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Long Range Afferents in Rat Spinal Cord. III. Failure of Impulse Transmission in Axons and Relief of the Failure after Rhizotomy of Dorsal Roots

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Long range afferents in rat spinal cord. III. Failure of impulse transmission in axons and relief of the failure after rhizotomy of dorsal roots

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SUMMARY

Dorsal root afferents entering the spinal cord form a T-junction with a caudal branch descending many segments and giving off side branches terminating in the dorsal horn. This anatomical finding contrasts with the physiological observation that the postsynaptic effects of arriving afferents in the dorsal horn are limited to a few segments on either side of the root carrying the input. This paper explores the possibility that one explanation for this paradox is that orthodromic impulse conduction fails to penetrate the long range parts of the caudal branch. The experiments show that when all roots are intact, very few fibres can be detected carrying orthodromic impulses as far as 20 mm caudal to the entry point. After section of neighbouring dorsal roots, however, large numbers of conducting fibres can be recorded at that point. Signs of orthodromic conduction begin 7 days after root section. These results were confirmed by another method which compared the relative refractory period of the membrane of the descending branch produced either after a local stimulus had evoked an action potential or after a rostral distant stimulus had produced an orthodromic action potential. Again, in the intact cord, the results indicate that impulses fail to penetrate long distances into the descending branches but that, as soon as 2 days after rhizotomy in the area of suspected conduction failure, orthodromic conduction is restored. It is proposed that the failure and release of conduction may depend on the control of membrane potential in the primary afferents, which would form a pre-presynaptic control mechanism.

1. INTRODUCTION

As myelinated sensory afferents enter the spinal cord, they branch at a T-junction to form a branch running rostrally and one running caudally. The existence of the rostral branch is of no surprise as many of these fibres eventually terminate in the dorsal column nuclei. However the presence of the caudal branch is more of a puzzle as there is no distant destination equivalent to the dorsal column nuclei. Substantial numbers of arriving fibres send caudal branches over long distances as shown by both anatomical and physiological methods reviewed in Wall & Shortland (1991). Twenty-seven percent of entering axons extend at least 10 mm caudally in the rat and 4.5% reach 25 mm which is 11 segments caudal to the entry point. As these axons descend caudally, they emit side branches which terminate in the grey matter of the dorsal horn (Shortland & Wall 1992). One would therefore expect that signs of the arrival of impulses in long range, descending afferents would be detected postsynaptically in dorsal horn cells. However, this has failed to be observed in studies using either extracellular recording of action potentials or intracellular recording of postsynaptic potentials (Brown 1981; Woolf & Fitzgerald 1986; Wilson *et al.* 1989; Woolf & King 1989; Shortland & Fitzgerald 1991). The receptive fields of cells in the dorsal horn fit precisely the proposal that the cells are only excited by afferents arriving over nearby dorsal roots. For example, in the S1 segment which we have particularly studied here, the receptive fields of cells show no signs of response to inputs rostral to the L5 dorsal root (Woolf & Fitzgerald 1986; Shortland & Fitzgerald 1991) and yet we show that there are substantial numbers of afferents present which originate from dorsal roots at least as far away as the L1 root (Wall & Shortland 1991; Shortland & Wall 1992). The only exception to this commonly reported discrete origin of dorsal horn cell functional input is the presence of a small group of cells in the extreme lateral edge of the dorsal horn (Devor & Wall 1976). However, these cells have only proximal receptive fields and we know that some of the long range afferents originate from very distal nerves such as the sural nerve (Wall & Shortland 1991). Furthermore, there is no evidence that these very large receptive fields are formed by monosynaptic contacts of afferents on the recorded cells. We are faced with a considerable paradox in that there is clear evidence for the presence of long range branches of afferent fibres although there is no evidence that cells in the region of the terminals of these fibres respond to them.

One possible explanation for this paradox is that

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orthodromic impulses fail to be conducted into the distant parts of these long range axons. This paper examines the orthodromic conduction of impulses into the caudal branch of long range afferents by two methods. Both methods show that substantial numbers of axons fail to conduct an orthodromic impulse to a point 20 mm caudal to their entry point in intact cords. This negative evidence becomes more convincing after a subsequent set of experiments which find that these axons do succeed in conducting impulses 20 mm caudally after axons from neighbouring dorsal roots have degenerated. In the discussion, we show that the proposal that orthodromic impulses may block at branch points is not without precedent. We also discuss the control of membrane potential as a possible mechanism for the blockade of orthodromic impulse transmission. An abstract of preliminary results from one of the two methods has been published (Wall & McMahon 1991).

2. METHODS

All experiments were done during the lights-on period on male Sprague-Dawley rats, weighing 200–350 g and maintained on a 12h:12h light:dark cycle. In all acute experiments, the animals were deeply anaesthetized with intraperitoneal urethane (1.25 g kg^{-1} body mass). One carotid artery and the trachea were cannulated and temperature, ECG and expired CO_2 were monitored and maintained within normal levels. The spinal cord was transected at T10 to eliminate descending effects on the lumbar and sacral cord and to prevent CSF flooding the exposed cord. In some animals, the amount of movement of the subsequently exposed cord was too great to allow microelectrode recording and stimulation. In this situation, the animals were decerebrated (for ethical reasons) and paralysed with gallamine triethiodide (Flaxedil) and artificially respired. The results were unaffected by this additional procedure.

(*a*) *Recording of orthodromic impulses in caudal branches of afferent axons* (**figure 1**)

A laminectomy exposed the cord from T12 to coccygeal segments and paraffin oil covered the entire exposed cord and roots. The axons of interest run in the medial dorsal columns (Shortland & Wall 1992). This area of cord tends to be obscured by the meandering mid dorsal vein and its tributaries. We selected an area clear of these obstructions for recording, most commonly L6 or S1, and then measured the required distance rostrally to identify a root entering the cord. The root so identified was either T12 or T13 or L1. That root was cut in the periphery and mounted on silver hooks for stimulation. Because these roots are short (5–8 mm), there was a considerable danger of stimulus spread to neighbouring roots or to the cord itself. For that reason various checks were made to insure that only myelinated fibres in the chosen root were being stimulated as described in the results. The stimulus applied to the root was limited to less than 50 μA for 200 μs at 1 Hz. The recording electrodes were of the tungsten, glass insulated, platinum plated Merrill-Ainsworth type (1972) with about 15 μm exposed tip. In all experiments, a preliminary search was made in dorsal columns 5–10 mm caudal to the stimulated root to observe the expected action potentials in the descending branches of the entering afferents. This was done to check the state of the animal and the equipment. Then the more caudal area for detailed search was selected. The microelectrode was lowered to touch the surface of the cord as close as possible to the midline and slowly penetrated to a depth of 200 μm. Later histological inspection showed that these tracks were always well within the dorsal columns. The subsequent search grid is described in the results section. The recording equipment was standard. Continuous filters were initially set to record between 500 Hz and 15 kHz and later tuned to optimize the observed spike.

(*b*) *Antidromic impulses and refractory periods measured on roots after microelectrode stimulation in caudal dorsal columns* (**figure 3**)

The method used here has been described in the previous papers (Wall & Shortland 1991; Shortland & Wall 1992). In these experiments, single units were recorded from dorsal root strands while antidromic impulses were evoked by microelectrode stimulation in dorsal columns are various distances from the entering root. The cord was exposed as for the orthodromic search just described. The selected root had thin strands dissected free which contained a few conducting myelinated fibres. The dissected fine strand which was connected proximally to the root and cord was mounted on a single fine silver hook. Recording electrodes led to conventional recording equipment with filters set from 1–15 kHz. The recording strands contained a number of conducting axons which could be recorded after stimulation of the root from which the strand was dissected (see figure 2, Wall & Shortland 1991). Of these units, one was selected for detailed examination by adjusting the position of the stimulating microelectrode in the dorsal columns and by adjusting the stimulus intensity. The stimulating microelectrodes were glass-coated tungsten with a 25 μm exposed tip. Preliminary tests were made close to the root entry zone. Then a distant caudal area of dorsal columns was selected for detailed search with the stimulating microelectrode. The stimulating microelectrode was placed on the cord surface with a search stimulus of 50 μA for 200 μs at 1 Hz, tip negative, and lowered into the cord until an antidromic impulse was recorded in the strand from the dorsal root. The microelectrode was then adjusted in position until a minimum stimulus strength location was reached. At this point, always within 200 μm of the cord surface and well within the dorsal columns, the impulse properties were examined provided that the threshold of the axon was below 10 μA. We had shown previously that, in these conditions, the axon lay less than 50 μm from the tip of the stimulating electrode (McMahon & Wall 1985). When the properties of the antidromic impulse had been studied

