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# Descending tracts of the lateral columns of the rat spinal cord: a study using the horseradish peroxidase and silver impregnation techniques*

FRANK P. ZEMLAN, LEE-MING KOW, JOAN I. MORRELL AND DONALD W. PFAFF

*The Rockefeller University*, 1230 *York Avenue, New York, New York* 10021

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## INTRODUCTION

Projections from the brain to the spinal cord are the basis for the neural control of reflex behaviours of the trunk and limbs. The lateral columns of the spinal cord, and particularly the anterolateral columns, appear necessary for the elicitation of nociceptive reflexes (Liebeskind & Paul, 1977), reproductive reflexes (Kow, Montgomery & Pfaff, 1977), control of flexor group muscles (Grillner, Hongo & Lund, 1966) and other reflexes and behaviours essential for the organism. Whereas much of this functional work has been done with the rat, no comprehensive study exists of the descending projections through the lateral columns in this species. In order to provide a neuroanatomical basis for studies of the supraspinal control of reproductive reflexes, descending projections through the lateral columns were investigated.

The present paper examines the cells of origin of descending lateral column fibres. Microtransections were made and horseradish peroxidase (HRP) applied to the severed axons to identify projections through different portions of the lateral columns (LaVail & LaVail, 1972; Kuypers, Kievit & Groen-Klevant, 1974; Nauta, Pritz & Lasek, 1974). HRP uptake by severed axons appears to result in more intense labelling of cell bodies than when the protein marker is taken up in undamaged terminal areas (Halperin & LaVail, 1975).

## MATERIAL AND METHODS

### *Animal preparation*

Female rats (Hormone Assay Labs, 300–400 g body weight) were anaesthetized with Equithesin and a unilateral microtransection of a subpopulation of lateral column fibres was made on the right side, at spinal levels $C_2$ or $T_{10}$, according to procedures described elsewhere (Kow *et al.* 1977). In 12 rats (ten with $T_{10}$ and two with $C_2$ transections), approximately 2 mg of HRP (Sigma, Type VI, Lot no. 25C and 124C) were applied to the severed axons with a microspatula. After application the excess HRP was removed and the transected area covered with gel foam. The overlying muscles were apposed and sutured and the skin closed. As retrograde transport of HRP occurs at about 72 to 120 mm/day (Kristensson, Olsson & Sjöstrand, 1971; LaVail & LaVail, 1972), operated rats were allowed to survive for 2–4 days. Another 13 rats were transected in a similar manner but no HRP

* Reprint requests should be addressed to Dr Frank P. Zemlan.

applied. These rats were allowed to survive for 2, 7 or 14 days and were processed according to the Fink–Heimer (1967) method of selective silver impregnation. All the 25 rats remained in good general health after operation.

*HRP staining procedure*

The procedure used for demonstrating the presence of peroxidase was modified from that of Graham & Karnovsky (1966) and Nauta *et al.* (1974). Animals were perfused with 0·9% saline, and then with a solution of 1% glutaraldehyde plus either 1% or 3% paraformaldehyde in phosphate buffer (0·05 M). The brain and spinal cord were removed and stored in fresh fixative for 4–6 hours at 4 °C. The spinal cord was removed in segments which were determined by overlaying vertebrae. The tissue was then rinsed several times with 0·05 M phosphate buffer and placed overnight in a solution of 30% sucrose in 0·05 M phosphate buffer at 4 °C. The following morning coronal frozen sections were cut at 100 μm into a solution of 5% sucrose in 0·05 M tris buffer (pH 7·71 at 5 °C). Alternate sections were saved caudal to the anterior commissure. After cutting, sections were rinsed in 0·05 M tris buffer (pH 7·6) at 25 °C and then placed in a 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution (50 mg DAB/100 ml 0·05 M tris buffer, pH 7·6 at 25 °C) and agitated for 30 minutes at room temperature. Then, 0·1 ml of 3% $H_2O_2$/100 ml DAB solution was added and agitated for 30 minutes. After rinsing in three changes (15 minutes each) of 0·05 M tris buffer (pH 7·6 at 25 °C) and three changes of distilled water (10 minutes each), sections were serially mounted on chrome–alum treated slides, dried and counterstained with cresyl violet. Although some workers have suggested that reaction product may be lost as a result of counterstaining (Kuypers *et al.* 1974) we did not find this to be true for our material. To check for possible loss of label, tissue was processed to form reaction product but not counterstained. The location of each HRP labelled cell was charted, then the tissue was counterstained and recharted. No loss of labelled cells was observed with light counterstaining.

*Transection site tissue processing and analysis*

The portion of the spinal cord containing the transection ($C_1$–$C_3$ or $T_9$–$T_{11}$) was taken and processed separately. After perfusion the tissue was removed and placed in fresh fixative. The tissue was embedded in gel–albumin and cut in coronal frozen sections at 25 μm. Sampling was one section every 100 μm. Sections were stained according to the method of Fink & Heimer (1967). The transection site was then reconstructed from the silver stained serial sections. The maximum ventral and dorsal extent of the transection was determined using two criteria, namely the extent of gliosis and the location of degenerating fibres in the spinal cord. The boundary between the anterolateral (AL) and dorsolateral (DL) columns was determined by a line drawn through the central canal that bisected the lateral columns.

