in Fig. 1.

impulse generated in the dorsal root and the impulse generated in p.b.s.t. had collided in the axon, somewhere between the recording electrodes on the ventral root filament and the p.b.s.t. At an interval of 50 ms the response reappeared (*E*). Stimulation of p.b.s.t. (*F*) alone then evoked the response again.

In sixteen cases in which nearly similar unitary potentials were found in a ventral root filament following stimulation of a dorsal root and a peripheral nerve (skin or

muscle), ten showed evidence of collision. The inability to test a sufficient range of intervals because of deterioration of the axon may account for the absence of collision in some cases, but it is also possible that nearly similar potentials on stimulation of a dorsal root or peripheral nerve were not due to activity in the same axon.

We have not observed, on stimulating dorsal roots, action potentials propagating over the whole length of the ventral root, such as dorsal root axons supplying the pia-arachnoid via the ventral root would be expected to give.

Effects of sectioning the spinal nerve at various distances from the dorsal root ganglion. In several experiments in which responses were recorded in dorsal root filaments following stimulation of a ventral root, we observed the effect of cutting the spinal nerve progressively closer to the distal pole of the dorsal root ganglion. An example may be seen in Fig. 7. This filament showed multiple action potentials at various

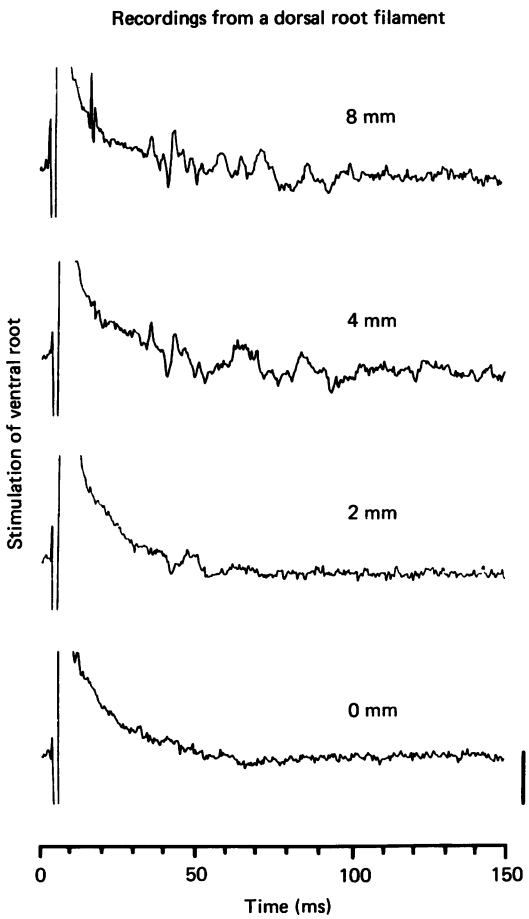

Fig. 7. Effects of sectioning the spinal nerve at progressively closer distances to the dorsal root ganglion on the responses elicited in a dorsal root filament by ventral root stimulation. Stimulation electrodes on ventral root S2 at 5 and 10 mm from the dura; recording electrodes on a dorsal root S2 filament at 4 and 7 mm from the dura. Calibration bar; 1 mV for 100 trials, i.e. 10 μV per trial.

latencies in response to the ventral root stimulation with the spinal nerve cut 8 mm from the ganglion. Following a section 4 mm from the ganglion the earliest response was lost but most of the ensuing action potentials remained. Sectioning at 2 mm from the ganglion abolished nearly all the responses and at the distal pole of the ganglion (0 mm) all responses were abolished. Several possible explanations may be proposed. The dorsal root afferent axons coursing to the ventral root might make their recurrent loop beginning at some distance (several millimetres) distal to the ganglion so that section of the spinal nerve, for example at 2 mm, would destroy the continuity of the axon between dorsal and ventral root (see vertical dashed line in Fig. 8*B*). Another possible explanation is that section at such a level might depolarize the axon sufficiently to block conduction at a more central locus. Also, sections within a few millimetres of the ganglion might interfere with its blood supply, depressing conduction in the structures contained therein.

