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A QUANTITATIVE STUDY OF THE CENTRAL PROJECTION PATTERNS OF UNMYELINATED VENTRAL ROOT AFFERENTS IN THE CAT

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SUMMARY

1. The ventral roots of the spinal cord contain a large number of unmyelinated primary afferent neurones. There is some controversy, however, about the function of these fibres and the route of their central projection. Here we have used electrophysiological techniques to quantify the central projection patterns of these neurones in the segment S2 of adult chloralose-anaesthesized cats.

2. A total of 1185 single unmyelinated units were recorded in small filaments isolated from intact and de-efferented ventral roots or intact dorsal roots of the segment S2 in nineteen cats. The projection patterns of these neurones were tested using supramaximal electrical stimulation of the pelvic and pudendal nerve (the main tributaries of the spinal nerve of this segment) and of the segmental companion root (dorsal or ventral as appropriate).

3. The principal finding of this study is that 85% of unmyelinated afferent axons in the ventral root are direct and exclusive projections. They constitute a separate class of afferents which is only capable of transmitting information from the periphery via the ventral roots. However, the proportion of these fibres that enter the central nervous system is unknown and it seems likely that some of them peter out as they approach the spinal cord and end blindly. The functional role of such afferents remains obscure.

4. For the remaining 15% of unmyelinated ventral root afferents, a projection into the segmental dorsal root was found. The majority of those fibres (about two-thirds) are primary afferent neurones innervating the pia mater. Some of these units had a small spot-like receptive field and responded to mechanical stimuli such as pressure and stretch of the root. They did not have axon projections in a peripheral nerve.

5. A few (5%) unmyelinated ventral root fibres are collateral branches of normal primary afferents projecting through the dorsal root. These trifurcating neurones are a small population which make up only some 0·5% of all dorsal root ganglion cells. The functional significance of this population too is unknown.

6. For none of the fibres that projected into both dorsal and ventral root was there positive evidence for the existence of looping axons that merely make a detour into one of the roots. Although the existence of loops cannot completely be excluded, our

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evidence suggests that they can constitute at most 5% of the unmyelinated ventral root afferents.

INTRODUCTION

Since the original descriptions of Bell and Magendie that dorsal and ventral spinal roots subserve different roles, the validity of the law of separation of sensory and motor function has been repeatedly questioned. It was already known to Magendie and has been extensively confirmed since then (see Coggeshall, 1980, 1986; Risling, Hildebrand & Dalsgaard, 1987) that afferent fibres do occur in the ventral roots. It is now established that most of them are unmyelinated (Coggeshall, Coulter & Willis, 1974; Appelbaum, Clifton, Coggeshall, Coulter, Vance & Willis, 1976; Clifton, Coggeshall, Vance & Willis, 1976; Floyd, Koley & Morrison, 1976). There remains, however, some considerable controversy about the nature of these fibres and the route of their central projections. Figure 1 shows diagrammatically a number of hypotheses that have been advanced.

The first possibility (Fig. 1*Ba*) is that ventral root afferents might directly enter the spinal cord, a view mainly based on horseradish peroxidase (HRP) tracing studies showing afferent terminals in the spinal cord after application to the ventral root (Light & Metz, 1978; Nadelhaft, de Groat & Morgan, 1980; Beattle, Bresnahan, Maw & Finn, 1987) as well as retrogradely labelled cells in the dorsal root ganglion after injection into the cord with a lesion of the dorsal roots (Maynard, Leonard, Coulter & Coggeshall, 1977; Yamamoto, Takahashi, Satomi & Ise, 1977). This view was also supported by immunohistochemical observations on presumptive sensory axons using this route (Kawatani, Erdman & de Groat, 1985; Gibson, Polak, Anand, Blank, Yiangon, Su, Terenghi, Katagiri, Morrison, Lumb, Inyama & Bloom, 1986).

However, these results have been challenged on the grounds that the number of unmyelinated fibres in the ventral root decreases towards the spinal cord (Risling & Hildebrand, 1982; Vergara, Oberpaur & Alvarez, 1986) and close ultrastructural examination of the transition zone failed to detect an appreciable number of axons entering the spinal cord (Risling & Hildebrand, 1982; Risling, Dalsgaard, Cukierman & Cuello, 1984). In contrast some fibres appeared to form U-turns, yet others terminated within the spinal pia mater, suggesting that a subpopulation of unmyelinated ventral root fibres may either be loops of dorsal root afferents (Fig. 1*Bd*, *e*), or regular sensory afferents innervating the pia or the ventral surface of the spinal cord (Fig. 1*Bc*; Dalsgaard, Risling & Cuello, 1982; Risling *et al.* 1984; Azerad, Hunt, Laporte, Pollin & Thiesson, 1986).

Another possible anatomical arrangement for unmyelinated ventral root afferents is that these fibres are collateral branches of dorsal root afferents (Fig. 1Bb). There is electrophysiological evidence for centrally bifurcating neurones projecting into both ventral and dorsal root (Kim, Shin & Chung, 1987). This finding has been supported by morphological experiments showing double-labelled ganglion cells after exposure of dorsal and ventral root to different fluorescent dyes (Chung & Kang, 1987; Fang, 1987).

In summary, two main categories of unmyelinated ventral root afferents have been described previously. They are either a type of neurone with an exclusive projection

into the ventral root (Fig. 1*Ba*), or they belong to a group of neurones which also project into the segmental dorsal root, namely loops or branches of dorsal root afferents and units innervating the pia mater (Fig. 1*Bb–e*). The latter anatomical arrangements could explain the phenomenon of recurrent sensitivity. It has been shown that stimulation of the ventral root can produce pain in man (Frykholm, Hyde, Norlén & Skoglund, 1953), elicits pseudoaffective responses in animals (Chung, Kim & Shin, 1986) or activates secondary neurones in the spinal cord (Chung, Lee, Kim & Coggeshall, 1985). Since all these phenomena are abolished by cutting or anaesthetizing the segmental dorsal root, but not by blocking the ventral root centrally to the stimulus, it is believed that a substantial number of ventral root fibres enter the spinal cord by the dorsal roots.

So far, none of these studies have assessed the relative occurrence of each subpopulation and therefore we have addressed this controversy with an electrophysiological approach which allowed us to quantitatively estimate the relative proportions of different anatomical arrangements. We chose to study the S2 ventral root in the cat which receives the main sacral sensory input from the pelvic viscera (Morgan, Nadelhaft & de Groat, 1981; Kawatani, Nagel & de Groat, 1986), since the sacral segments contain a high proportion of ventral root afferents (Coggeshall *et al.* 1974; Appelbaum *et al.* 1976), many of which may have a visceral receptive field (Clifton *et al.* 1976; Floyd *et al.* 1976).

Although our results provide evidence that some unmyelinated ventral root fibres are branches of dorsal root afferents or peripheral processes of pial afferents, the main finding of this paper is that these populations are small, amounting together to no more than 15% of all unmyelinated ventral root afferents. The majority of sensory C fibres in this ventral root appear to be an entirely separate population with no projections in the dorsal root.

METHODS

Anaesthesia and animal maintenance

Nineteen adult cats of both sexes weighing 2·9–4·4 kg were used in the present study. The animals were anesthetized with α-chloralose (50 mg kg^{-1}, I.P.) following induction with ketamine (Ketanest®; 15 mg kg^{-1}, I.M.). Supplementary doses of 5–10 mg kg^{-1} α-chloralose were given intravenously to maintain deep anaesthesia as judged by the persistence of miotic pupils and the absence of heart rate and blood pressure fluctuations. The total amount of anaesthetic during an average duration of 22 h of experimentation was 6·0 $\pm$ 1·0 mg kg^{-1} h^{-1} (mean $\pm$ standard deviation; S.D.). Blood pressure and heart rate were continuously recorded after cannulation of the carotid artery and the mean arterial pressure always exceeded 80 mmHg. Drugs were injected into the external jugular vein; the urinary bladder was catheterized transurethrally to monitor urine excretion. Animals were paralysed with pancuronium (Pancuronium®, 0·15 $\pm$ 0·04 mg kg^{-1} h^{-1}; mean $\pm$ S.D.) and artificially ventilated through a tracheal cannula, keeping the end-expiratory P_{CO_2} at 3–4%. Body core temperature was measured intra-oesophageally and maintained close to 38 °C by an electrical heating pad.