*Tissue processing and analysis of descending fibres*

The locations of the projection areas of descending lateral column fibres were determined by microtransection of the AL or DL columns and subsequent silver impregnation of degenerating fibres (Fink & Heimer, 1967). A discussion of the criteria for distinguishing fibre from preterminal degeneration is presented elsewhere (Zemlan, Leonard, Kow & Pfaff, 1978). The cytoarchitectural lamination of the spinal cord, as developed by Rexed (1952, 1954) and adapted for the rat (Steiner & Turner, 1972), was determined for each charted tissue section.

*Analysis of HRP material*

All sections were scanned at ×100 and ×450. Occasionally, doubtfully labelled cells as seen under bright-field were re-examined with dark-field illumination to verify the labelling. The criterion for a labelled cell was the appearance of many small, regularly shaped, evenly distributed granules in the cell body and the proximal portions of the processes. While endogenous DAB reactivity has been reported in the brain (Keefer & Christ, 1976; Wong-Riley, 1974), reactive structures like red blood cells, microphages and macrophages are easily distinguished from neurons on morphological grounds. Endogenous DAB reactive cells in the brain can also be distinguished from exogenously labelled cells by their morphology, as endogenously reactive cells are for the most part glial cells (Keefer & Christ, 1976), which are small as opposed to labelled neurons which often have labelled processes and therefore present quite a different appearance. In addition, endogenous DAB reactive cells contain stained granules which are distinctly larger than those seen in exogenously labelled neurons. Finally, in the present work, only neurons with clearly defined cell bodies were considered.

After the initial determination of the location of labelled cells within a given structure the delineation of the structure was compared with those named in various atlases (Rexed, 1952, 1954; Valverde, 1962; König & Klippel, 1963; Zeman & Innes, 1963; Berman, 1968; Palkovits & Jacobowitz, 1974).

The HRP animals described in detail in the Results section were selected on the basis of the transection site employed. Of the ten animals transected at $T_{10}$, lateral column transections were complete in three. Three other animals had relatively well circumscribed AL column transections: as the locations of labelled cell groups in these animals were in good agreement, only the data from animals P21 and P23 are presented. Three more animals had well circumscribed lesions of the middle portion of the lateral columns (approximately the dorsal half of the AL column and the ventral half of the DL column): the data from P24 is presented in detail. One animal had a transection limited only to the DL column, and the data from P27 is presented in detail. Two animals were transected at $C_2$, one transection included all the AL column plus 30–40% of the adjacent DL column; the other transection included all the AL column and 75% of the DL column.

## RESULTS

The first three animals presented show descending projections through the ventral, middle or dorsal portions of the lateral columns following transection at $T_{10}$. The fourth case is representative of animals with a transection at $C_2$. The descriptions of these animals cover the central nervous system from the site of transection rostrally as far as the mesencephalic–diencephalic junction. Rostral to this, labelled cells were observed only in the hypothalamus; these are described separately.

### *Localization of cells of origin*

*Hindbrain and spinal cord*

#### *Rat P21*

Animal P21 was transected at $T_{10}$ and was allowed to survive for 3 days after transection and HRP application. The transection site (Fig. 1*h*) extended