Anatomical observations

Examination of serial sections from the cut dorsal root and companion spinal ganglion revealed that HRP applied to the crushed end of the distal stump of the root was retrogradely transported along this route. Transport distance along the dorsal roots varied between 25 and 30 mm depending on the ganglion used (L7 being generally shorter than S1). The method of HRP application used in this study provided an intense and immediate impregnation with crystals of enzyme, followed by a sustained delivery with the slow-release gel.

Transport times between 5 and 8 days were chosen to match the slowest axonal flow, in order to allow the marker to go as far as possible along the ventral root.

Plate 1 *A* shows a section through a L7 dorsal root ganglion in which ganglion cells are densely labelled following retrograde transport of HRP along the dorsal root axons. The degree of filling was independent of the size of the cell body and the reaction product remained inside the cell bodies for at least 8 days. The reaction product was also observed in appreciable amounts in the adjacent portion of the spinal nerve, indicating that transganglionic transport of HRP had occurred (Pl. 1 *B*).

Labelling of axons by HRP was also observed in the ventral root close to the ganglion. Plate 1 *C* shows a certain number of labelled axons of various sizes scattered among unlabelled axons, the latter being presumably motor in origin. Some axonal U-turns were observed there, indicating that axons, after a course in the ventral root toward the spinal cord, had taken a reverse direction (see Pl. 1 *A*). Symmetrically disposed axonal U-turns were also observed in the spinal nerve near the ganglion (see Pl. 1 *B*). The labelled axons appeared to be small, but the granular type of labelling attained with the TMB procedure makes it difficult to appreciate the exact size of the axons. For the same reason, it is very difficult to observe with light microscopy the exact course of very fine axons, presumably non-myelinated, observed at some distance from the ganglion. In the 4–6 mm of the ventral root adjacent to the ganglion, most of the reaction product was gathered at the periphery of the root. This labelling disappeared progressively and nearly completely in the more central part of the root; however, at the entrance of the ventral root in the spinal cord, a few faintly labelled axons were observed.

DISCUSSION

The early experiments of Magendie (1822) and Bernard (1858) showed that manipulation of centrally cut ventral roots elicited painful reactions in animals in which the companion dorsal roots were intact. This 'recurrent' sensibility was attributed to afferent axons which entered the ventral root and then turned on a recurrent course to pass via the dorsal root to the spinal cord. Recent studies have shown the presence of a considerable number of non-myelinated axons in ventral roots (Coggeshall, Coulter & Willis, 1974). The number of such axons is greater in the distal portion of the L7 ventral root than more proximally (Risling & Hildebrand, 1982). While some non-myelinated axons have been found to make U-turns in ventral roots, others enter the spinal pia mater (Risling *et al.* 1984). Light & Metz (1978) also showed, by HRP staining, that some afferent axons enter the spinal cord via the ventral roots. Electrophysiological evidence for U-turns of myelinated fibres in rat ventral roots has also been reported by Bostock (1981). Wee, Emery & Blanchard (1985) have recently shown that most of the non-myelinated axons in ventral root L6 of cat degenerated central to, but not distal to, a section of ventral root and that they degenerated following dorsal root ganglionectomy; non-myelinated axons were widely distributed in ventral roots of various segments including those which contained, or did not contain, efferent outflow from the sympathetic and parasympathetic system.

The number of non-myelinated axons in ventral roots increases post-natally (Risling, Hildebrand & Aldskogius, 1981), and is further increased in the kitten following section of the sciatic nerve (Risling, Hildebrand & Cullheim, 1984).

The receptive fields of afferent non-myelinated axons in ventral roots have also been investigated in L7 and S1 (Coggeshall & Ito, 1977) and in S2, S3 and Cd1 (Clifton, Coggeshall, Vance & Willis, 1976). Their receptors are found in viscera and in somatic structures, both in skin and deep structures.