Dissection

The sacral spinal cord and cauda equina were exposed by an extensive lumbosacral laminectomy. Following a long mid-line incision of the dura, the left dorsal root ganglion of the segment S2 was identified. The left part of the dura was reflected to clearly expose the entire length of the roots. Using a dissecting microscope the ventral and dorsal roots were traced from their dura sleeves to

the spinal cord and gently freed from surrounding arachnoidea, leaving the vascular supply of the roots intact. The roots were either lifted on a pair of stimulating electrodes or prepared for recordings (Fig. 1*A*). We did not observe any obvious intradural nervous connections between the ventral or dorsal roots after they had emerged from their dura sleeves.

Using a lateral approach, the pelvic and pudendal nerves, which are the major afferent nerves

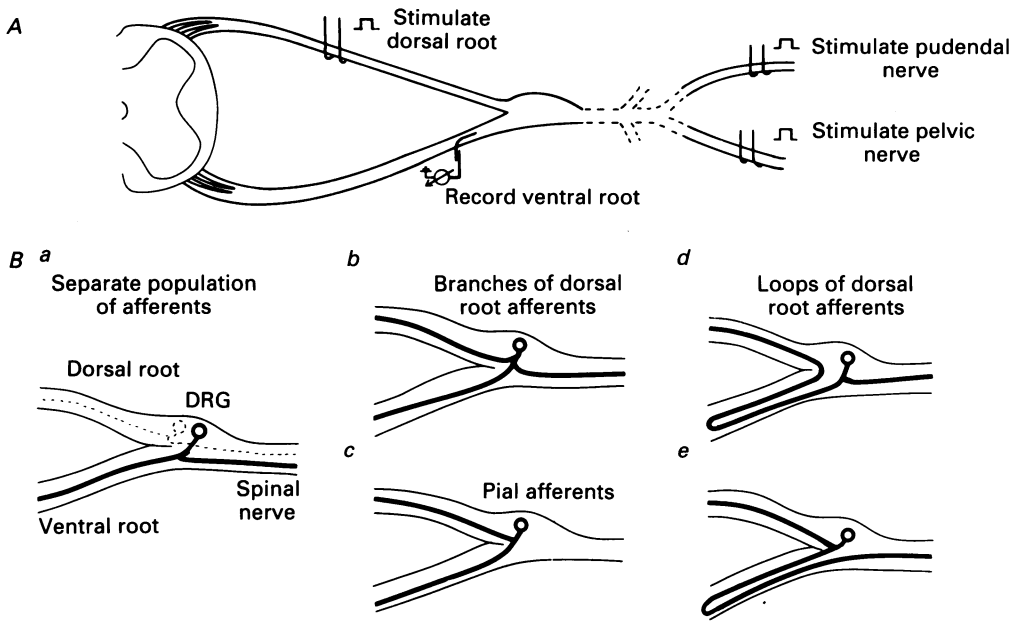

Fig. 1. Experimental set-up and possible anatomical configurations of ventral root afferents. *A*, schematic drawing of the experimental set-up. Fibres were recorded from the ventral root and all microdissected strands were tested with supramaximal electrical stimuli delivered to the pelvic and pudendal nerve and to the segmental companion dorsal root. The recording electrode was positioned close to the dorsal root ganglion (DRG). Afferents were also recorded from the dorsal root at mid-root level with the stimulating electrodes on the companion ventral root (again close to the DRG) and on the peripheral nerves. *B* lists possible anatomical configurations of ventral root afferents. They may either constitute a separate population of afferents with an exclusive projection into the ventral root (*Ba*) or possess some form of dorsal root projection (*Bb–e*). Thus, unmyelinated axons in the ventral root might be the third collateral branch of dorsal root fibres (*Bb*) or the peripheral process of pial afferents (*Bc*). Alternatively they could be the loops of the central (*Bd*) or peripheral (*Be*) processes of dorsal root afferents.

of this segment (see below), were dissected, isolated from surrounding tissue with plastic sheaths and mounted for bipolar electrical stimulation on pairs of platinum electrodes. Subsequently, the whole exposure was covered with warm paraffin oil in a pool made with skin flaps. Particular care was taken to avoid shunting of electrodes by cerebrospinal or extracellular fluid, which had to be repeatedly removed at all stimulation sites during the course of an experiment.

Recording and stimulation technique

Centrally cut filaments from either dorsal or ventral roots were repeatedly split with sharpened watchmaker's forceps on a Perspex platform until single-unit activity could be clearly recorded. Filaments were prepared from several different parts of the root and a large portion of the root was sampled in each experiment. Signals were amplified by a low-noise differential AC preamplifier (input resistance 10 mΩ) and filtered with bandwidths of 70–120 Hz to 1·2–1·5 kHz. Nerves and roots were electrically stimulated with 0·5 ms square-wave pulses at 0·2–0·5 Hz and variable intensities up to 30 V.

Fibres conducting at less than 2·5 m s^{-1}, as estimated over the entire conduction distance, were considered to be unmyelinated. The data obtained from those units were used for the numerical analysis of the various anatomical configurations of ventral root afferents.

The data were directly photographed from the oscilloscope (Nihon Kohden camera PC-2A, Kogyo Co. Ltd, Japan) and stored on magnetic tape (SE 7000, EMI) for off-line analysis.

Recordings from ventral root fibres

Intact ventral roots (experiment A)

Attempts were made to sample the whole population of afferents entering the ventral root. Recordings were made from the distal third of the ventral root at an average distance of 11 ± 5 mm (mean $\pm$ S.D.) from the centre of the dorsal root ganglion in seven cats.

De-efferented ventral roots (experiment B)

In the second series the ventral root of two animals had been chronically de-efferented 4 days prior to the acute experiment to avoid possible contamination of our sample by unmyelinated preganglionic axons. These operations were performed under anaesthesia provided by ketamine (Ketanest®; 15 mg kg^{-1}, I.M.) and diazepam (Valium®; 0·2 mg kg^{-1}, I.M.) for induction and supplementary doses of methohexital (Brevimytal®; 10–20 mg per dose, I.M.). With antiseptic precautions a lumbosacral hemilaminectomy was performed to expose the sacral spinal cord and cauda equina. Following the incision of the dura the ventral root S2 was cut close to its exit from the spinal cord. A ligature was placed around the end of the peripheral stump to facilitate the identification of the root in the terminal experiment. The defect in the dura was covered with a plastic sheath and muscle and skin were closed in layers. Post-operatively, the cats received several subcutaneous injections of pentazocin (Fortral®; 0·3 mg kg^{-1} per dose) during the following 2 days and the recovery was uneventful. Particularly, micturition and defaecation were retained in both animals. In the two acute, terminal experiments, recordings were obtained from the ventral root 9 and 15 mm proximal to the centre of the dorsal root ganglion.

In all nine experiments (A and B) strands were dissected from the ventral root and tested with electrical stimulation of the segmental dorsal root to determine the presence or absence of axon projections. The dorsal root stimulation electrode was positioned at mid-root level, half-way between the dorsal root ganglion and the spinal cord. Pelvic and pudendal nerve stimulation was applied to all strands in order to assess the number of unmyelinated afferents in each filament. The conduction distance from the recording site to pelvic or pudendal nerve stimulation electrodes varied between 30–45 mm and 40–65 mm, respectively.

Recordings from dorsal root fibres (experiment C)

The process of dissecting filaments from the ventral root would inevitably have destroyed most potential loops of dorsal root afferents into the segmental ventral root (Fig. 1*Bd,e*). Therefore, in ten animals recordings were also obtained from the dorsal root. Here, the recording site was half-way between the dorsal root ganglion and the spinal cord. The stimulation electrodes were positioned on the ventral root as close to the dorsal root ganglion as possible. The average distance of the cathode to the proximal pole of the dorsal root ganglion was $3{\cdot}5 \pm 0{\cdot}5$ mm (mean $\pm$ S.D.). This close position to the dorsal root ganglion was chosen in order to detect all potential loops of dorsal root afferents into the ventral root. Great care was taken to avoid current spread to the dorsal root or its ganglion. This precaution was necessary to avoid unwanted stimulus escape to any fibres that did not enter the ventral root proper. In four experiments both pelvic and pudendal nerve were stimulated and in six experiments only the pelvic nerve was stimulated. Conduction distance between recording and stimulation electrodes ranged between 45–60 mm for the pelvic and 65–75 mm for the pudendal nerve.