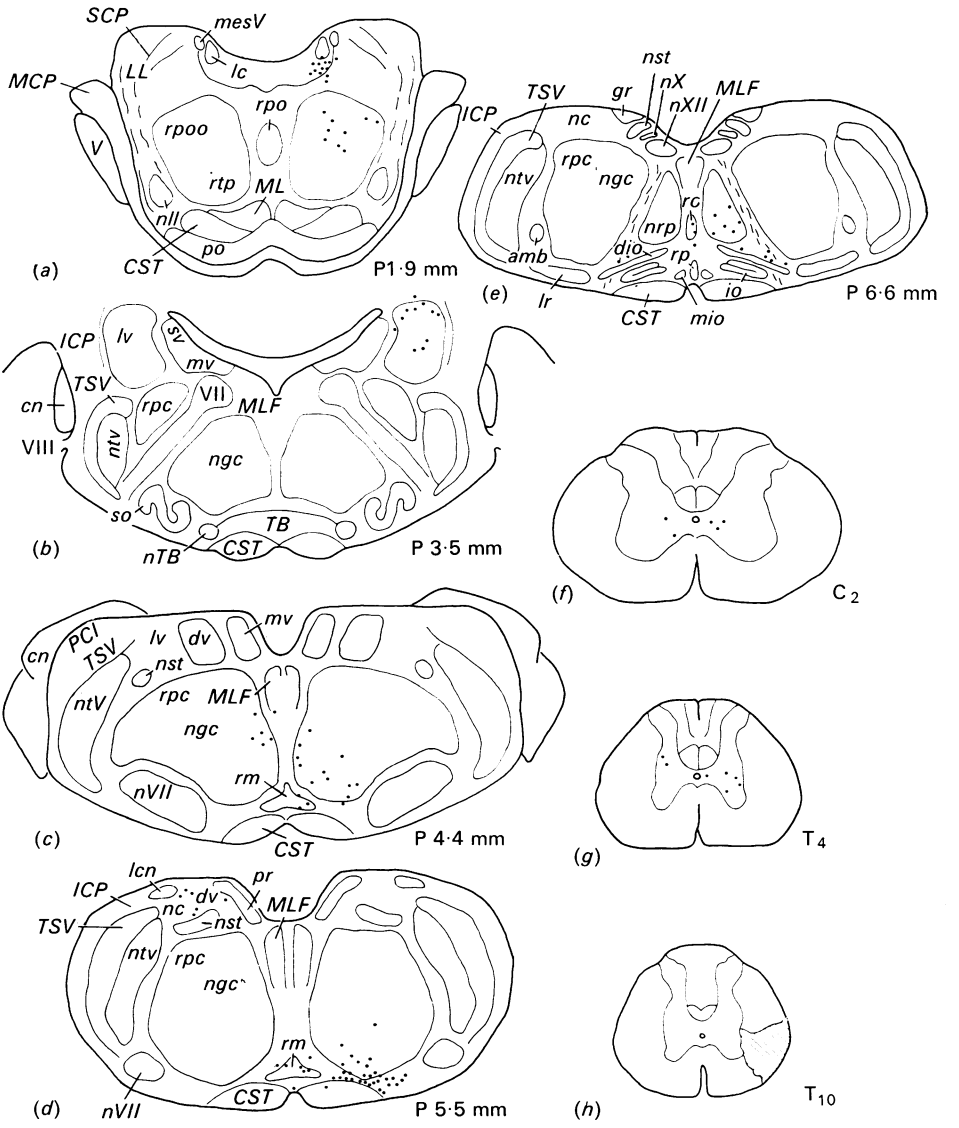


Fig. 1. Location of labelled cells in animal P21 following a transection of the right anterolateral column at $T_{10}$ with subsequent HRP application to the severed axons. The HRP labelled cells are the number per 100 $\mu$m thick section. Stereotaxic co-ordinates (König & Klippel, 1963) are indicated at the bottom right of each brain section.

ventromedially almost to the border of the ventral and AL columns, with little degeneration and no gliosis, observed in the ventral columns. Dorsally, all the AL column appeared transected, with invasion of the adjacent portion of the DL column only in its lateral extent.

*Pons*

*Pontine reticular formation.* Labelled cells were observed in the pontine reticular formation (rf) (Fig. 1*a*). Labelled cells were seen throughout the posterior portion of

nucleus reticularis pontis oralis (rpoo). Few labelled cells were seen posteriorly in nucleus reticularis pontis caudalis (rpoc).

*Locus coeruleus and subcoeruleus.* Labelled cells were rarely seen in locus coeruleus (lc) itself, but were observed in nucleus subcoeruleus (Fig. 1*a*). The size of HRP labelled cells was intermediate between that of the large cells of the nucleus of mes V and the medium sized cells of lc proper.

*Medulla*

*Vestibular complex.* Cells containing reaction product were limited to the ipsilateral lateral vestibular nucleus (lv, Fig. 1*b*). Labelled cells first appeared as the lv enlarged dorsally towards the cerebellum, and occurred mostly in the dorsal half of the lv across the entire mediolateral extent of the nucleus. While cells of varying size were labelled, most of the labelled cells were the large Deiters' cells. No labelled cells were observed in the posterior portion of the lv.

*Medullary reticular formation.* This group of labelled cells was the most numerous of any group seen in P21 (Fig. 1*c*, *d*, *e*). Anteriorly, labelled cells were scattered among the fibres of the trapezoid body, and extended into the medial portion of the ipsilateral nucleus gigantocellularis (ngc). Posteriorly, this group of labelled cells became more numerous and were located in the ventral portion of ngc (Figs. 1*d*, 7*e*). At the level of the inferior olive (io) labelled cells were observed at the dorsal margin, but not in the inferior olive itself (Fig. 1*e*). At this posterior level, labelled cells were more scattered, but still ventrally located, extending into ngc across the fibres of the hypoglossal nerve, and across the medial extension of ngc – the nucleus reticularis paramedianus (nrp). These scattered cells became less numerous in the posterior portion of ngc. A few scattered cells were observed in nucleus reticularis ventralis and, to a lesser extent bilaterally in nucleus reticularis dorsalis.

*Nucleus raphe magnus.* Labelled cells were observed throughout nucleus raphe magnus (rm). A few cells containing reaction product were observed at the anterior extent of the nucleus, just posterior to the trapezoid body (Fig. 1*c*). Posteriorly, cells were more numerous (Figs. 1*d*, 7*a*). Other raphe structures contained no labelled cells except for a few labelled cells in nucleus raphe pallidus.

*Nucleus cuneatus.* A small number of labelled cells were seen in the contralateral nucleus cuneatus (Fig. 1*d*). The cells showing reaction product were observed in the anterior portion of the nucleus. Labelled cells continued posteriorly and were occasionally seen medially in the adjoining nucleus prepositus and laterally in the lateral cuneate nucleus. The posterior extent of this labelled cell group was at the anterior level of nucleus gracilis.