Afferent axons which run in ventral roots may have several types of trajectory as schematically shown in Fig. 8: a large number appear to travel centrally for a limited distance in a ventral root, turn along a recurrent path, and then course to the spinal cord in a dorsal root. Evidence for such a pattern has been noted above and is confirmed in the present study.

In another pattern afferent axons travel centrally in ventral roots to enter the spinal cord (cf. Light & Metz, 1978) and may lack processes in dorsal roots. These axons are very likely responsible for the central effects observed by Voorhoeve & Zwaagstra (1984) after arterial injection of bradykinin in cat low-spinal preparations with complete bilateral rhizotomy. An example of such an axon has been shown in the present study.

Another pattern is that of an axon innervating the pia mater (Risling, Dalsgaard & Cuello, 1984), and passing into the cord via the dorsal root. We did not encounter examples of such axons in our series.

Afferent axons are typically considered to have one peripheral process, passing via the spinal nerve to the periphery, and one central process, coursing to the cord via the dorsal root, both issuing from the stem axon that leads from the cell body in the dorsal root ganglion. However, counts of dorsal root axons and dorsal root ganglion

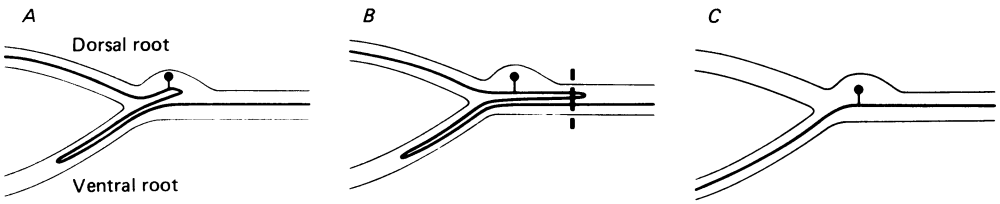

Fig. 8. *A*, dorsal root afferent axon with a recurrent course in the ventral root. *B*, possible course of a recurrent axon within the proximal part of the spinal nerve (see p. 239). *C*, afferent axon entering the spinal cord through the ventral root.

cells show that the former are considerably more numerous than the latter (Chung & Coggeshall, 1984). Hence, some cell bodies must have more than one central process. The increase in numbers of non-myelinated axons in ventral roots in the post-natal period (Risling *et al.* 1981) or following sciatic nerve section (Risling *et al.* 1984) could result from sprouting of dorsal root neurones. These additional processes could take a recurrent course in ventral roots but the possibility must also be considered that some might end blindly. It would be difficult to distinguish between these possibilities in electrophysiological experiments.

Dorsal root C fibres are known to have a smaller diameter central to the dorsal root ganglion than distal (Gasser, 1955). Whether the recurrent processes of dorsal root ganglion cells that travel in ventral roots take origin from the central or distal axonal branch is not known. The effects of sectioning the spinal nerve at points progressively nearer the ganglion (see Fig. 7) can be taken to indicate that the distal process may make the recurrent loop (see Fig. 8*B*) but this evidence is not conclusive. However, axons making U-turns have been observed in the spinal nerve near the ganglion (see Pl. 1*B*).

Most of the afferent axons in ventral roots appear to be of small diameter and to have a nociceptive function. This is in accord with the well known recurrent sensitivity of ventral roots, in which excitation of the distal stump of cut ventral root produces painful reactions. In man the pain produced by such stimulation appears referred to deep structures (Frykholm, Hyde, Norlen & Skoglund, 1953). Coggeshall & Ito (1977) found that nociceptive receptive fields of sensory fibres in L7 and S1 were in skin and deep structures, as well as viscera. In the present study we have found that non-myelinated axons in ventral roots may go to either skin or muscle nerves. Clifton *et al.* (1976) also found that those afferent ventral root axons in S2, S3 and Cd1 going to somatic structures innervated skin and deep structures. Dorsal horn cells that were activated by stimulation of ventral roots were also found to be activated by strong mechanical stimulation of the skin (Chung *et al.* 1985). In this case, however, the responses could have been due to afferent fibres other than those in ventral roots which also converged on the second-order neurone.