Quantitative anatomical calculations

For the quantitative analysis of the different anatomical configurations of ventral root afferents some correction factors have to be introduced to compare the different experimental approaches of this study. The introduction of these factors does not change the key conclusions of the present paper, but helps to estimate the accurate percentage for each anatomical arrangement.

Percentage of the activated peripheral afferent input. As evidenced by quantitative anatomical studies using retrograde tracing methods the peripheral nerves which were stimulated in this study

contribute approximately 30% (pelvic) and 40% (pudendal) to the total sensory input of the S2 segment (Morgan *et al.* 1981; Chung & Coggeshall, 1984; Kawatani *et al.* 1986). We did not attempt to stimulate other nerves which project to this segment, such as the dorsal ramus of the spinal nerve or other branches of the lumbosacral plexus. This means for the quantitative analysis that we were activating at most 70% of the segmental peripheral input.

Ratio of afferent and efferent fibres projecting through intact ventral roots (experiment A). The pudendal nerve is a mixed somatic nerve that contains axons of motoneurones, but not of parasympathetic preganglionic neurones (Kawatani *et al.* 1986). Hence, it is reasonable to assume that all unmyelinated ventral root fibres projecting into the pudendal nerve are afferent. However, there is a large number of unmyelinated preganglionic fibres that project through the ventral roots into the pelvic nerves which is the only parasympathetic nerve of the sacral segments. Electronmicroscopic studies have shown that half of the unmyelinated fibres in the sacral ventral roots are afferent (Coggeshall *et al.* 1974; Appelbaum *et al.* 1976). But whereas all unmyelinated efferent fibres (i.e. one-half of the unmyelinated fibre population) would project into the pelvic nerve, only 30% of the remaining afferent half would do so. This means that approximately 25% of those unmyelinated ventral root fibres which project into the pelvic nerve are afferent and 75% efferent (see also Nadelhaft *et al.* 1980).

Contribution of unmyelinated ventral root afferents to the total number of unmyelinated afferents in a segment. Unmyelinated ventral root afferents comprise 5% of the total number of unmyelinated fibres in a segment. This is a conservative and deliberately low estimated calculation. The figure is derived from comparison of electronmicroscopic counts of unmyelinated afferents in both dorsal and ventral lumbosacral roots (Coggeshall *et al.* 1974; Chung & Coggeshall, 1984; Risling *et al.* 1987).

Dichotomizing neurones. It has been shown that very few unmyelinated primary afferent neurones send axon processes into both pelvic and pudendal nerve (Häbler, Jänig & Koltzenburg, 1988). For the numerical analysis those fibres were counted as one peripherally activated unit in the present study. None of the dichotomizing neurones which were recorded in the ventral and dorsal root projected centrally into the companion root.

Statistical evaluation

For a statistical comparison of the conduction velocity of different types of unmyelinated ventral root fibres the Mann–Whitney U test was used. The χ^2 test or Fisher's exact test were employed as appropriate to calculate differences of probability for the occurrence of fibre arrangements between different experimental approaches.

RESULTS

A total of 1185 clearly identified single units conducting at less than 2·5 m s^{-1} were recorded that could be electrically driven from either peripheral nerve or companion spinal root. These units had a defined threshold with an all-or-none characteristic and a stable latency on electrical stimulation with single shocks.

Ventral root recordings (experiments A and B)

Intact ventral roots (experiment A)

When recording from intact ventral roots, all filaments were tested with supramaximal electrical stimuli delivered to the peripheral nerves and the segmental dorsal root. A total of 219 unmyelinated units was recorded: 208 fibres could be electrically activated from the periphery, out of which 26 projected into the pudendal and 182 into the pelvic nerve. Out of all filaments tested, ten contained twelve unmyelinated units that were excited from the companion dorsal root. Three

of these units were recorded in two strands that contained neither pelvic nor pudendal units. The remaining nine unmyelinated axon roots were recorded in eight strands which also contained two to four unmyelinated units which were activated from the pelvic or pudendal nerve. On these occasions a particular effort was made to find evidence for trifurcating neurones that had a branch in a peripheral nerve and central processes in ventral and dorsal roots. In most instances the shape of the action potentials evoked from the dorsal root and peripheral nerve was too dissimilar to make such arrangement plausible (see Fig. 3), but suspicious cases were always subjected to a collision test. Never did we find trifurcating neurones projecting into the dorsal root as well as into the pelvic nerve. Only once did we record from an afferent in the ventral root that sent an axon into both the pudendal nerve and dorsal root. In this instance both processes seemed to be unmyelinated, but the peripheral axon conducted at 1·2 m s^{-1}, three times as fast as the central process in the dorsal root at 0·4 m s^{-1}.

Our peripheral search stimuli activated only some 70% of the total peripheral segmental afferent input (see Methods). Thus our numbers of trifurcating neurones are an underestimate of the true proportion of this type of unit in this segment. None the less, even allowing for the unstimulated peripheral nerves such as the dorsal ramus of the spinal nerve and other branches of the lumbosacral plexus, the number of trifurcating afferents still has to be very small.

Since the pudendal nerve is a somatic nerve, it is reasonable to assume that all twenty-six unmyelinated ventral root fibres were afferent in function. On the other hand 75% of the pelvic units were unmyelinated preganglionic parasympathetic neurones that were antidromically stimulated (see Methods). Thus only every fourth of the 182 recorded unmyelinated fibres, that is forty-five units, can be considered to be afferent in function (Table 1, columns 2–5).

Parenthetically we might mention that we also recorded from two thin myelinated fibres conducting at 5·0 and 5·5 m s^{-1} that projected into the dorsal root, but did not possess a branch in the pelvic or pudendal nerve.

De-efferented ventral roots (experiment B)

In order to avoid contamination of our sample with preganglionic axons, the ventral root had been severed 4 days prior to the acute experiment in two cats; thus all of the units that could be activated from the periphery in these animals were afferent.

Unmyelinated fibres. A total of seventy-six unmyelinated fibres were excited electrically from the pelvic or pudendal nerve. None of these fibres had a dorsal root projection. Stimulation of the dorsal roots activated another six C fibres, but for none of these could we find a peripheral process (Table 1, column 8).

Myelinated afferents. We have concentrated mainly on the unmyelinated ventral root afferents. It would be difficult to make the same quantitative measurements for the myelinated population, because afferents in this fibre group cannot be so easily differentiated from the efferent fibres in the ventral roots of intact animals. De-efferentation of the ventral root, however, overcomes these problems and therefore we include the results from this fibre group here.

We recorded from twenty-two afferents conducting in the Aδ range with a conduction velocity between 2·5 and 25 m s^{-1}. Electrical stimulation of the pelvic and pudendal nerve excited twenty-one fibres, but none of them projected into the segmental dorsal root. A single thin myelinated axon could be stimulated from the dorsal root, though not from the peripheral nerves. This suggests that the anatomical arrangements of thin myelinated and unmyelinated afferents are similar in the ventral roots.