*Spinal cord*

Cells labelled with protein marker were rare throughout the posterior medulla, and this scarcity continued throughout the cervical portion of the cord. At cervical levels, there were 1·2 labelled cells per 100 $\mu$m of tissue. These cells were located bilaterally in an area of the grey matter corresponding to Rexed's laminae VII and VIII (Fig. 1*f*).

At the midthoracic level cells were more numerous, about 7·5 cells per 100 $\mu$m of tissue being fairly evenly divided between the ipsilateral (4·0 cells/100 $\mu$m) and contralateral (3·5 cells/100 $\mu$m) sides of the cord. On the contralateral side cells were located at the base of the dorsal horn, while on the ipsilateral side cells were located in laminae VII and VIII (Fig. 1*g*).

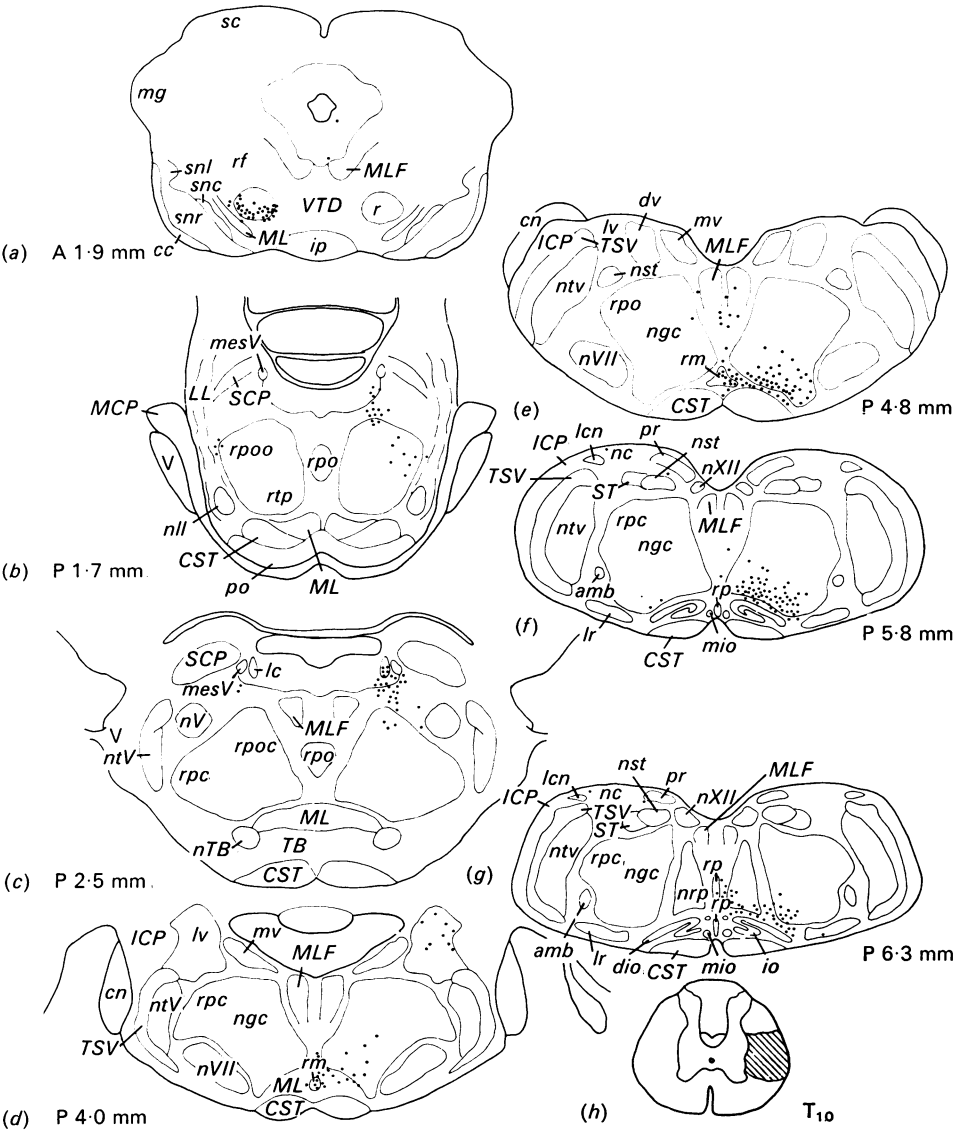


Fig. 2. Location of labelled cells in animal P24 following a transection of the middle portion of the right lateral column at $T_{10}$ with subsequent HRP application to the severed axons. The HRP labelled cells are the number per 100 $\mu$m thick section.

## *Rat P24*

Animal P24 was transected at $T_{10}$ and allowed to survive for 2 days. The transection extended through the dorsal half of the AL column and the ventral half of the DL column. The dorsal portion of the DL column appeared relatively free of degeneration (Fig. 2*h*).