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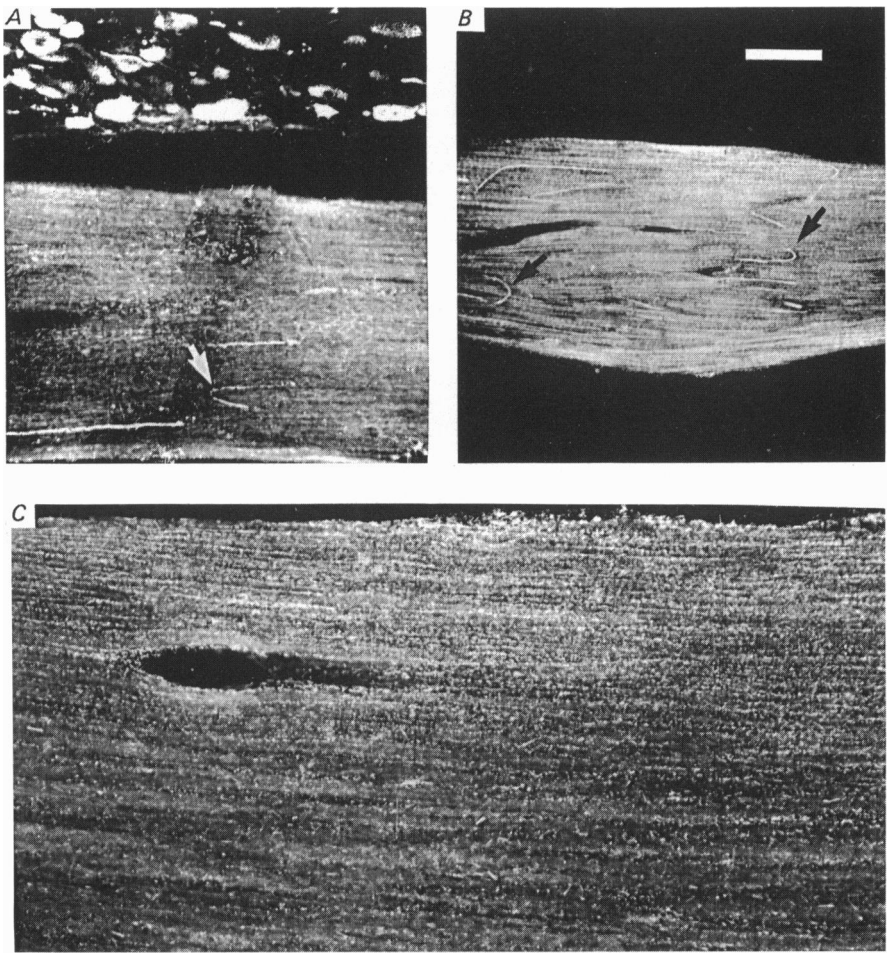
A
B
C

EXPLANATION OF PLATE

Axons in a L7 ventral root and spinal nerve labelled after HRP application at the crushed end of the distal stump of the L7 dorsal root. *A*, 40 μm longitudinal section of the dorsal root ganglion and of the most distal part of the adjacent ventral root. Densely labelled cells are seen in the upper part of the photomicrograph and labelled axons in the ventral root, in the lower part. The arrow points to a U-turn made by one of the axons. Dark field. Calibration in *A* and *B*, 40 μm; in *C*, 20 μm. *B*, 40 μm longitudinal section of the spinal nerve, where transganglionic HRP transport had occurred. Note the U-loops of labelled sensory axons (see arrows) returning toward the ganglion. Dark field. The section is close to the surface of the nerve. *C*, 40 μm longitudinal section of the same ventral root as in *A*, 6 mm central to the ganglion. Note the presence of many labelled axons especially near the edge of the root. Dark field. In each microphotograph the left part of the section is toward the spinal cord.