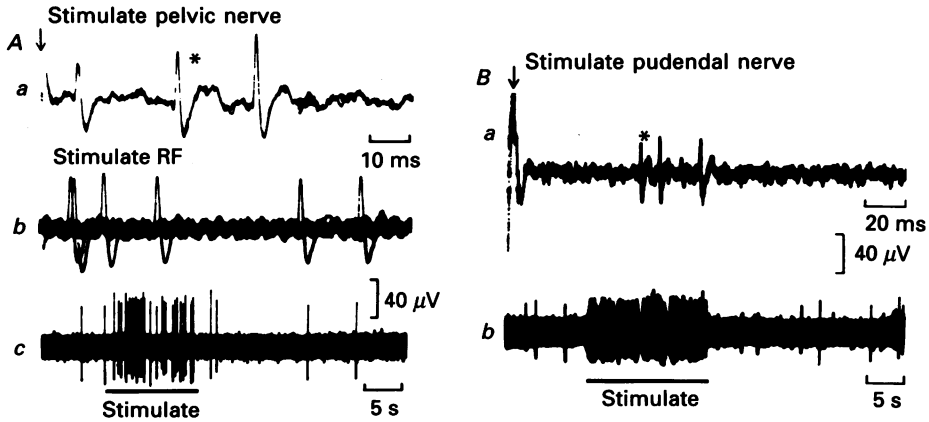

Fig. 2. Examples of unmyelinated afferents of the pelvic (*A*) or pudendal nerve (*B*) recorded from two different filaments of intact ventral roots. *A a*, identification of an unmyelinated fibre (*) with electrical (*A a*) and natural stimulation (*A b*) (both traces are several superimposed sweeps). This unit was activated by an innocuous shearing stimulus applied to its receptive field (RF) in the anal mucosa (*A c*). *B*, example of three unmyelinated afferents which were activated by electrical stimulation of the pudendal nerve (*B a*) (several sweeps superimposed) in a different filament. One of the afferents (*) could be excited by innocuous brushing of its receptive field in the hairy perigenital skin.

Receptive fields

We will not report in detail on the functional properties of ventral root afferents, although we did find receptive fields for both thin myelinated and unmyelinated fibres in intact and lesioned ventral roots.

Pelvic units. The receptive fields of pelvic units were found in the urinary bladder, urethra, colon and anal canal. These are organs that are also innervated by pelvic dorsal root afferents. A representative example of a pelvic unit innervating the mucosa of the anal canal is shown in Fig. 2*A*. The record shows that both unmyelinated units (Fig. 2*A a*) are easily discernible and their action potentials were in fact larger than that of a thin myelinated fibre which was also present in the same filament. The example illustrates that it was clearly possible to compare, on the basis of an identical shape of the action potential, the naturally and electrically evoked activity on a single unit basis (Fig. 2A, a, b). This unit, which had no appreciable resting activity prior to testing, responded to an innocuous shearing stimulus applied to the anal mucosa (Fig. 2*A c*), and the initial response was followed by some after-discharge. The functional receptor characteristics of pelvic units were conspicuously similar to those of dorsal root afferents. Few unmyelinated pelvic

afferents of the dorsal root are mechanosensitive (Häbler, Jänig & Koltzenburg, 1989; Jänig & Koltzenburg, 1989) and this was also found to be true for ventral root units.

Pudendal units. Several low-threshold mechanosensitive unmyelinated pudendal units were observed in our sample. Electrical stimulation of the pudendal nerve less often activated thin myelinated fibres than did stimulation of the pelvic nerve, due to the presence of preganglionic fibres in the latter. On the other hand thick myelinated fibres were regularly found after stimulation of the pudendal nerve, but only rarely when the pelvic nerve was stimulated. In the example shown (Fig. 2*B*) three single unmyelinated units were recorded, one of which responded to light touch of the receptive field in the perigenital hairy skin. Generally, the response characteristics of these unmyelinated units were similar to those of pudendal dorsal root afferents (H.-J. Häbler, W. Jänig & M. Koltzenburg, unpublished observations). However, no attempt was made for a detailed study of the receptor properties of pudendal ventral root afferents, particularly those with high mechanical thresholds.

Other pelvic or pudendal units had no detectable mechanosensitive receptive field, but displayed resting activity, suggesting that they were afferent in function. This corroborates that a considerable portion of our samples of pelvic and pudendal units in the ventral root were indeed afferent.

Spontaneously active units with no peripheral projection

During the course of the experiments on intact and de-efferented ventral roots we occasionally recorded from spontaneously active fibres. Since these fibres could neither be activated from the segmental dorsal root, nor from the pelvic or pudendal nerve, they are not incorporated into the quantitative analysis. As judged by the configuration of the action potential, many appeared to be unmyelinated. One possibility is that they are ventral root afferents with on-going activity which did not project into the pelvic or pudendal nerve. Alternatively they could comprise a group of sympathetic postganglionic fibres that reach their target organs by this route.

Dorsal root recordings (experiment C)

Although recordings from the ventral root would have sampled all types of unmyelinated root fibres, the microdissection technique would nearly always have destroyed potential loops (Fig. 1*Bd*, *e*). In this case, however, the central part of such a neurone would appear as a single unit activated from the companion root with no peripheral counterpart. In fact, our sample contains several such fibres (see above) and they could either be central parts of cut loops or antidromically activated pial afferents.

Moreover, since we were stimulating only some 70% of the peripheral segmental input, a third alternative would be that they are branches of trifurcating neurones which happened to project into a peripheral nerve other than the pelvic or pudendal.

To overcome these ambiguities we recorded in a third series of experiments (experiment C) from the dorsal root and tested all filaments with supramaximal ventral root stimulation which would have excited potential loops, branches or pial afferents. A deliberately low calculation is that 5% of the total number of unmyelinated fibres of a lumbosacral segment of the cat are found in the ventral root

(see Methods). Providing that ventral root fibres have an arrangement other than direct projections we would expect this percentage of fibres within a sample of dorsal root afferents (Fig. 1*Bb–e*).

We isolated 884 unmyelinated units from the dorsal root out of which 873 fibres could be activated only from the periphery. From these 526 were obtained in four experiments with stimulation electrodes on both the pelvic and pudendal nerve. The remaining 347 units were from six experiments where only the pelvic nerve was stimulated.

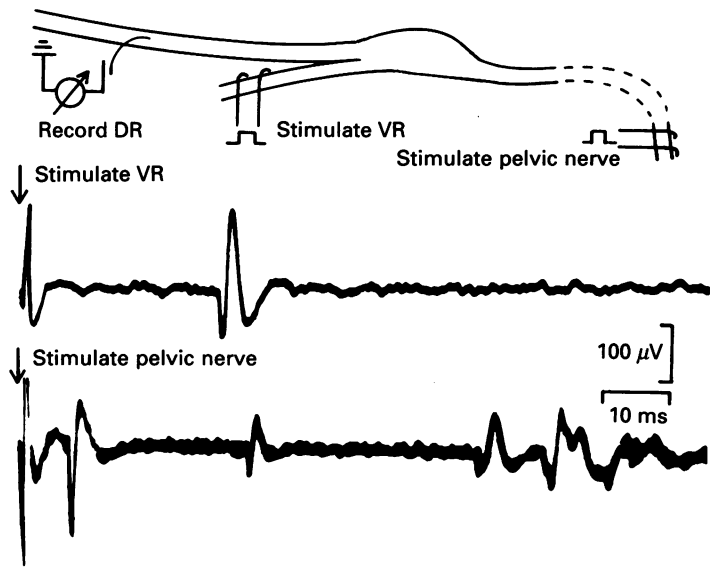

Fig. 3. Example of an unmyelinated axon recorded in the dorsal root (DR) that was activated by electrical stimulation of the ventral root (VR) and did not project with a collateral branch into the pelvic nerve. Upper panel shows the experimental set-up. Electrical stimulation of the pelvic nerve excited one unmyelinated fibre (middle trace). The same filament contained unmyelinated units and one myelinated axon from the pelvic nerve (lower trace). However, the configurations of the action potentials elicited from stimulation of the ventral root was too dissimilar to make the presence of a collateral for the ventral root fibre likely. Both traces are several superimposed sweeps.

Out of all strands tested, again including those strands that did not include peripheral units, only eleven filaments contained fourteen unmyelinated fibres that could be electrically excited from the ventral root. This is clearly much less than the 5% of dorsal fibres that would be expected if all ventral root afferents were collateral branches of dorsal root afferents. Whenever a unit could be activated from the ventral root particular care was taken not to miss any evidence for trifurcation. For eleven units we were unable to find a peripheral branch; in fact, in all of these cases the configuration of the action potential as illustrated in Fig. 3 was clearly too dissimilar to account for such a possibility.