### *Mesencephalon*

*Red nucleus.* Numerous well labelled medium and large cells were observed through the ventral portion of the red nucleus (r), as well as at the posterior pole

of the nucleus where the giant cells of the magnocellular portion are located (Fig. 2*a*). A few labelled cells were seen in the parvocellular portion, and in the anterior extent of the magnocellular portion.

*Pons*

*Pontine reticular formation.* Labelled cells were predominantly in the nucleus pontis oralis (Fig. 2*b*), with few in the nucleus pontis caudalis (Fig. 2*c*). Of the various sized cells of the pontine reticular formation, labelling occurred mainly in the medium to large cells.

A few scattered cells occurred in the most anterior portion of rpoo with the number of labelled cells increasing posteriorly. Although labelled cells occurred bilaterally, a larger number were observed ipsilaterally. Additionally, a small but distinct group of labelled cells occurred at the border of rpoo and the lateral lemniscus.

*Locus coeruleus and subcoeruleus.* Labelled cells occurred in the ipsilateral nucleus subcoeruleus and locus coeruleus (Fig. 2*b*, *c*). They were more numerous following a midlateral transection than after an AL column transection. These cells first appeared at the level of the anterior lc (Figs. 2*b*, 7*d*). This relatively large cluster of cells reached a maximum ventrodorsal extent of about 0·7–0·8 mm. The cells varied in size, however, none being as large as those of the nucleus of mes V.

*Medulla*

*Vestibular complex.* A few cells were seen in the dorsal portion of the ipsilateral lv at the level of the anterior half of the nucleus of VII (Fig. 2*d*). No labelled cells were observed elsewhere in the vestibular complex.

*Medullary reticular formation.* Beginning at the anterior extent of ngc and continuing for about 3·0 mm posteriorly, every section contained many labelled cells. The heaviest concentration of such cells was at the ventral margin of ngc (Figs. 2*d*, *e*). Posteriorly, labelled cells maintained the same position in the reticular formation and were observed through the ventral portion of nrp and ngc (Fig. 2*g*). A few were observed in nucleus reticularis ventralis, mostly in its ventral half. On average, 5·0 cells were observed per 100 $\mu$m of tissue, and were about equally divided between the ipsilateral (2·9 cells/100 $\mu$m) and contralateral (2·1 cells/100 $\mu$m) sides.

*Raphe nuclei.* Labelled cells were observed in the ipsilateral nucleus raphe magnus (Fig. 2*e*). A few appeared at the most anterior extent of rm, but as the nucleus enlarged posteriorly the number increased. A few cells were also observed along the midline in the anterior portion of nucleus raphe pallidus.

*Nucleus cuneatus.* A small number of labelled cells were observed in nucleus cuneatus (nc), predominantly contralateral to the transection, and in the anterior portion of nc, the lateral cuneate nucleus and nucleus prepositus.

*Rat P27*

Animal P27 was transected at $T_{10}$ and was allowed to survive for 2 days. The transection site was triangular in shape and included slightly less than the dorsal half of the DL column (Fig. 3*i*).

*Pons*

*Pontine reticular formation.* Only one group of cells was observed in the pontine reticular formation: this was at the anterior-most level of rpoo, at the border of