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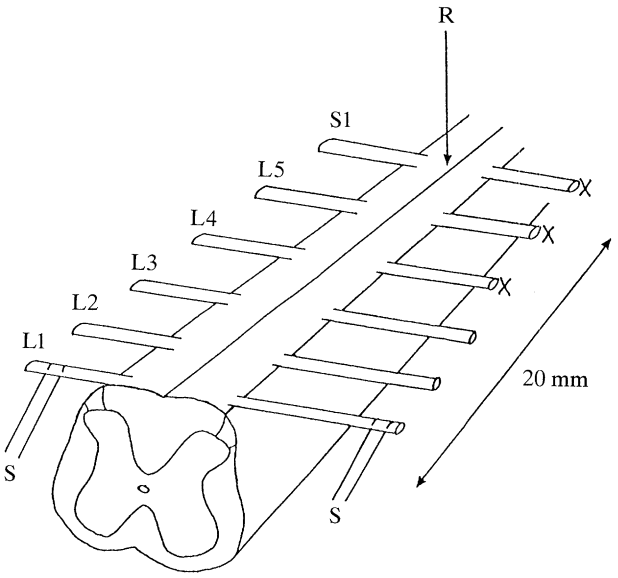

Figure 1. Diagram of the arrangement for recording orthodromic impulses in primary afferents in dorsal columns. S marks the stimulating electrodes placed on the cut dorsal roots. R indicates the recording microelectrode probing the superficial medial dorsal column caudal to the stimulated root. In control experiments all roots were intact other than the roots for stimulation. In other experiments dorsal roots L4, L5, L6 and S1 and S2 had been cut on one side at various times before the acute experiment.

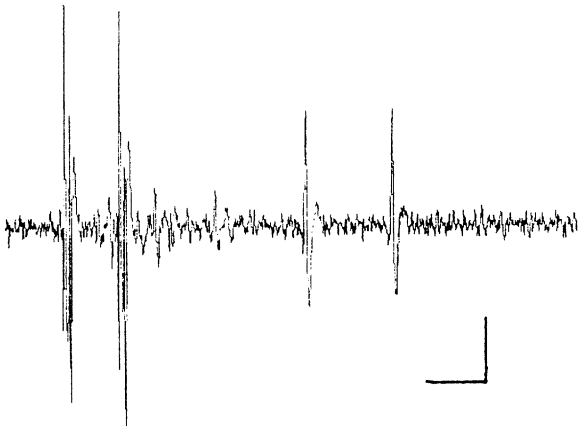

Figure 2. Example of recording of an orthodromic unit. Two stimuli were applied 2 ms apart to the L1 root. The stimulus artefacts are visible on the left. A recording microelectrode had been placed 20 mm caudal to the stimulating root in the dorsal column. Two identical action potentials are visible on the right. This record is the average of 8 superimposed traces. The horizontal bar represents 2 ms and the vertical 100 μV. Note slowing of conduction velocity of the second impulse (see text).

(see § 3), an orthodromic impulse was produced by stimulating the recording root (St 1, figure 3).

(*c*) *Root section*

Under intraperitoneal Nembutal anaesthesia (40 mg kg^{-1}) a hemilaminectomy of the caudal lumbar vertebrae was carried out. The dura was incised and dorsal roots L4, L5, L6, S1 and S2 were dissected free on one side and cut at their exit point from the dura. The dural opening was covered with gelatin foam and muscle and skin were closed in layers with silk sutures. All animals recovered uneventfully and were examined the next day and immediately before the acute experiments. While gently holding the animal, the deafferented foot and leg was pinched with toothed forceps to detect any sign of withdrawal or vocalization. When this was observed, it was assumed that the root sections were not complete and the animals were given a lethal anaesthetic overdose. Because root section may lead to autotomy (Basbaum 1974) all operated animals were inspected each day and, if self-inflicted damage was observed on the anaesthetic toes, the animals were immediately killed with an overdose of barbiturate anaesthesia.

(*d*) *Histology*

At the end of each acute experiment, the animal was killed with an overdose of anaesthetic. The microelectrode search area was marked with an insect pin. The entire exposed region was then widely dissected to identify which roots had been used for recording and stimulation, which roots had been chronically sectioned and which segments had been searched with the microelectrodes. The relevant segments were then dissected free and placed in formalin in saline. Sections were cut through the search area with a freezing microtome at a thickness of 50 μm. They were then stained with the myelin staining dye Solarchrome Cyanin.

3. RESULTS

(*a*) *Evidence that impulses recorded in one axon originated from stimuli to the same axon*

In assessing the experiments reported here, it is crucial that the impulses on which we report were recorded in one axon and originated within that axon as a consequence of stimulation of the same axon. It was necessary to eliminate impulses produced by post-synaptic events such as the dorsal root reflex which were observed and not studied further. All action potentials reported had a fixed all-or-none shape which appeared suddenly as the stimulus strength was raised above threshold. A sample of the action potential was saved on a memory trace and superimposed on all subsequent recordings from that unit to assure that observations applied to the same single unit. All units reported were in myelinated fibres since the conduction velocity within the axon in the cord was greater than 2 m s^{-1}, as judged by the latency between stimulus and response and the distance between the stimulus site and the recording point. All units had a fixed latency between stimulus and response and would respond twice to two stimuli separated by 2 ms (figure 2).

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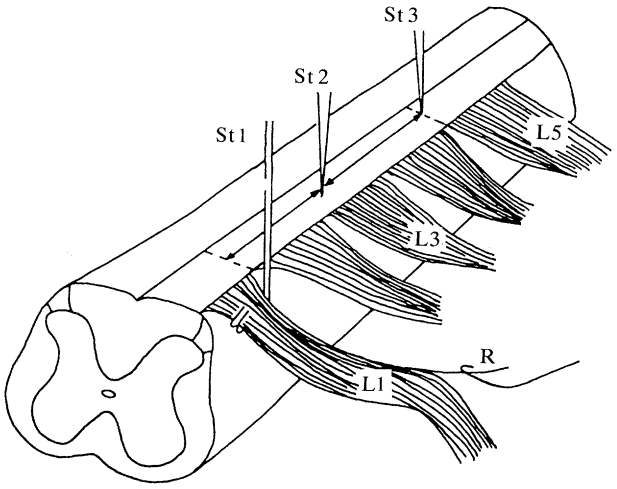

Figure 3. Diagram of the arrangement for recording antidromic action potentials and interacting these with orthodromic action potentials. The stimulating microelectrodes were placed in the dorsal columns at various distances caudal to the recording root, St2 and 3. The recording of single antidromic action potentials was made on strands of dorsal root placed on a silver hook, R. Orthodromic action potentials were generated from a pair of hook electrodes, St1, placed around the proximal part of the root which contained the recording strand.

(i) *Orthodromic impulses* (figure 1)

In these experiments, stimulation was applied to roots T12 or T13 or L1 and recordings made with microelectrodes in the superficial dorsal columns at various distances caudal to the root entry. The variation in selection of the root to be stimulated allowed an area of dorsal column to be selected for recording which was not obscured by overlying blood vessels (see § 2). As these roots are relatively short (5–8 mm), it was necessary to check that the stimulus had not spread from the root lying on the silver wire hooks to the spinal cord or to nearby roots. For each unit reported, the threshold for root stimulation was below 50 μA for 200 μs. As a final check, a unit was selected at the end of each experiment and shown to disappear if the stimulated root was crushed proximal to the stimulus point.

The first measure to ensure that a continuous axon ran from stimulus to recording points was to report only on units whose latency varied by less than 0.1 ms when stimuli were applied at twice threshold at 1 Hz. A more rigorous criterion was to show that each axon responded twice when two stimuli at 2 times threshold were applied at a 2 ms interval (figure 2). It is possible that we thereby rejected a small number of axons as antidromic studies reported below show that there are a few axons with refractory periods longer than 2 ms. However, we decided to accept this loss to ensure that all axons were in continuity since no transynaptic responses are known to repeat reliably at 500 Hz even in pathological states. When one impulse follows another at a 2 ms interval, it is riding on the after potentials remaining from the first impulse. As seen in figure 2, this slows the conduction velocity of the second impulse. For 21 afferents this slowing varied from 5–20%, average 10.5 ± 3.8 (s.d.).