Collateral branches

For only three of the fourteen units projecting into the ventral root could a third collateral branch be found in a peripheral nerve and in each case this was in the

pudendal nerve. Figure 4 shows one example of an unmyelinated branch in the ventral root and a thin myelinated peripheral process. As expected in all three cases the collision test worked bidirectionally. Conduction velocities for the ventral root branches were 0·4, 1·1 and 2·3 m s^{-1}, whereas the corresponding values for the peripheral branch were always faster at 0·8, 2·8 and 5·3 m s^{-1}, respectively. Never did we observe in dorsal root recordings a myelinated branch that projected into the ventral root. The electrical threshold for ventral root fibres ranged from 1·6 to 4·0 V and was somewhat higher for the pudendal nerve.

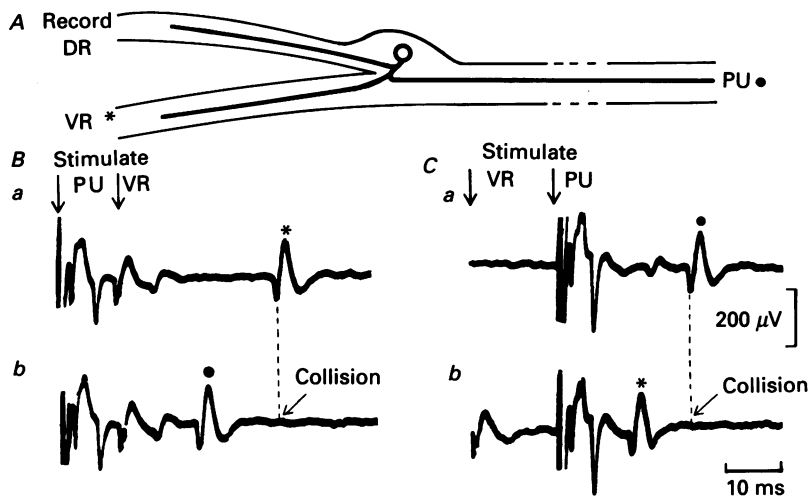

Fig. 4. Example of an afferent neurone that projected into the ventral root, dorsal root and pudendal nerve. *A*, experimental set-up and anatomical arrangement of the trifurcating neurone. The traces in *B* and *C* show the collision test for this neurone. *Ba*, electrical stimulation of the ventral root (VR) and subthreshold stimulation of the pudendal nerve (PU) excited the ventral root branch of this unit (*). When the stimulation intensity of the pudendal nerve was increased the action potential from the branch in the pudendal nerve was evoked (●) and collided with that elicited from the ventral root (*Bb*). This collision test worked bidirectionally (*C*) and the action potential elicited by stimulation of the ventral root collided with the action potential evoked from the pudendal nerve. In *Ca* the ventral root was not stimulated and thus there is no stimulus artifact and stimulation of the pudendal nerve activated the unit. The latency jump in *Cb* occurred when the ventral root was stimulated supramaximally indicating collision. All traces are several superimposed sweeps.

In all cases the ventral root processes represented a true collateral branch rather than a loop of the central or peripheral axon of the afferent (Fig. 1 Bd,e), because the latencies on electrical stimulation were invariably much shorter for the peripheral processes than for the ones in the ventral root.

Afferents of the spinal pia mater

For the remaining eleven units we searched systematically along the ventral and dorsal root for a mechanosensitive receptive field. In order not to interfere with the recording conditions the search was limited to the distal two-thirds of both roots. Our stimuli consisted of gently stroking the roots with a glass rod, slightly pulling the roots or stretching small parts of the root between two glass rods. Before the

application of mechanical stimuli none of the units exhibited resting activity. Four units were activated by one of these stimuli and a representative example is shown in Fig. 5. It illustrates that electrically and naturally evoked activity was clearly discernible (Fig. 5*A* and *C*). This unit responded vigorously when one of its receptive fields was activated. For this unit two separate receptive fields could be found close to the dorsal root ganglion on both the ventral and dorsal root (Fig. 5*B*). Following the initial activation the unit developed some resting activity (Fig. 5*D*).

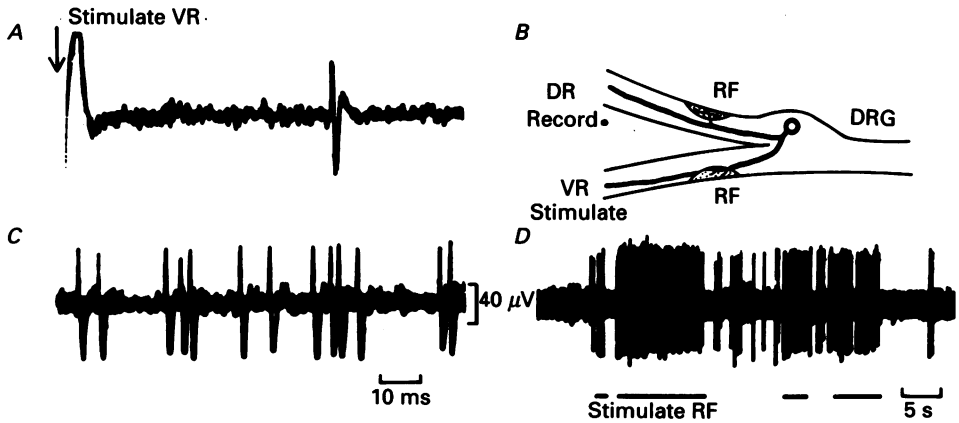

Fig. 5. Electrical and natural identification of an afferent unit innervating the pia of the spinal roots. *B*, experimental design and suggested anatomical arrangement of the afferent unit with a receptive field (RF) in the distal parts of the ventral (VR) and dorsal root (DR). The receptive field of the unit on the ventral roots was proximal to the pair of stimulating electrodes. *A*, electrical stimulation of the ventral root excited an unmyelinated unit that was also activated by stretching the receptive field between two glass rods (*C*). Both traces are several superimposed sweeps. *D*, stimulation of the receptive field on the ventral root by stretching the piece of ventral root between two glass rods vigorously excited the afferent unit and elicited after-discharges.

Conduction velocity

There was a small, but significant, difference in the conduction velocity distributions of the unmyelinated fibre groups studied (Fig. 6). The regular dorsal root afferents from the pelvic and pudendal nerve were the fastest population with a median conduction velocity of 0·80 m s^{-1} ($n = 401$). This was statistically different ($P < 0{\cdot}01$, U test) from ventral root afferents whose median for the conduction velocity was 0·66 m s^{-1} ($n = 102$). To calculate the conduction velocity of the ventral root afferents only values from pudendal units in intact animals ($n = 26$) and all unmyelinated fibres from de-efferented ($n = 76$) animals were used. Those axons conducted slowest which were recorded either in the ventral or dorsal root and projected into the companion root. Their median was 0·51 m s^{-1} ($n = 30$) assuming a straight conduction distance to and from the centre of the dorsal root ganglion. This value differed significantly from the conduction velocity of those afferent axons in the dorsal and ventral roots that could be stimulated by the pelvic or pudendal nerve ($P < 0{\cdot}01$, U test). The distribution of conduction velocities appeared to be a normal distribution for ventral root afferents and fibres projecting between roots.

Virtually all afferents conducted below 1·5 and 1·0 m s^{-1}, respectively. The distribution of dorsal root afferents was slightly skewed, however, as the large majority of unmyelinated afferents also conducted below 1·5 m s^{-1} and very few above 2·0 m s^{-1}. The relative lack of afferents conducting above 2·0 m s^{-1} suggests that there is a gap between a population of unmyelinated and thin myelinated fibres.