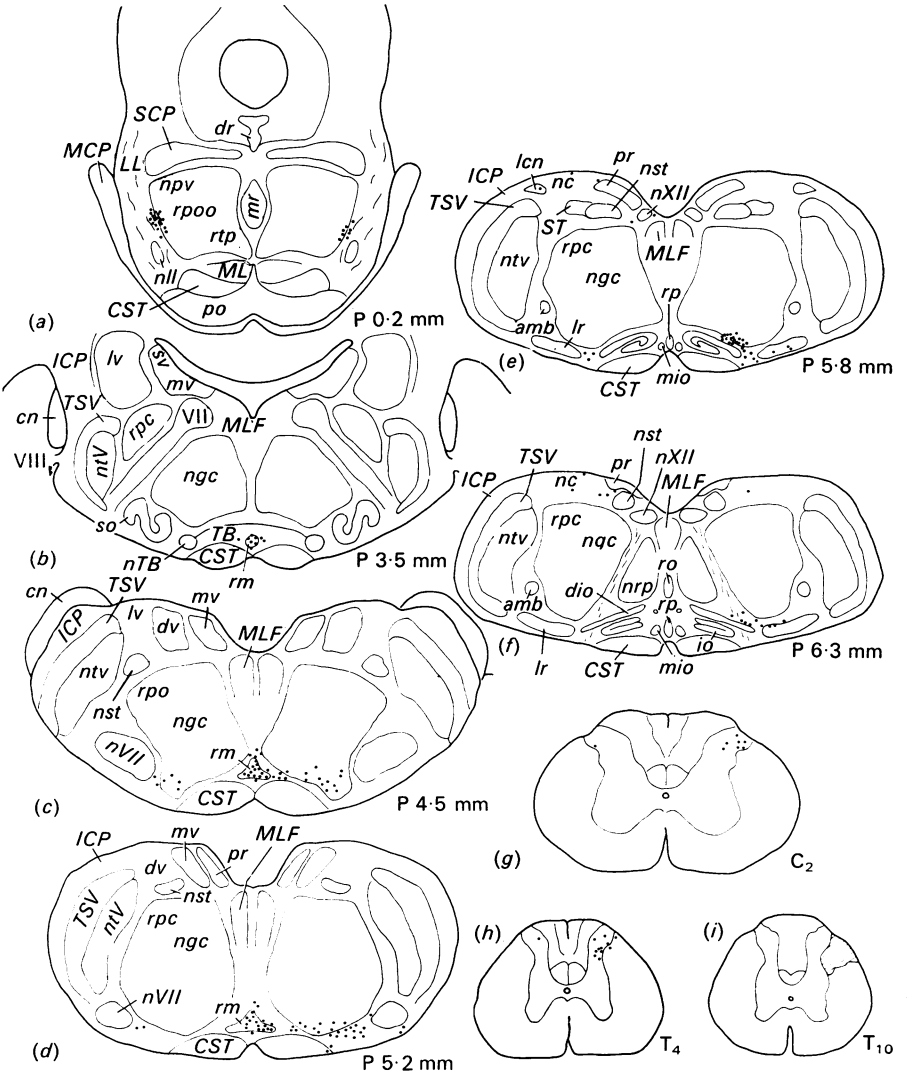


Fig. 3. Location of labelled cells in animal P27 following a transection of the right dorsolateral column at $T_{10}$ and subsequent HRP application to the severed axons. The HRP labelled cells are the number per 100 $\mu$m thick section.

the latter nucleus and the lateral lemniscus (LL), in the far lateral pontine rf (Fig. 3*a*). While this group appeared distinct in cross section, it was small, and extended for only about 0·5 mm in the A–P plane. Labelled cells were present bilaterally, but were more numerous contralateral to the transection.

## *Medulla*

*Medullary reticular formation.* Labelled cells were observed in the ipsilateral ngc at the ventral border of the nucleus between rm and the nucleus of VII (Figs. 3*c*, *d*). This position was maintained throughout ngc (Fig. 3*e*). At posterior levels of ngc the number of labelled cells decreased (Fig. 3*f*). Compared with a midlateral

transection, a DL column transection resulted in fewer labelled cells in ngc, and such cells occupied a more compact position in the ventral portion of the medulla.

An average of 2·3 labelled cells per 100 $\mu$m of tissue was observed in the ventral portion of nucleus reticularis ventralis, mostly on the ipsilateral side (2·0 cells/100 $\mu$m). An average of 6–7 labelled cells was observed per 100 $\mu$m of tissue in the anterior portion of the lr (Fig. 3*f*).

*Raphe nuclei.* Labelled cells in the nucleus raphe magnus were most numerous following a DL column transection. A few first appeared in the posterior portion of the pons in the trapezoid body, and probably represent the anterior extent of rm (Fig. 3*b*). Posteriorly, the number of labelled cells increased (Figs. 3*c*, *d*). Besides rm, other raphe nuclei were devoid of labelled cells except for a few scattered cells in nucleus raphe pallidus.

*Nucleus cuneatus.* A small number of labelled cells was observed in the contralateral nucleus cuneatus (Figs. 3*e*, *f*), confined to the anterior portion of the nucleus, and at this level these cells were also observed in the adjoining nuclei, the lateral cuneate nucleus and nucleus prepositus. Also, a small number of cells occurred medially, directly anterior to the nucleus of XII, but only contralateral to the transection.

In animal P27 the red nucleus was not clearly defined, making positive identification of the location of labelled cells difficult.

*Spinal cord*

An average of 4·6 cells per 100 $\mu$m of tissue was observed at cervical levels (Fig. 3*g*) appearing mostly in laminae III, IV and V. At the midthoracic level there were about 13·3 cells per 100 $\mu$m of tissue, mostly ipsilateral (Fig. 3*h*). At a level directly anterior to the transection ($T_8$ and $T_9$) there were about 15·0 cells per 100 $\mu$m of tissue, mostly ipsilateral, in laminae III, IV and V.

*Rat P37*

Animal P37 was transected at $C_2$ and was allowed to survive for 2 days. The transection site included all of the AL column with only a slight incursion into the adjacent portion of the ventral column. Dorsally, most of the DL column was transected, only a quarter of its dorsal portion being spared.

The distribution of labelled cells after cervical transection was similar to that observed after thoracic transection, with the following additions. A small number of labelled cells were observed ipsilaterally in the anterior portion of the interstitial nucleus of Cajal, in the nucleus linearis pars rostralis, in the deep layers of the superior colliculus, and throughout the descending vestibular nucleus (dv).

*Diencephalon*

*Rat P23*

A representative animal, P23, was transected at $T_{10}$ and allowed to survive for three days after transection and HRP application. The transection included almost all the AL column, sparing only a small portion which was adjacent to the ventral column. Dorsally, the transection included a small 'crescent' of DL column tissue (Fig. 4*c*).