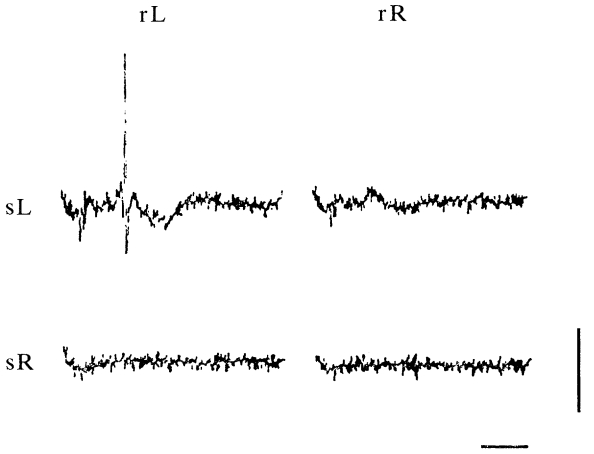

Figure 4. Orthodromic impulse in a single axon in the dorsal columns 20 mm caudal to the stimulated root. These single sweeps were made in the situation shown in figure 1. Stimulation was on the L1 dorsal root and occurs 1 ms after the beginning of the trace. The top left hand trace shows one of the rare orthodromic impulses recorded in intact cord 20 mm caudal to the ipsilateral stimulated root. The other traces show that no impulses were recorded when the contralateral root was stimulated, sR rL. In this animal no conducting fibres were detected at this distance when the opposite root was stimulated and recordings were made on the side ipsilateral to that root, sR rR. The horizontal bar represents 2 ms and the vertical bar 100 μV.

(*b*) *Orthodromic transmission from roots to dorsal columns with roots intact*

The search stimulus (50 μA, 200 μs, 1 Hz) was applied to cut dorsal roots either T12, T13 or L1 on the left and right sides (figure 1). The recording microelectrode was slowly lowered into the dorsal columns caudal to the stimulated root from the surface to a maximum depth of 200 μm. The advance was arrested at 10 μm intervals. The search tracks were made on both sides starting from close to the midline and extending 200 μm lateral to the midline. This search area was limited to the dorsal columns. After preliminary experiments, a routine search method was established so that comparable areas were examined in each animal. The area was from the midline to 200 μm lateral and for each track from the surface to 200 μm deep. The total number of recorded axons per penetration track was noted. Deeper probes entered grey matter where postsynaptic spikes could be recorded but were not studied. Each succeeding trace was visually inspected for the appearance of a brief axon spike and, when one was suspected, an average of eight sweeps were made so that the spike would emerge clear of the base line (figures 2 and 4).

As expected, there was no problem when recording in dorsal columns 5–10 mm caudal to the stimulated root. Many spikes originating from the stimulated root were recorded on each track in this area of the dorsal columns. This trial was done in each animal to confirm that the animal and set up were in an adequate condition.

As the search tracks were moved more and more caudally, fewer and fewer axon spikes were seen. By

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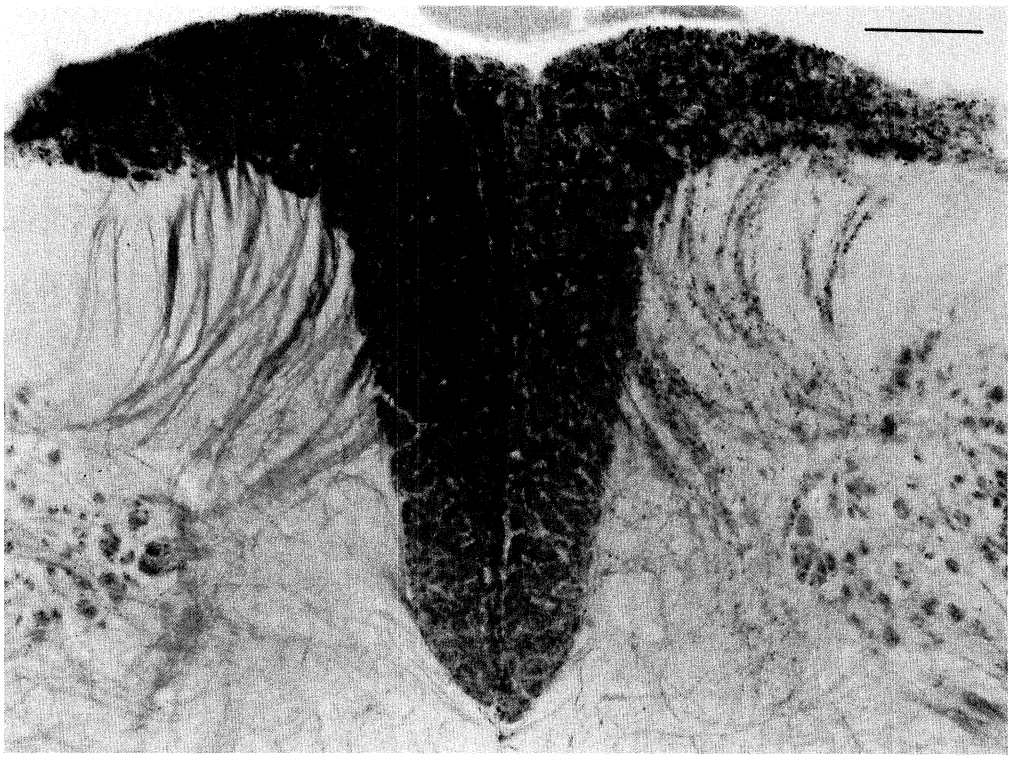

Figure 5. Cross section through dorsal horns at the border of the L6–S1 segments. This 50 μm section was stained with Solarchrome cyanin which stains myelin. On the right side dorsal roots L4, 5, 6 and S1 and S2 had been sectioned 17 days before. A decrease of staining in dorsal columns and a fragmentation of penetrating afferents is observed on the side of the root sections. Bar: 200 μm.

20 mm caudal to the stimulated root (in the region of the S1 segment) very few spikes could be detected. We then devoted six animals for a search to determine the precise number. In 70 search tracks, only four axons were detected. An example of one is shown in figure 4. No contralateral axons were recorded. It is interesting to note that two of these four rare axons were very close to each other and could be recorded from a single location suggesting the possibility of bundles of these occasional long range, orthodromically conducting axons.

(*c*) *Orthodromic conduction in dorsal columns after rhizotomy of caudal roots*

At this stage we were faced with the puzzle that we had been able to record very few fibres conducting impulses 20 mm caudal to the stimulated root while anatomical studies and antidromic conduction studies (Wall & Shortland 1991; Shortland & Wall 1992) showed that substantial numbers of descending axons existed 20 mm caudal to their entry point. This suggested the possibility that there was a failure of orthodromic conduction in intact cords. We thought it possible for reasons discussed later that a block might be relieved by the degeneration of neighbouring fibres in the segments through which the long range descending axons ran. We therefore cut dorsal roots L4–S2 as described in the § 2. The dorsal columns were then searched at 20 mm caudal to the root entry for orthodromically conducting fibres using exactly the same method as had been used in the six animals with intact roots. We succeeded in doing this in 14 animals which had survived 1–19 days after the root section. Sections of the recorded segment (figure 5) revealed fragmented myelinated fibres in the dorsal horn on the side of root section and a decreased density of staining for myelin in the dorsal columns. The fragmentation was apparent at three days after root section and was still evident over the 19 days covered by this series, although there was no gross atrophy of dorsal columns or dorsal horns. At much later times (more than 30 days) the fragments were no longer apparent and marked atrophy of the dorsal columns was evident (figure 6).

In intact animals, the number of conducting axons per search track 20 mm caudal to the entry point was 0.06 (70 tracks). The results in the cords where roots had been sectioned is shown in figure 7. When the root stimulated and the dorsal column searched were on the same side as the sectioned roots, there was an obvious increase in the number of recorded conducting axons from day 7 onwards. In days 1–6, 0.04 axons were recorded per search track (50 tracks). In the next 6 days, days 7–12, 1.1 axons per track were recorded (40 tracks). In the longest survival period, 13–19 days, 1.7 axons per tract were recorded (42 tracks). The regression of the index of orthodromic conduction was highly significant ($p < 0.01$).