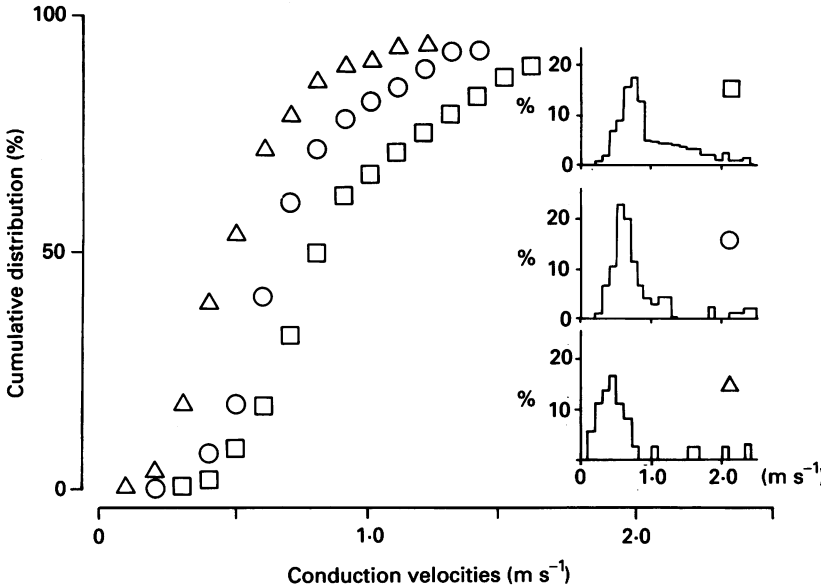

Fig. 6. Distribution of the conduction velocities of unmyelinated dorsal and ventral root afferents and fibres projecting between roots. The values are presented as a cumulative distribution and as histograms (insets). The data are based on 401 dorsal root afferents (□), 102 ventral root afferents (○) and 30 axons (△) that were recorded in either dorsal or ventral roots and projected into the segmental companion root. The sample of unmyelinated ventral root afferents includes the pudendal units from intact animals, and all unmyelinated fibres from animals with de-efferented ventral roots.

DISCUSSION

This study has used an electrophysiological method to quantify the proportions of ventral root afferents with various branching or looping configurations. Although each particular recording and stimulating arrangement is unable to distinguish unequivocally between all the different anatomical arrangements, by combining the results of different experimental approaches we quantitatively estimated the representation of each anatomical type (Table 2). These different arrangements are considered in detail below, but the key conclusion of this paper is that 85 % of ventral root afferents belong to a group of unmyelinated afferents with no projections into the dorsal root. Thus, they constitute a separate class of afferent neurone which is only capable of transmitting information via the ventral roots.

TABLE 1. Summary of the experimental results

Type of experiment (1)	Peripheral origin of units (2)	Number of units activated (3)	Estimated percentage of preganglionics (4)	Estimated number of afferents (5)	Percentage of segmental input tested (6)	Estimated total number of afferents available (7)		Number of afferents activated from companion root (8)	
Experiment A									
Intact ventral root ($n = 7$)	Pudendal	26	0%	26 } 71	70%	(71/70%)	101	Total	12
	Pelvic	182	75%	45 (182/25%)				No peripheral branch	11
								Peripheral branch	1
Experiment B									
De-efferented ventral root ($n = 2$)	Pudendal or pelvic	76	0%	76	70%	(76/70%)	109	Total	6
								No peripheral branch	6
								Peripheral branch	0
Experiment C									
Dorsal root ($n = 10$)	Pudendal or pelvic	526	0%	526	70%	(526/70%)	751 } 1908	Total	14
	Pelvic only	347	0%	347	30%	(347/30%)	1157	No peripheral branch	11
								Peripheral branch	3

For each experimental approach (column 1), the numbers of recorded unmyelinated fibres that responded to electrical stimulation of a peripheral nerve (column 3) are given separately for pelvic or pudendal units or are pooled (column 2). For each experimental approach the estimated percentage of unmyelinated preganglionics that was present in the sample is given (column 4). This correction factor has been used to estimate the actual number of recorded afferents (column 5). Only the number of pelvic units studied in intact ventral root (experiment A) had to be corrected, as this sample contained unmyelinated preganglionic parasympathetic axons. To the total segmental afferent input of the segment S2 the pelvic nerve contributes 30% and the pudendal nerve 40%. These values are used to estimate the total number of afferents that would have been present in the filaments, if the entire segmental input were tested. The correction factor (column 6) was 70%, if both pelvic or pudendal nerve stimulation was tested or 30% in those experiments when only the pelvic was stimulated. The estimated total number of unmyelinated afferents (column 7) can be used for the numerical comparison with the number of units that projected between roots (column 8).

Branch point conduction block is unlikely to distort the results

One assumption of this study is that the electrophysiological methods will reveal the true configuration of afferent branches and loops. However, it could be argued that transmission block of action potentials at branch points might distort our

TABLE 2. Estimates of the relative numbers of ventral root afferent fibres in the S2 root with different anatomical configurations

Afferents with no dorsal root branch	Trifurcating afferents	Pial afferents	Loops of dorsal root afferents
85%	5%	> 5%	< 5%, if any

Synopsis of the relative percentage of each anatomical arrangement is the combination of the results from all three experimental approaches.

interpretation. In fact it has been speculated that sensory neurones could use this mechanism to selectively channel the transmission of action potentials into different daughter branches (Coggeshall, 1986). For the results of the present study we do not consider this to be likely for a number of reasons: firstly, branch point conduction block is not relevant for looping axons (Fig. 1*Bd*,*e*). Secondly, a branch point block would be expected to occur when an action potential runs from a smaller axon into a larger one (Parnas, 1979). The only anatomical arrangement for which this consideration is relevant is trifurcating afferent neurones (Fig. 1*Bb*). Ultrastructural examination of unmyelinated afferents has shown that the diameter is generally smaller in the ventral than in the dorsal root (Fadić, Vergara & Alvarez, 1985; Vergara *et al.* 1986). These findings have been confirmed by electrophysiological means in the present study showing a lower conduction velocity for afferent axons of the ventral root as compared to the dorsal root (Fig. 6). Thus a branch point conduction block should be expected to occur more often for recordings from dorsal roots (experiment C) than from ventral roots (experiment A). Yet, our measurements do not support this. In dorsal root recordings (experiment C) three out of fourteen units (21%) that projected into the companion root were found to be trifurcating neurones, whereas the ratio for ventral root recordings (experiment A) was one out of twelve (8%) (Table 1). These values are not statistically different ($P > 0{\cdot}03$; Fisher's exact test) and suggest that if conduction block was occurring it would have to be as prevalent in dorsal to ventral root conduction as vice versa.

Numerical calculations

Table 1 shows the numbers of units recorded in each type of experiment, in response to stimulation of peripheral nerves or the companion root (dorsal or ventral, as appropriate). In all three experimental approaches all potential projections between roots of this segment were available for electrical stimulation from the companion root. On the other hand only a fraction of the total peripheral input to the segment S2 was activated by an electrical stimulus applied to the peripheral nerves. Quantitative anatomical studies have shown for the segment S2 that 30% of the neurones project into the pelvic and 40% into the pudendal nerve (Morgan *et al.*

1981; Chung & Coggeshall, 1984; Kawantani *et al.* 1986). Since at most 70% of the peripheral segmental input was available for electrical stimulation, it is necessary to extrapolate to the total number of afferents that were present in the recording strands in order to make a comparison with the numbers of units which were driven from the companion root. This figure gives the true number of neurones with peripheral processes which were potentially available for activation from the companion root. The calculation, undertaken in Table 1, is based on the reasonable assumption that recording technique does not introduce a great sampling bias for unmyelinated afferents projecting through particular peripheral (e.g. pelvic or pudendal) nerves.

Since we deliberately recorded filaments from several different parts of the roots and as we sampled a large portion of the root in each experiment, it seems unlikely that our recording procedure missed systematically any particular anatomical configuration. Careful ultrastural studies have shown that many unmyelinated fibres tend to assume a superficial position in root fascicles close to or in the transition zone of the ventral root and spinal cord proper (Risling *et al.* 1984). However, such preferential grouping of fibres has been shown not to exist close to the dorsal root ganglion where our recording or stimulation electrodes were positioned.

Conduction velocity of unmyelinated afferents

The diameter for unmyelinated axons of dorsal root ganglion cells is largest in the peripheral nerve, smallest in the ventral roots, with dorsal roots assuming an intermediate position (Fadić *et al.* 1985; Lee, Chung, Chung & Coggeshall, 1986; Vergara *et al.* 1986). This has been fully corroborated by the present study which shows that conduction velocity of ventral root afferents is significantly lower than that of dorsal root afferents. The lowest value was estimated for the direct projections of ventral and dorsal root.