A small number of labelled cells was observed throughout both the magnocellular and parvocellular portions of the ipsilateral paraventricular nucleus in the

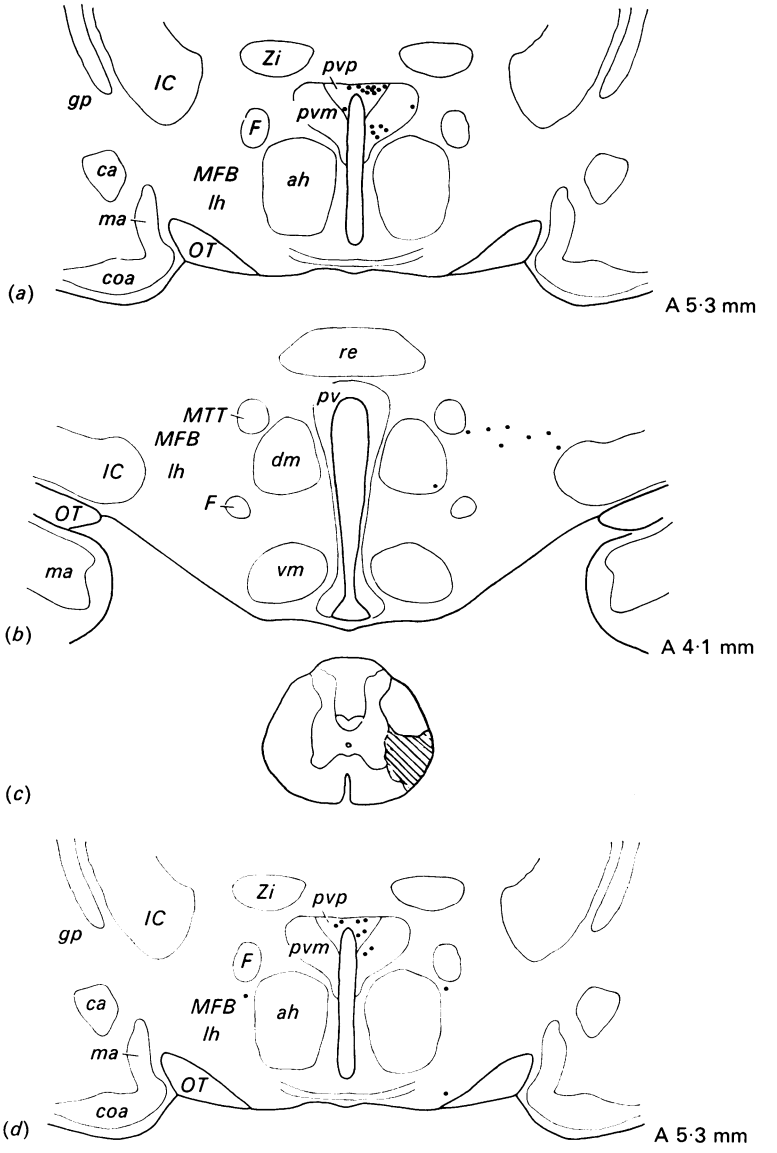


Fig. 4(*a*), (*b*), (*c*). Location of diencephalic labelled cells in animal P23 following a transection of the right anterolateral column at $T_{10}$ with subsequent HRP application to the severed axons. This is a composite figure, in which the charted HRP labelled cells are the total number seen through the indicated anatomical region. (*d*) Location of diencephalic labelled cells following a transection of the right dorsolateral column in animal P27. The total number of observed labelled cells are shown (for transection site see Fig. 3*i*).

hypothalamus (Figs. 4*a*, 7*b*). Posteriorly, a few scattered cells were observed in the lateral hypothalamus (Fig. 4*b*).

### *Rat P27*

In animal P27, with a transection confined to the DL columns, only a few scattered cells were observed in the magnocellular and parvocellular portions of the paraventricular nucleus, predominantly ipsilateral (Figs. 4*d*, 7*c*). A few additional

cells were observed in the medial aspect of the lateral hypothalamus slightly posterior to the level of the paraventricular nucleus.

### *Distribution of lateral column fibres in the spinal cord*

The location of fibre and preterminal degeneration in the spinal cord below the level of transection is illustrated with two animals which demonstate the pattern seen following a predominantly AL or DL column transection.

#### *Rat P34*

Animal P34 was transected at $T_{10}$ and allowed to survive for 7 days. The transection site included most of the AL column, only the ventromedial portion bordering the adjacent ventral column being spared. A small amount of the adjacent DL column was also transected (Fig. 5*a*).

At the level of $T_{13}$–$L_1$, degenerating fibres were seen to have left the transected AL column and expanded throughout the ipsilateral DL column (Fig. 5*b*). Degenerating fibres entered the grey matter, with preterminal degeneration observed from lamina VII dorsally to lamina III. Some degenerating fibres coursing through the ipsilateral zona intermedia gained access to the contralateral side. From this point scattered preterminal and fibre degeneration was observed through laminae V, VI, VII and VIII, contralaterally.

In the white matter of the cord at $L_3$, degeneration was more circumscribed and did not extend as far into the DL and ventral columns as at more anterior levels (Fig. 5*c*). Degenerating fibres passed from the white to the grey matter, predominantly adjacent to the transected AL column. Fibres continued medially through the zona intermedia and gained access to the contralateral side, where scattered preterminal degeneration was seen throughout lamina VII. A similar pattern of degeneration was observed through the posterior lumbar and sacral portions of the spinal cord (Fig. 5*d*, *e*).