On the side of the cord opposite the root sections, no anatomical changes were apparent and yet there were small changes in the ability of long range descending axons to carry orthodromic impulses. In these experiments, the stimulated root and the record-

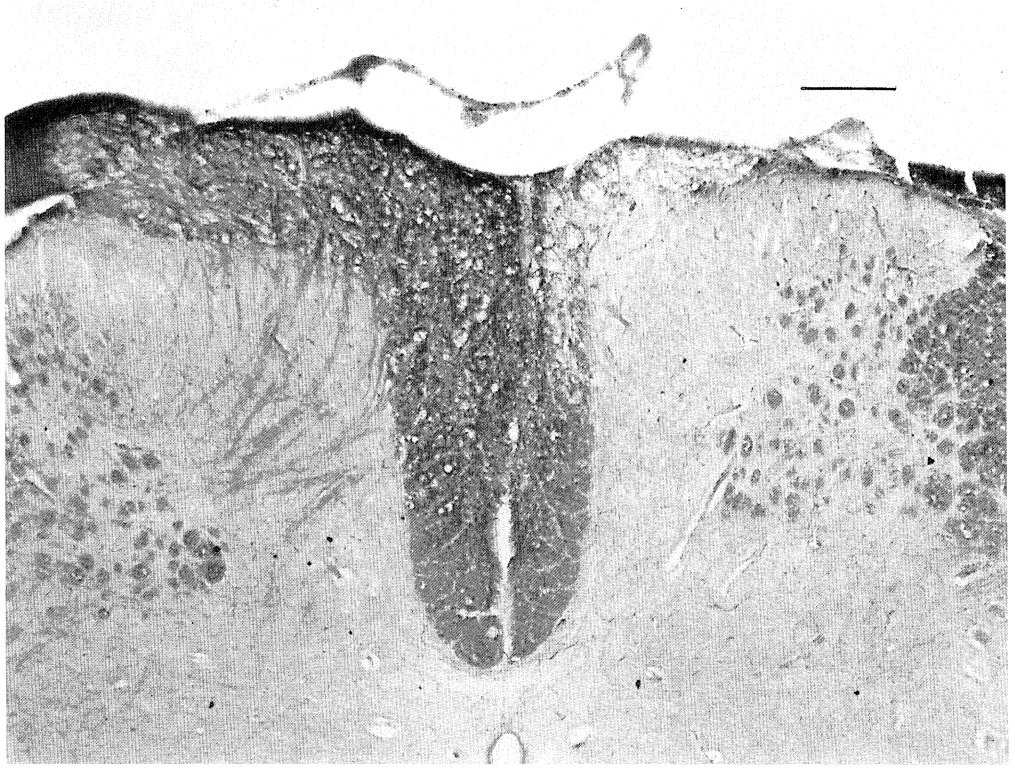

Figure 6. Cross section through dorsal horns in the S1 segment prepared as in figure 5. On the right side the roots had been cut 75 days previously. On the side of the root section, no penetrating afferents are seen and the dorsal columns are faint. Bar: 200 μm.

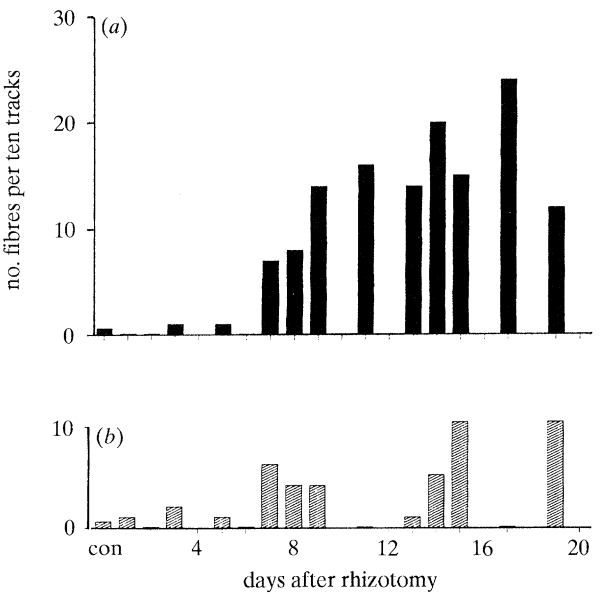

Figure 7. Numbers of orthodromically conducted axons detected 20 mm caudal to the spinal cord entry point of the stimulated root. In six intact cords with 70 search tracks only four conducting axons were detected i.e. 0.6 axons per ten tracks. Dorsal roots L4–S2 were cut on the left side. Cords were searched 1–19 days after root section to detect the ability of the left L1 root to transmit orthodromic impulses 20 mm caudally to the ipsilateral cord. There was no obvious change over the first six postoperative days after which there was a considerable increase. In the lower part of this figure the same experiment was carried out when the stimulated root and the recorded dorsal columns were on the right side contralateral to the sectioned roots. A minor increase in conducting axons was apparent on this side contralateral to the rhizotomies.

ing tracks were located on the side contralateral to the rhizotomies. In all other respects the experiments were identical to those just reported. During the first six days after root section, 0.08 conducting axons per search track were observed (50 tracks). This figure is comparable with and statistically indistinguishable from the 0.06 axons observed in intact cords (70 tracks). However, during days 7–13 after root section, the number rose to 0.3 conducting axons per tract (40 tracks). During days 14–19, the number was 0.4 (42 tracks). Again, these increases were statistically significant ($p < 0.05$).

A possible criticism of these results in which the number of conducting axons apparently increased after rhizotomy would be that the degeneration of axons produced by rhizotomy simply made it easier to detect existing intact conducting axons. In § 4, we give reasons why this criticism is unlikely. However, to avoid the criticism, we developed a second method which overcomes the possibility that the apparent increase of orthodromic conduction could be caused by a sampling error.

(*d*) *The detection of orthodromic conduction by measuring refractory periods*

This method is made possible by the fact that antidromic conduction is reliable while orthodromic conduction is in question. We can therefore examine each axon to determine if an orthodromic action potential has penetrated as far as the point from which an antidromic action potential is initiated. We did this by studying each axon in two conditions: the first was

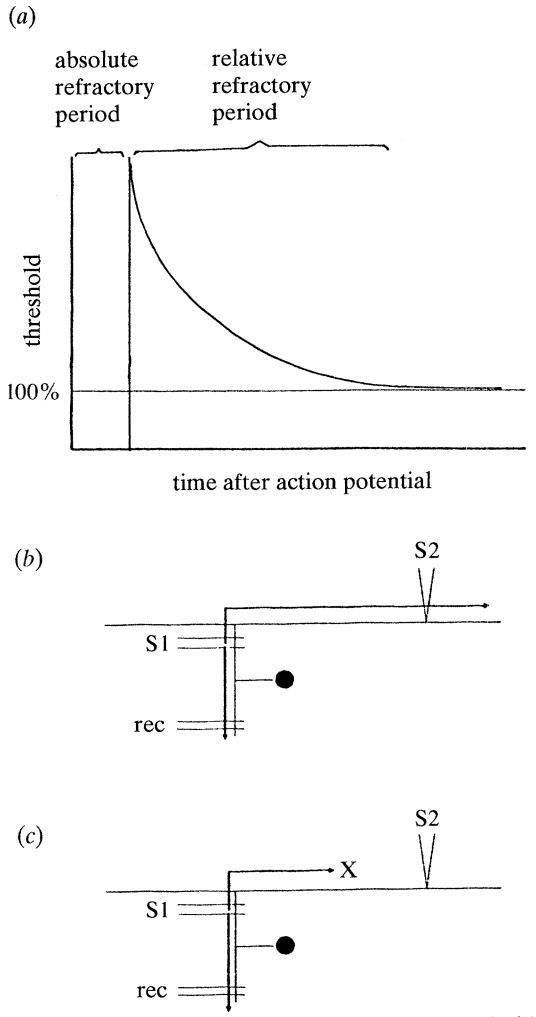

Figure 8. (*a*) Schematic representation of the excitability changes seen in a neuronal membrane in the wake of an action potential. For a short period after the occurrence of an action potential (typically 1 ms or so), the membrane cannot be re-excited by any applied stimulus. This is the absolute refractory period. Following this, there is an additional period when a second action potential can be initiated, but only with suprathreshold stimuli. This is the relative refractory period. (*b*,*c*) The use of relative refractory periods to detect orthodromic conduction failure. (*b*) An impulse set up by an electrode on the root (S1) propagates for long distances into the central branches of the axon. In this case, the orthodromic action potential will reach S2 after which there will be an absolute and partial refractory period before S2 can initiate an action potential. (*c*) An impulse starting from the (S1) electrode does not conduct as far as the S2 stimulating electrode. In this case, the membrane under the S2 electrode will not be depolarized and any suprathreshold stimulus here will excite the axon with equal ease, i.e. there will be no relative refractory period.

the ability to generate two antidromic potentials, whereas the second was the ability to generate an antidromic potential after the axon had carried an orthodromic potential. If an orthodromic action potential swept over the point from which an antidromic potential was to be initiated, it would leave behind it refractory periods which would limit for a time the ability to initiate the antidromic impulse. This, briefly, was the evidence for orthodromic conduction we were seeking and which we will now describe in detail.