The reason to choose a conduction velocity of 2·5 m s^{-1} as a cut-off point between thin myelinated and unmyelinated fibres follows convention. However, our measurements show that virtually all ventral root afferents with a projection in the peripheral nerve conducted at less than 1·5 m s^{-1} and that the distribution of these conduction velocities follows a normal distribution (Fig. 6). Thus it is likely that we have not missed a substantial proportion of unmyelinated afferents by selection of inappropriately low cut-off point. The same applies to the dorsal root fibres whose distribution of the conduction velocity is slightly skewed, although there are only very few axons conducting faster than 2·0 m s^{-1}.

Most ventral root afferents do not project into the dorsal root (Fig. 1Ba)

Recordings from intact ventral (experiment A) or dorsal roots (experiment C) allow separate calculations of the relative occurrence of two main categories of unmyelinated ventral root afferents. They either fall into a group of neurones which project also into dorsal roots, namely loops or branches of dorsal root afferents, or units innervating the pia mater (Fig. 1*Bb–c*). Alternatively they comprise a group of units with direct and exclusive projections into the ventral root (Fig. 1*Ba*). All three types of experiments that we have performed agree that the latter possibility is by far the most prevalent anatomical arrangement.

Quantitative analysis. Filaments recorded from intact ventral roots (experiment A) contained an estimated total of 101 afferents (Table 1, column 7). Supposing that all ventral root affferents had a projection into the dorsal root we would expect to activate as many units from the dorsal root as from the peripheral nerve. These units should either appear as collateral branches of ventral root fibres (Fig. 1*Bb*) or, alternatively, as single units that were either antidromically activated pial afferents (Fig. 1*Bc*) or part of loops (Fig. 1*Bd*,*e*). However, only twelve units (Table 1, column 8) projected into the dorsal root. Thus for eighty-nine units no projection into the dorsal root could be detected and therefore 88% of the units are estimated to have an exclusive ventral root projection. The remaining 12% of the fibres were either branches (see below), antidromically excited pial afferents or parts of cut loops.

Based on recordings from chronically de-efferented ventral roots (experiment B) a similar conclusion is drawn. For 103 neurones only six units with dorsal root projections were recorded. Hence 94% of the units were calculated to possess no projection in the segmental dorsal root ($P > 0{\cdot}1$; χ^2 test compared to values for experiment A).

Finally, the results from dorsal root recordings (experiment C) yield a percentage of 85% for this group. In this experiment roughly 1900 units were estimated to be excited from the periphery (Table 1, column 7). Taking into account the conservative estimation that 5% of the unmyelinated afferent fibres of a segment are found in the ventral roots (Coggeshall *et al.* 1974; Chung & Coggeshall, 1984; Risling *et al.* 1987) we should have recorded from ninety-five ventral root afferents, providing all neurones had a dorsal root projection. However, only fourteen neurones did project into the ventral root (Table 1, column 8). Therefore, the maximal percentage for unmyelinated ventral root neurones that could have been loops, branches or pial afferents is of the order of 15%.

Thus all experimental approaches arrive independently at the conclusion that 15% or less of the unmyelinated ventral root afferents have an anatomical configuration other than a direct projection.

Do ventral root afferents enter the spinal cord? Whether the afferent fibres in the ventral root without projection into the dorsal root will eventually enter the spinal cord is beyond the scope of the technique used. Extensive ultrastructural studies have demonstrated that unmyelinated axons do not enter the spinal cord in appreciable numbers (Risling & Hildebrand, 1982; Risling *et al.* 1984). It is unclear whether the same applies for thin myelinated afferent fibres which are also present in the ventral root (see Results; Coggeshall *et al.* 1974; Clifton *et al.* 1976; Floyd *et al.* 1976). On one hand, physiological studies have repeatedly failed to elicit pseudoaffective responses or to activate secondary spinal interneurones by stimulation of the central stump of the cut ventral root (Chung *et al.* 1985, 1986). This shows that very few afferents enter the spinal cord through the ventral root or that these afferents do not have a prominent central effect. On the other hand, anatomical evidence still favours the view that at least some afferent fibres use this route as entry (Maynard *et al.* 1977; Yamamoto *et al.* 1977; Light & Metz, 1978; Nadelhaft *et al.* 1980; Kawatani *et al.* 1985; Gibson *et al.* 1986; Beattie *et al.* 1987).

What is the function of unmyelinated ventral root afferents that do not enter the spinal cord? If unmyelinated ventral root afferents fail to enter the spinal cord, there is a

question as to their function. It could be argued that most of these axons were misled during ontogeny and therefore could not form their appropriate central connections. This notion is difficult to reconcile with the fact that the number of unmyelinated axon profiles continues to rise in lumbar ventral root for several months postnatally (Risling, Hildebrand & Aldskogius, 1981).

Another possibility is that many of those unmyelinated fibres which appear in the ventral root postnatally are recurrent sprouts of the peripheral process of dorsal root ganglion cells that might have failed to find their appropriate peripheral target tissue. This idea is supported by the dramatic invasion of ventral roots and pia mater by unmyelinated fibres after neonatal peripheral nerve transsection (see Risling *et al.* 1987). Assuming unmyelinated ventral root fibres in adult animals were the recurrent sprouts of dorsal root afferents, our technique would have revealed such arrangement. Under these circumstances all ventral root afferents would be excited by electrical stimulation of the dorsal root.

Electron microscopy has shown that there is a steady decline of the numbers of unmyelinated axon profiles along the course of the ventral root (Risling & Hildebrand, 1982) and a dramatic drop in the transition zone between root and spinal cord proper. Therefore an explanation is that many unmyelinated afferents peter out along the course of the ventral root or reach the ventral surface of the spinal cord. This would not be an idiosyncratic feature of the ventral root afferents, since blind-ending unmyelinated axons which do not enter the spinal cord have also been described in the dorsal roots (Carlstedt, 1977). To assign a proper functional role to such a type of afferent neurone appears to be a purely speculative task. Providing the central terminals would release some active substance on stimulation of the peripheral receptor, one could further speculate that this might elicit changes of pial blood flow or exert some trophic influence. Substances would possibly also be released into the cerebrospinal fluid and might have more generalized effects. In fact, the release of neuropeptides such as substance P has been measured on the pial surface of the dorsal horn following noxious stimulation of the hindlimb (Duggan, Hendry, Morton, Hutchison & Zhao, 1988). However, it is also possible that the neurones have mainly a peripheral role and no central effect. The release of neuroactive substances from the peripheral endings would result in vasodilatation and neurogenic oedema without concomitantly eliciting the central action that is thought to follow the activation of unmyelinated dorsal root afferents.

Some ventral root afferents do enter the spinal cord

Although most ventral roots afferents apparently do not enter the spinal cord there is good evidence that some afferent ventral root fibres are connected to the CNS by some form of dorsal root projection (Fig. 1*Bb–e*). This includes branches or loops of dorsal root afferents and pial afferents. Activation of this fibre group could elicit the phenomenon of recurrent sensitivity (Frykholm *et al.* 1953; Chung *et al.* 1985, 1986). The results of the present study indicate that they are a small minority of the ventral root afferents and their relative contribution are discussed below.

Double-labelling studies have provided conflicting results when fluorescent dyes were applied to ventral and dorsal roots of rats (Chung & Kang, 1987; Fang, 1987). The population of double-labelled dorsal root ganglion neurones would comprise

trifurcating neurones (Fig. 1*Bb*), but would also include pial afferents (Fig. 1*Bc*) and potentially some loop arrangements (Fig. 1*Be*). However, the quantitative estimation for this type of ventral root afferents varies from more than a third (Chung & Kang, 1987) to only few (Fang, 1987). In these studies the number of neurones labelled from the ventral root ranged from 2% (Chung & Kang, 1987) to 9% (Fang, 1987) of all dorsal root ganglion cells.