(i) *Condition one: refractory periods of an antidromic action potential after a preceding antidromic action potential* (figure 8*a*)

When an axon has been stimulated at one point, there is a period when it is impossible to generate a second potential no matter how intense the stimulus. This is the absolute refractory period and for myelinated central axons lasts about 1 ms. After this, there is a period when it is possible to generate a second action potential if the stimulus is more intense than the resting threshold intensity of stimulus. This is the relative refractory period and lasts 1–2 ms in central myelinated axons (see figure 8*a*). After the relative refractory period, the axon returns close to its optimal resting excitability.

In practice, we recorded action potentials in single units in filaments dissected from dorsal roots, and stimulated the units at various locations in the dorsal columns (figure 3; Wall & Shortland 1991). First, after adjusting the location of the stimulating microelectrode (S2 in figure 8*b*,*c*), the threshold to provoke an antidromic potential was determined. This was always less than 10 μA for a 200 ms duration square-wave current pulse delivered at 1 Hz. Next, a second stimulus was applied through the same microelectrode and the interval between the two stimuli was lengthened until two impulses were recorded on the root. When the second stimulus was two times threshold, it was necessary to separate the two stimuli by 1.33 ± 0.50 (s.d.) ms ($n = 51$ fibres) before a second impulse appeared. When the second stimulus was three times threshold, the necessary interval fell to 0.99 ± 0.37 ms. This second refractory period is significantly shorter than the first ($p < 0.001$). In the results which follow we measured the refractory period at five times threshold (which was found to be close to the absolute refractory period) and then at 1.1 times threshold. We used 1.1 times threshold because the results were unstable if we attempted to use the minimal threshold. The difference between the two refractory periods (that is, using five-times and 1.1-times threshold stimuli) was noted for each fibre, and designated the relative refractory period.

(ii) *Condition two: refractory periods for an antidromic potential after an orthodromic potential*

We reasoned that the refractory periods for a second impulse to be generated at any point in an axon should be exactly the same whether the first impulse had been generated at that point by local stimulation, or had been conducted to that point by orthodromic impulse transmission (the case illustrated in figure 8*b*). However, if an orthodromic impulse failed to reach the point from which an antidromic impulse was generated, the nerve membrane at that point would not have been fully depolarized by the action potential and therefore the refractory period for the antidromic impulse would differ from that produced when two antidromic impulses had been generated at the same point. The case is illustrated in figure 8*c*.

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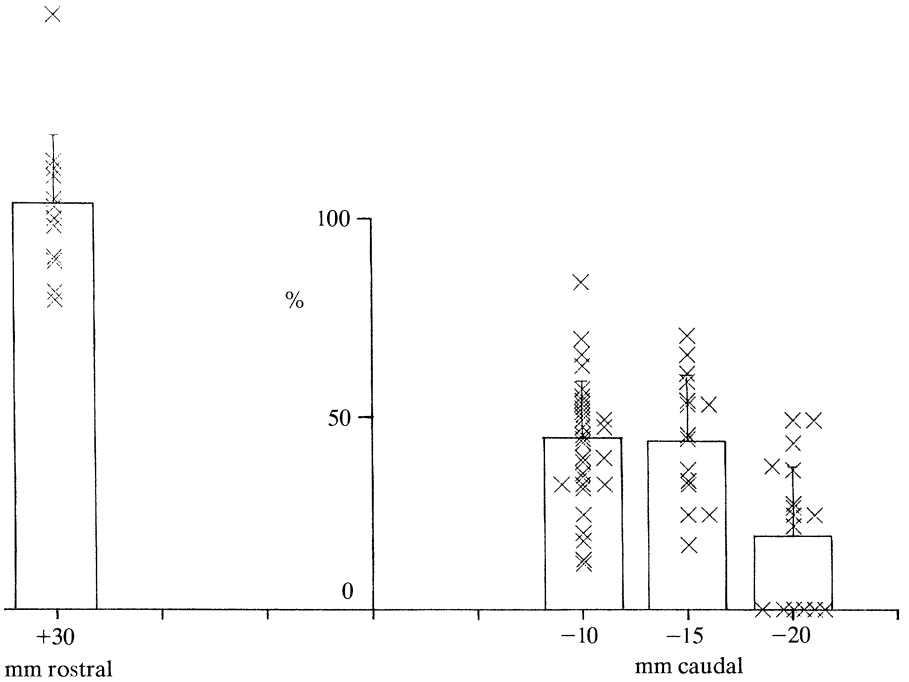

Figure 9. Ratio of relative refractory period following the arrival of an orthodromic impulse in sensory afferents and the relative refractory period following local stimulation. The second impulse in each case was an antidromic impulse stimulated by a microelectrode in the dorsal columns (figure 3), and recorded on a dorsal root. On the left, the test was done at 30 mm rostral to the root on which the antidromic impulse was recorded. These axons heading for the dorsal column nuclei were evidently carrying an orthodromic impulse as the relative refractory period was the same whether it followed the orthodromic impulse or whether it followed a locally generated impulse. However, when axons were tested 10, 15 and 20 mm caudal to the root entry the relative refractory period following the orthodromic impulses was much smaller than that following local stimulation. At 20 mm, many fibres showed no relative refractory period suggesting that the orthodromic impulse had not reached the vicinity of the stimulating electrode. The crosses show the values for individual fibres.

We set up this test as shown in figure 3. First, an axon was stimulated in the root at St1, to generate an orthodromic impulse. Then the stimulating microelectrode in the dorsal columns delivered a pulse to the axon. The interval between the root stimulus which had generated the orthodromic impulse and the dorsal column stimulus was increased until a second impulse was recorded on the root filament. There was a period during which no second impulse could be recorded at any stimulus intensity through the microelectrode. This period contains two components. First, the orthodromic impulse is travelling along the axon and, if an antidromic impulse is generated in the dorsal columns, it will collide with orthodromic impulse and will not be recorded in the root filament. This is the collision time. If the orthodromic impulse passes the dorsal column stimulating point, it will leave the membrane in a state of absolute refractoriness. We could not separate these two components (the collision time and the absolute refractory period) because there was no way in which we could measure the orthodromic conduction time. However, once the orthodromic impulse has passed the stimulus point, there will be a period of relative refractoriness which is independent of orthodromic conduction time. For these reasons, we report here only on the relative refractory time for generating an antidromic impulse after the orthodromic impulse. This relative refractory period was measured as the time difference between the stimulus at five times threshold and at 1.1 times threshold which could generate the second impulse.

Thus, for each unit studied, we determined two relative refractory periods, one after a locally generated action potential and one after an orthodromic impulse. If the orthodromic impulse had reached and passed the dorsal column stimulating point, the two partial refractory periods should be the same (condition shown in figure 8*b*). If, on the other hand, the orthodromic impulse had failed to be transmitted into the region of the dorsal column stimulating electrode, there would be no partial refractory period (figure 8*c*). We recorded the results as the ratio of the observed antidromic-following-orthodromic relative refractory period, over the observed antidromic-following-antidromic relative refractory period. The ratio should be 100% if the fibre had conducted orthodromically to the stimulus site, and should be 0% if the impulse had failed to transmit into the region of the membrane from which the antidromic impulse originated.

(iii) *Relative refractory periods in axons running rostrally from the root entry* (figure 9)

Some long running rostral branches extend as far as the dorsal column nuclei and must carry orthodromic impulses, as they are responsible for the monosynaptic responses in cells of the dorsal column nuclei. We therefore tested the validity of the relative refractory period method on these rostral axons. Axons were

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identified in mid thoracic dorsal columns 30 mm rostral to their entry point over the L1 dorsal root. Microelectrode stimulation in the distant dorsal columns produced an antidromic impulse in a thin filament of the dorsal root (figure 3). First the relative refractory period was measured by delivering two stimuli through the microelectrode with the second stimulus either at 1.1 times threshold or 5 times threshold. The difference between these two intervals was the local relative refractory period. Next an orthodromic impulse was generated by stimulating the root containing the axon (figure 3, St1). After this stimulus had generated orthodromic impulses, the minimal time at which 1.1-times-threshold and five-times-threshold microelectrode dorsal-column stimulation could generate an antidromic impulse was measured. The difference between these two times was the orthodromic-antidromic relative refractory period. Twelve axons were tested in this way and the two relative refractory periods, local and orthodromic, were approximately the same. In figure 9, the crosses indicate the ratio of the two relative refractory periods for each axon. The average orthodromic relative refractory period was 105% (s.d. 19) of the local relative refractory period. This was the expected evidence that the orthodromic impulse had passed the stimulus point.

(iv) *Relative refractory periods in axons running caudally from the root entry* (figure 9)

These experiments were done exactly as described in the previous paragraph except that the stimulating microelectrode was placed at various distances caudal to the root entry (figure 3). When the microelectrode stimulus was placed 8–12 mm caudal (three segments) to the root entry (32 fibres), the local and orthodromic relative refractory periods were not the same. Here the orthodromic relative refractory period averaged only 44% ($\pm$16) of the local relative refractory period. A similar result was obtained from 15 fibres tested 13–17 mm (four to five segments) caudal to the root entry: 46% $\pm$ 16. When the microelectrode stimulus was moved even further caudally, 18–22 mm (six segments) from the root entry, the orthodromic relative refractory period in 16 fibres was only 19% $\pm$ 19 of the local relative refractory period. These caudal branches of afferent axons differed significantly from the rostral branches ($p < 0.05$) (unpaired t-test). In seven of these 16 caudal fibres, no orthodromic relative refractory period could be measured at all. For these fibres there was therefore no evidence that the orthodromic action potential had travelled even to within electrotonic range of the stimulation point which was 20 mm caudal to the entry point. For those fibres with a relative refractory period which was shorter than expected, we will propose in the discussion that the orthodromic impulse had approached but had failed to reach the microelectrode stimulus point.

(v) *Relative refractory periods in caudal axons after rhizotomy*

Because these experiments, in common with the previous type of experiment, provided evidence for a failure of orthodromic conduction in long range descending axons, we decided to study the effect of rhizotomy. The rhizotomies were done as before to cut L4–S2 dorsal roots. The animals were tested for peripheral anaesthesia as before and the roots were examined to assure complete section. The first group of animals were tested 70–80 days after rhizotomy. At this stage (figure 6) there was considerable atrophy of the dorsal columns but there was no problem in locating an area of the medial dorsal column from which a stimulating microelectrode could evoke antidromic action potentials in the distant rostral root. All experiments after rhizotomy examined the cord 18–22 mm caudal to the recording root and on the same side as the roots had been sectioned. Twenty-seven antidromically conducting axons were recorded in these long surviving animals and the orthodromic relative refractory period was 103% $\pm$ 19 of the local relative refractory period. This figure is significantly larger than the 19% $\pm$ 19 which had been observed in axons stimulated in this area with intact spinal roots ($p < 0.01$) (unpaired t-test).

Finally, a timed series was carried out on nine animals stimulating axons 20 mm caudal to the entry point on days 1–14 after the roots had been cut (figure 11). Seven fibres were examined on day 1, nine on day 2, eight on day 3, five on day 4, ten on day 5, five on day 6, seven on day 9, ten on day 13 and ten on day 14. On day 1, the situation varied little from the control animals with the orthodromic relative refractory period being 20% of the local relative refractory period. Four of seven axons showed no orthodromic relative refractory period, as though the orthodromic action potential had failed to penetrate within electrotonic range of the stimulus point. By day 2 only one of nine axons failed to demonstrate any orthodromic relative refractory period and the average had reached 49%. From this day on, there was a steady rise to reach over 80% on days 9, 13 and 14. In the period

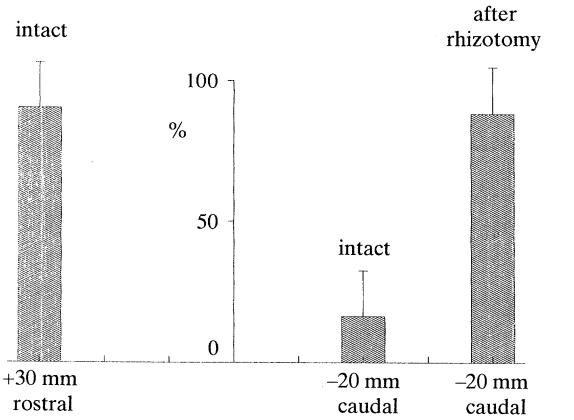

Figure 10. The effect of chronic rhizotomy on the ratio of refractory periods. Animals had dorsal roots L4–S2 cut and survived 70–90 days. In these animals the ratio of the two relative refractory periods was measured 20 mm caudal to the entry point. The ratio approached 100% in these animals suggesting that like the rostral branches they were now carrying orthodromic impulses. The error bars show the s.d.

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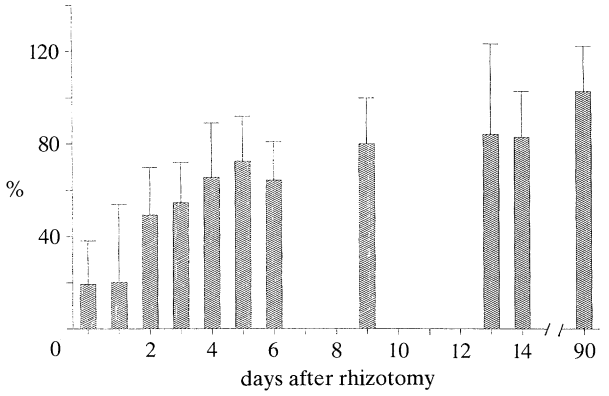

Figure 11. Timecourse of the effect of rhizotomy on the ratio of relative refractory periods. The results show that by two days there were signs of an increased ratio which increased further over the following days. The error bars show the s.d.

from 14 days to 75 there was a slight further increase from 83% ± 20 to 103% ± 19.

4. DISCUSSION

The results based on two different methods suggest that orthodromic impulses do not necessarily penetrate the far reaches of the caudally directed branch of afferent nerve fibres after they enter the spinal cord. There is no evidence of conduction block in the branch running rostrally toward the dorsal column nuclei. The experiments also show that the probability of impulse transmission caudally increases considerably if the nearby afferents have degenerated following root section.

There is a potential design fault in any experiment using our first method of direct recording of axons carrying orthodromic impulses. The results could be produced by a sampling error which favoured an area of conducting axons in one situation. The first way of avoiding this possibility was to search in a rigidly defined grid area which was identical in all animals. Inadvertent error is less likely in view of the 30-fold difference in the frequency of detecting conducting fibres in the rhizotomy versus intact animals (1.7 versus 0.06 conducting axons per search track). A second possible error could be that conducting fibres are easier to detect when surrounded by the degenerating fibres produced by the rhizotomy. There is no known way in which this would take place unless there had been gross atrophy which concentrated the remaining intact fibres in one area. However, there is no such atrophy during the major period of observation (figure 5) and atrophy can only be observed at later stages (figure 6). The possibility of error due to surrounding degeneration is even less likely as we observed a sevenfold increase of conducting fibres on the side opposite the cut dorsal roots (0.4 versus 0.06 conducting axons per search track). This unexpected increase was observed in axons surrounded by intact fibres in which no signs of degeneration have been reported. The unexpected nature of the result also mitigates against observer bias. Because the findings are novel and since the method could be criticized, we turned to the second more difficult technique. This method tested all fibres without selection and produced comparable results to those produced by the first technique.

The implication of the results is that impulses may block in their transmission along axons. There is a very substantial literature on conduction block at bifurcation points in invertebrate nervous systems (Grossman *et al.* 1974; Spira *et al.* 1976; Parnas & Segev 1979; Gu 1991). It has been shown in experiments and in models that the failure of impulse transmission at these branch points can be attributed to a combination of geometry, channel distribution, external ions and the presence of nearby axo-axonic synapses.