Few ventral root afferents are trifurcated (Fig. 1Bb)

Trifurcating afferent neurones were detectable in all three types of experiment. In animals with intact ventral roots (experiment A), only one branched fibre was seen from an estimated sample of 101 peripherally activated central root afferents (1·0%). In animals with chronically de-efferented ventral roots (experiment B), none of the 109 ventral root afferents had this branching pattern. In dorsal root recordings (experiment C), 1908 afferents were estimated to be in the recorded strands and only three had branches in the ventral root (0·16%). Of course there are only about 5–10% as many ventral root afferents as dorsal root afferents in the segment S2. Allowing for this, trifurcating neurones represent up to 3·3% of all recorded ventral root afferents in this type of experiment. As the peripheral search stimulus maximally activated 70% of the afferent input to this segment additional allowance has to be made for trifurcating neurones that did not project into the pelvic or pudendal nerve. Thus the true proportion of trifurcating axons amongst ventral root afferents will be of the order of 5%. Therefore the three kinds of experiments taken together suggest that this form of anatomical arrangement is relatively rare. It leaves a margin of 10% for the other anatomical constellations, namely loops of dorsal root afferents or pial afferents.

Trifurcating dorsal root ganglion cells with unmyelinated axons have been reported in previous electrophysiological studies using averaging techniques (Kim *et al.* 1987). These workers selectively studied those ventral root fibres with a dorsal root projection. Using collision tests they found that this subpopulation contained a good third with an additional projection into a peripheral nerve. This is in good agreement with the results of the present study (Table 2.).

Amongst the total number of dorsal root ganglion cells the proportion of trifurcating neurones is small. Those neurones projecting into ventral and dorsal root comprise less than 0·5% of the cells. Interestingly, a similar quota is found for those primary afferents of the segment S2 which project into both pelvic and pudendal nerve (Häbler *et al.* 1988). While the functional significance of such a small population of neurones remains obscure, one may speculate that the dichotomizing branches of those neurones were misled during ontogeny.

Some ventral root afferents innervate the spinal pia mater (Fig. 1Bc)

Direct evidence for this type of neurone could only be obtained in recordings from the dorsal root. In four of fourteen neurones that projected into the ventral root a mechanosensitive receptive field on the pia mater had been found. This also means that at least 4% of the ventral root afferents belong to this type of neurone. For two reasons it is obvious that the true proportion of pial afferents must be higher: firstly, the search stimulus for a receptive field was limited to the distal parts of the ventral

root. Secondly, only mechanical stimuli were tested and it is possible that not all of the pial afferents respond to this stimulus. Candidates for other pial afferents could be amongst the population of neurones that projected into the ventral root, but could not be activated by electrical stimulation of the peripheral nerves. Thus, pial afferents could maximally contribute roughly 12% to all unmyelinated ventral root fibres.

Recordings from the ventral root are in keeping with this conclusion. In these experiments pial afferents would appear as antidromically activated axons without a third branch in a peripheral nerve. In fact most neurones that projected into the dorsal root fall into this category: in the first series of recordings from the intact ventral root (experiment A), eleven axons were activated from the dorsal root. At the same time an estimated 101 ventral root afferents with peripheral branches were recorded. Therefore the upper limit for pial afferents, as a proportion of ventral root afferents, can be calculated to be 11%.

Previous histological studies have revealed the presence of unmyelinated fibres that supply the spinal pia mater (see Coggeshall, 1986; Risling *et al.* 1987). Since a subpopulation of these fibres contain the peptide substance P, it is likely that they are the peripheral branches of primary afferent neurones and have a sensory role (Dalsgaard *et al.* 1982; Risling *et al.* 1984). Some of these axons have been found in close association with pial blood vessels (Risling *et al.* 1984). This invites speculation that they could influence pial blood vessels, notably producing vasodilatation, by the peripheral release of their peptides (Edvinsson, McCulloch & Uddman, 1982; Moskowitz, Brody & Liu-Chen, 1983).

Another small subpopulation of unmyelinated ventral root fibres possesses catecholamine fluorescence (Stevens, Hodge & Apkarian, 1983) or neuropeptide Y-like immunoreactivity (Risling, Dalsgaard & Terenius, 1985) and surrounds pial blood vessels. Thus, it is likely that some unmyelinated axons in the ventral root are the terminal branches of postganglionic sympathetic fibres that innervate pial blood vessels. The finding that some, possibly unmyelinated, fibres recorded in the ventral root displayed on-going activity, but lacked a process in the peripheral nerves tested, is in keeping with an efferent sympathetic function of these fibres. However, this population is very small, because sympathectomy failed to produce an appreciable reduction of unmyelinated axon profiles in the ventral root (Coggeshall *et al.* 1974).

Loops of ventral root afferents are rare, if not absent (Fig. 1Bd, e)

As mentioned above, the number of dorsal root axons with loops extending even a short way into the ventral root must be very limited. In fact, we have not found direct evidence for one example of this configuration and the results of the present study provide at best indirect evidence for this arrangement. The only candidates for this configuration are those neurones for whieh neither a receptive field on the pia mater nor a collateral peripheral branch has been detected. In the recordings from the dorsal root (experiment C) only seven out of the fourteen neurones could fall into this category. Yet, for none of these neurones could a branch in the peripheral nerve be found. Since a branch point conduction block does not apply for these arrangements (see above) and unless the assumption is made that looping axons project exclusively into nerves other than the pelvic or pudendal this configuration

appears to be very rare, if not absent. It is worth noting that none of the three dorsal root fibres with positively identified collateral branches in peripheral nerve and ventral root was a looped axon, because the latency of activation following electrical stimulation was always longer from the ventral root than from the peripheral nerve. Thus it seems more plausible that the seven neurones were either trifurcating neurones that happened not to project into the pelvic or pudendal nerve or were pial afferents for which the receptive field could not be demonstrated. The results of the ventral root recordings agree entirely with these conclusions. The maximal figures for potential loops as derived from experiment A (intact ventral root) and B (de-efferented root) are less than 11% (11/101) or 6% (6/109) respectively. These figures, however, also include trifurcating neurones whose peripheral process was not excited by the peripheral stimulation and also antidromically activated axons of pial afferents.

The finding that few ventral root afferents are loops of dorsal root afferents is in agreement with previous studies. Azerad *et al.* (1986) bipolarly recorded potentials over a distance of tens of millimetres at several locations along ventral root filaments that responded to electrical stimulation of the dorsal root using an averaging technique (similar to an arrangement shown in Fig. 1*A*). They recorded potentials close to the dorsal root ganglion which were absent at more proximal recording sites. However, such a result would also be expected for trifurcating neurones (Fig. 1*Bb*) that peter out along their projection in the ventral root or terminate to innervate the pia mater (Fig. 1*Bc*).

Very persuasive evidence for the existence of looping configurations comes from anatomical studies (Risling *et al.* 1984; Azerad *et al.* 1986). Using different methods these studies agree that some fibres in the ventral root form hairpin loops. However, for technical reasons these fibres could be followed only over a short distance and it is therefore unclear from where these fibres originate or indeed to where they project. Explanations that reconcile these anatomical findings with the present electrophysiological results are that looping dorsal root fibres are very rare or that fibres that form U-turns in the ventral root do not enter the segmental dorsal root. Instead these anatomical structures might possibly be part of the terminal arborization of pial afferents. Further, it has been reported that there is no great drop in the unmyelinated axon count in the distal stump following transection of the ventral roots (Coggeshall *et al.* 1974). However, this should be expected when either the central (Fig. 1*Bd*) or peripheral (Fig. 1*Be*) part of the loop would be isolated from its cell body and would degenerate.

Conclusions

In conclusion (Table 2), the majority of unmyelinated ventral root afferents constitute a separate population of primary afferent neurones with an exclusive projection into the ventral root. It is unlikely that these afferents enter the spinal cord; instead they may peter out along their course in the ventral root. The function of these neurones remains obscure. Few ventral root afferents are the collateral central processes of trifurcating dorsal root afferents that send axons into both roots and peripheral nerve, although the branch in the ventral roots probably does not enter the spinal cord. Most ventral root units that do project into the dorsal root are

primary afferents innervating the pia mater. It appears that they use the ventral root as a pathway to reach their appropriate target tissue. Loops of dorsal root afferents into the ventral root are rare, if not absent.

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