The vertebrate literature contains very much less reference to the possibility of conduction block. However there are sufficient examples to show that this option has been and should be considered. From the time of Barron & Matthews (1935) there have been reports of intermittent conduction. Chung *et al.* (1970) review the vertebrate literature on failure of bifurcation points to transmit trains of impulses and add their own data. Howland *et al.* (1955) gave evidence for transmission block in axons entering the dorsal horn after neighbouring afferents had transmitted an afferent volley. Bostock & Grafe (1985) report block to occur in areas from which the myelin had been removed by toxins. Stoney (1990) showed that even the T-junction in the dorsal root ganglion was a point of conduction failure. Zhang & Jackson (1993) patch clamped the terminals of axons in the posterior pituitary and found that the application of GABA activates a chloride channel which inhibits the generation of action potentials. As reviewed by Redman (1990) some impulses in afferent fibres fail to produce any postsynaptic potential. This is attributed by some to failure of neurotransmitter release and by others to failure of conduction of impulses in the fibres of the terminal arborization. Lüscher *et al.* (1983) suggested that some axons in the terminal arborization of IA afferents were steadily blocked in the normal resting state and that they began to conduct only after their membrane potential increased after a high frequency afferent barrage. Evidently, it is not novel to propose that impulse transmission may fail in some fractions of a terminal arborization in vertebrates.

The second type of experiment we used in this paper involved measuring the relative refractory period of an axon after it had carried an orthodromic action potential. Where we could discover no relative refractory period at all, it seems reasonable to conclude that the orthodromic action potential had failed to move into the vicinity of the stimulating electrode. However, we need to explain the more common situation where we could detect a relative refractory period but it was much shorter than that which followed the local generation of an action potential. If an action potential were to become stationary in its movement along an axon, there would be an electrotonic spread of the action potential's depolarization ahead of the blocked action potential. This depolarization would make the membrane ahead of the action

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potential more excitable and would produce an apparent shortening of the refractory period. We do not know the length constant, lambda, of electronic spread in central axons. However, we know that it must be many millimetres for myelinated fibres because of Lloyd's studies (1972) of the active dorsal root potential. The first large negative phase of this potential is recorded at a distance on dorsal roots which have carried an orthodromic volley. It is produced by the electrotonic spread from cord to root produced by membrane potential changes in fibres in the dorsal columns. As this large potential can be recorded many millimetres distal to the root entry zone and persists for many milliseconds, it is reasonable to assume that the space constant of the electrotonic spread in central axons is at least as large as that measured in peripheral nerves. Therefore we propose as a testable hypothesis that when no partial refractory period was detected, the orthodromic impulse had blocked at a distance greater than the space constant while a shortened refractory period indicated a closer approach of the blocked action potential to the stimulus point.

There are three problems which need explanation: (i) Why should orthodromic impulses fail to conduct?; (ii) Why is the probability of antidromic conduction superior to orthodromic conduction?; (iii) Why does orthodromic conduction improve after rhizotomy? There are two groups of factors which could be involved in all three questions. The first group would exist entirely within the axon examined and would include diameter, tapering, branching, myelination and membrane properties such as sodium channels. The second group would be extraneural and would include the ionic milieu, glia and the presence of axo-axonic synapses. The side branches of the axons we studied reach into the grey matter where there are many such axo-axonic contacts (reviewed in Hayes & Carlton 1992). These synapses exist not only on the terminal boutons but also on branch points. They are within the electrotonic distance of the descending axons examined in this paper, i.e. less than 100 μm.

The answer to the first question on why an othodromic impulse blocks can not be because the membrane in front is not excitable since we know it can transmit an antidromic impulse. It is therefore likely to be related to the membrane potential (Lüscher *et al.* 1983) and to the state of the membrane channels (Zhang & Jackson 1993). The origin of the second question enters because we report here two examples of a bias in favour of antidromic transmission. There was no problem is generating antidromic impulses in a dorsal root from a distant caudal point in the dorsal columns although very few action potentials could be detected running orthodromically from the root to the distant area. Second, in single axons which were conducting antidromically from 20 mm caudal to the root, relative refractory periods failed to show that the stimulus point was invaded by orthodromic impulses. Earlier studies of compound action potentials had shown that there was preferential conduction in the antidromic direction (Wall *et al.* 1956) in primary afferents running from roots to dorsal columns. This form of asymmetrical conduction has been studied intracellularly in the giant axons of the cockroach (Spira *et al.* 1976; Parnas & Segev 1979). They attributed the effect as being due to extracellular accumulation of potassium ions which depolarized the membrane and inactivated asymmetrically distributed sodium channels and to synaptic inputs which shunt the membrane current in the area of the block.

To answer the third question, we must first ask what happens to cut axons when dorsal roots are cut. A sequence of three types of change is observed in the terminals of the cut axons. First there is a change in neurofilaments with a clumping of synaptic vesicles apparent within 18 h peaking at 3 days and completed by 1 week (Ralston & Ralston 1979). Next there is electron lucent degeneration seen by 2 days peaking at 5–7 days and complete by 4 weeks (Ralston & Ralston 1979, 1982; Knyihar-Csillik *et al.* 1982). Finally there is electron-dense degeneration of synapses seen within 24 h peaking at 3–10 days and gone by 4 weeks (Heimer & Wall 1968; Ralston & Ralston 1978, 1982; Knyihar-Csillik *et al.* 1982). The complete clearance of debris takes many weeks (Kapadia & LaMotte 1987). Changes continue for at least 60 days (Coimbra *et al.* 1974). After rhizotomy, postsynaptic morphological changes may also occur. We observed here changes in the ability of intact axons to conduct orthodromically beginning 2–6 days after rhizotomy and continuing to increase over long periods of time. It will be noted that this improvement in orthodromic conduction appeared by two days with the relative refractory period method (figure 11) but took 7 days for direct recordings to improve (figure 7). This would be consistent with the proposal that the relative refractory period measure detects impulses in the axon at a distance from the stimulus site while the orthodromic recording method requires the presence of an action potential orthodromically propagated to the site of recording. This would fit the proposal that the orthodromic impulse block is gradually relieved so that impulses may advance small distances at first and only later complete their travel over the entire length of the axons under examination.

We must next consider the possibility that cutting dorsal roots alters the intrinsic properties of the neighbouring (intact) afferents so as to increase their ability to carry orthodromic activity. There are at least two different ways in which this could arise. On the one hand, the membrane properties of the intact afferents might change, for instance by the altered expression of ion channels. On the other, the geometry of the afferent might change, resulting in more favourable conditions for impulse conduction. There is little clear evidence for changes in intact sensory neurons after adjacent ones are damaged. Certainly there is no evidence that the fibres we observed conducting after rhizotomy could have been new sprouts emitted by the intact fibres. The only well-documented chemical change is the altered expression of tubulin in sensory neurons contralateral to rhizotomy (Wong & Oblinger 1990). In contrast, since the initial report of Liu & Chambers (1958) there have

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been repeated claims that rhizotomy in adults may alter the morphology of the neighbouring (so-called 'spared') roots, both in terms of numbers and of central distribution. However, such claims remain controversial (see McMahon (1992) for discussion) and it is somewhat surprising that as the resolution of anatomical techniques has increased, the degree of reported sprouting has decreased. None the less, it is feasible that following the degeneration of some afferent terminals (after rhizotomy), remaining terminals more successfully compete for factor(s) produced within the spinal cord, as has been suggested for peripheral axons (Voyvodic 1991).

The mechanism by which orthodromic conduction block is achieved, and how it is relieved by rhizotomy, is now the target of experiments to be reported elsewhere. It seems likely that controls of the membrane potential of conducting axons is the crucial factor. Induced primary afferent depolarization blocks transmission acutely in many afferents. Abolition of tonic primary afferent depolarization by GABA antagonists immediately relieves block. Peripheral nerve section decreases the ability of the spinal cord to generate primary afferent depolarization (Wall & Devor 1981). This decrease is apparent strongly on the side of the nerve section and weakly on the contralateral side. This finding supports the results reported here that root section has a marked effect on ipsilateral conduction and a smaller contralateral effect. If the orthodromic block of impulses is relieved by rhizotomy, one would predict that postsynaptic cells would be detected responding to distant intact roots. This has been observed (Basbaum & Wall 1976). Future work will concentrate on factors which control membrane potential and therefore conduction in long range afferents and on the postsynaptic consequences of the relief of conduction block.

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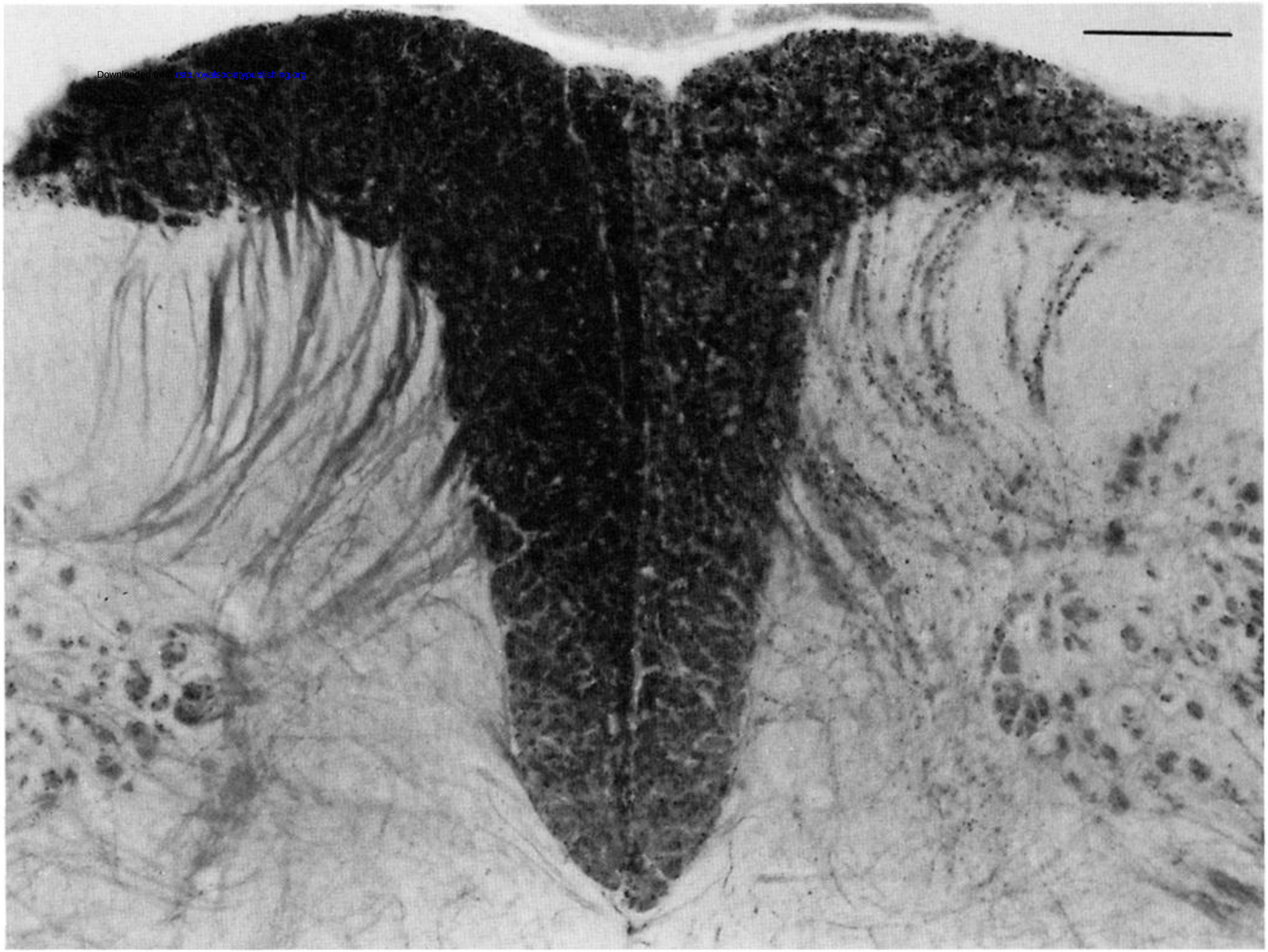
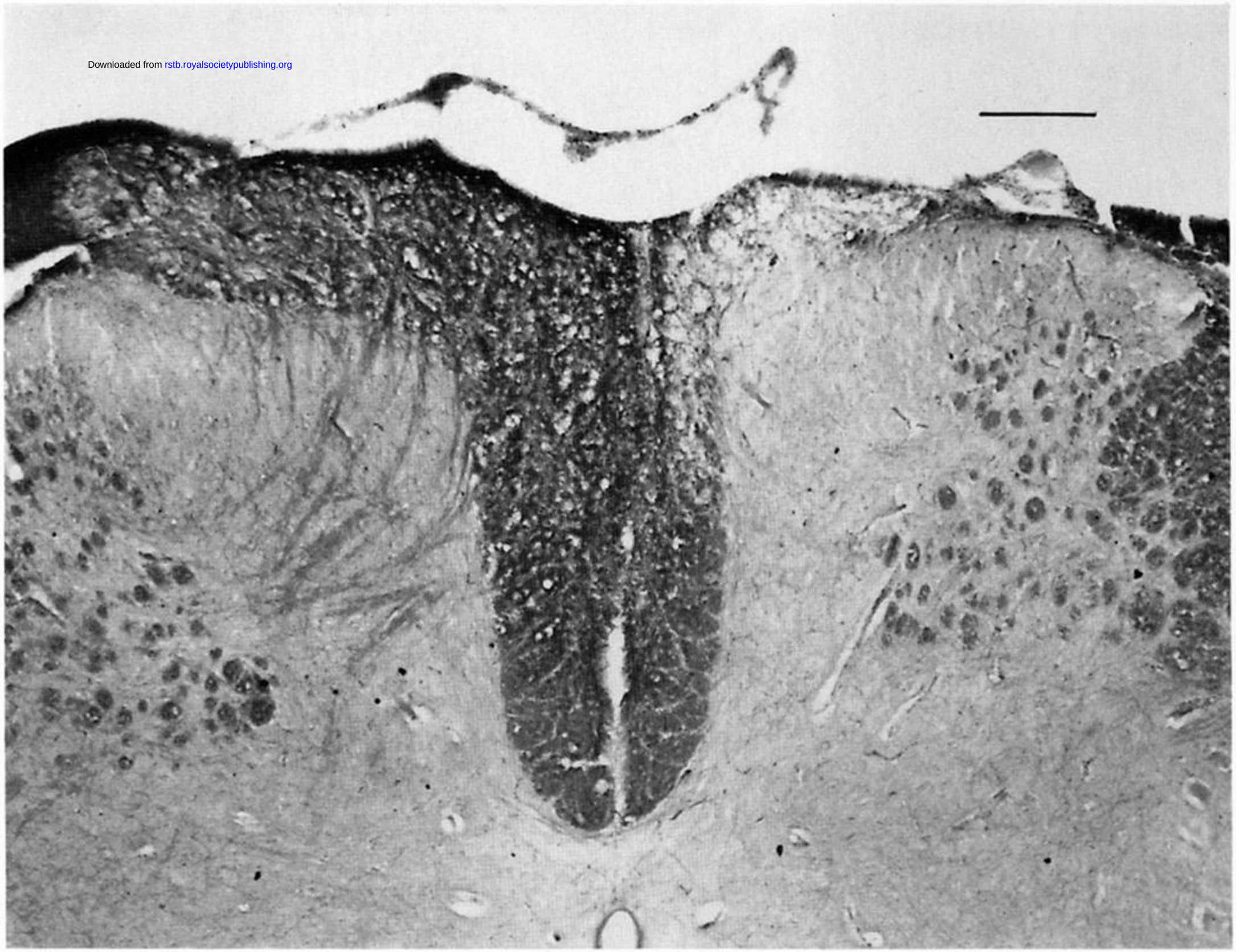


igure 5. Cross section through dorsal horns at the border of the L6–S1 segments. This 50 µm section was stained ith Solarchrome cyanin which stains myelin. On the right side dorsal roots L4, 5, 6 and S1 and S2 had been ctioned 17 days before. A decrease of staining in dorsal columns and a fragmentation of penetrating afferents is bserved on the side of the root sections. Bar: 200 µm.



igure 6. Cross section through dorsal horns in the S1 segment prepared as in figure 5. On the right side the roots ad been cut 75 days previously. On the side of the root section, no penetrating afferents are seen and the dorsal olumns are faint. Bar: 200 μm.