#### *Rat P9*

Animal P9 was transected at $T_{10}$ and allowed to survive for 7 days. The transection included most of the right DL column (Fig. 6*a*).

At anterior levels of the lumbar enlargement degenerating fibres in the white matter were largely confined to the ipsilateral DL column (Fig. 6*b*). Degenerating fibres were seen to sweep from the DL column and enter the adjacent grey matter, filling the lateral aspects of laminae V, VI and, to a lesser extent, VII. Preterminal degeneration was observed in laminae V and VI, but the majority of fibres continued to the medial portion of the zona intermedia and lamina VIII. The small amount of degeneration seen in the medial and lateral motor nuclei at this level appeared to be predominantly fibres of passage. A moderate amount of preterminal degeneration occurred in the contralateral lamina VIII with some scattered degeneration in laminae VI and VII. More posteriorly ($L_4$) a similar pattern of degeneration was observed (Fig. 6*c*). Degeneration was observed throughout most of the grey matter ipsilateral to the transection (laminae V through VIII), and was prevalent contralaterally, particularly in lamina VIII. A migration of fibres from the transected DL column to other portions of the white matter continued throughout the lumbar cord, and by sacral levels degeneration was intense in the dorsal portion of the contralateral DL columns.

At $L_6$, two major contralateral projections from the transected DL column

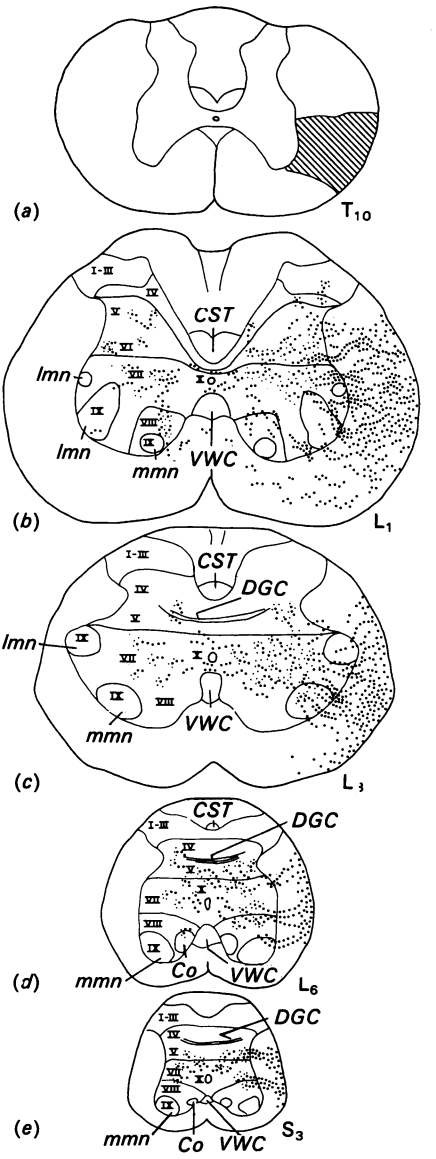


Fig. 5

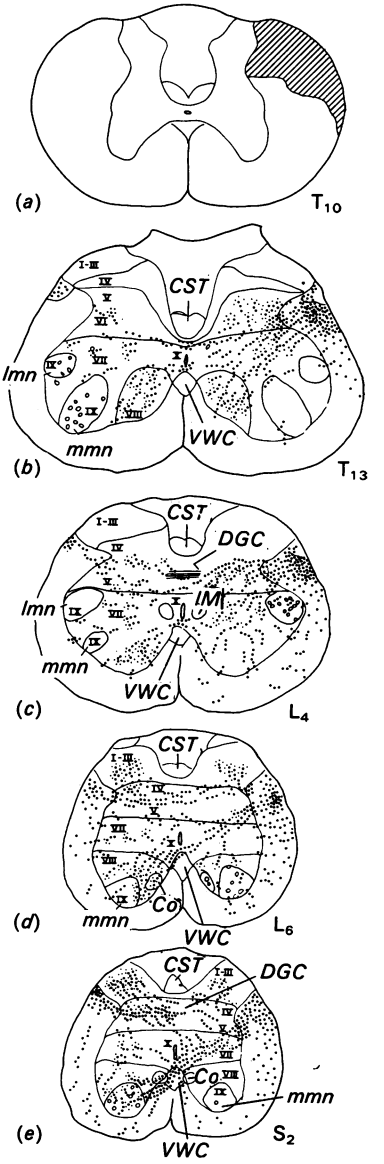


Fig. 6

Fig. 5. Location of fibre (large dots) and preterminal degeneration (small dots) caudal to the level of transection. The right anterolateral column was transected at $T_{10}$. Cytoarchitectonic lamination indicated with Roman numerals.

Fig. 6. Location of fibre (large dots) and preterminal degeneration (small dots) caudal to the level of transection. The right dorsolateral column was transected at $T_{10}$. Cytoarchitectonic lamination indicated with Roman numerals.

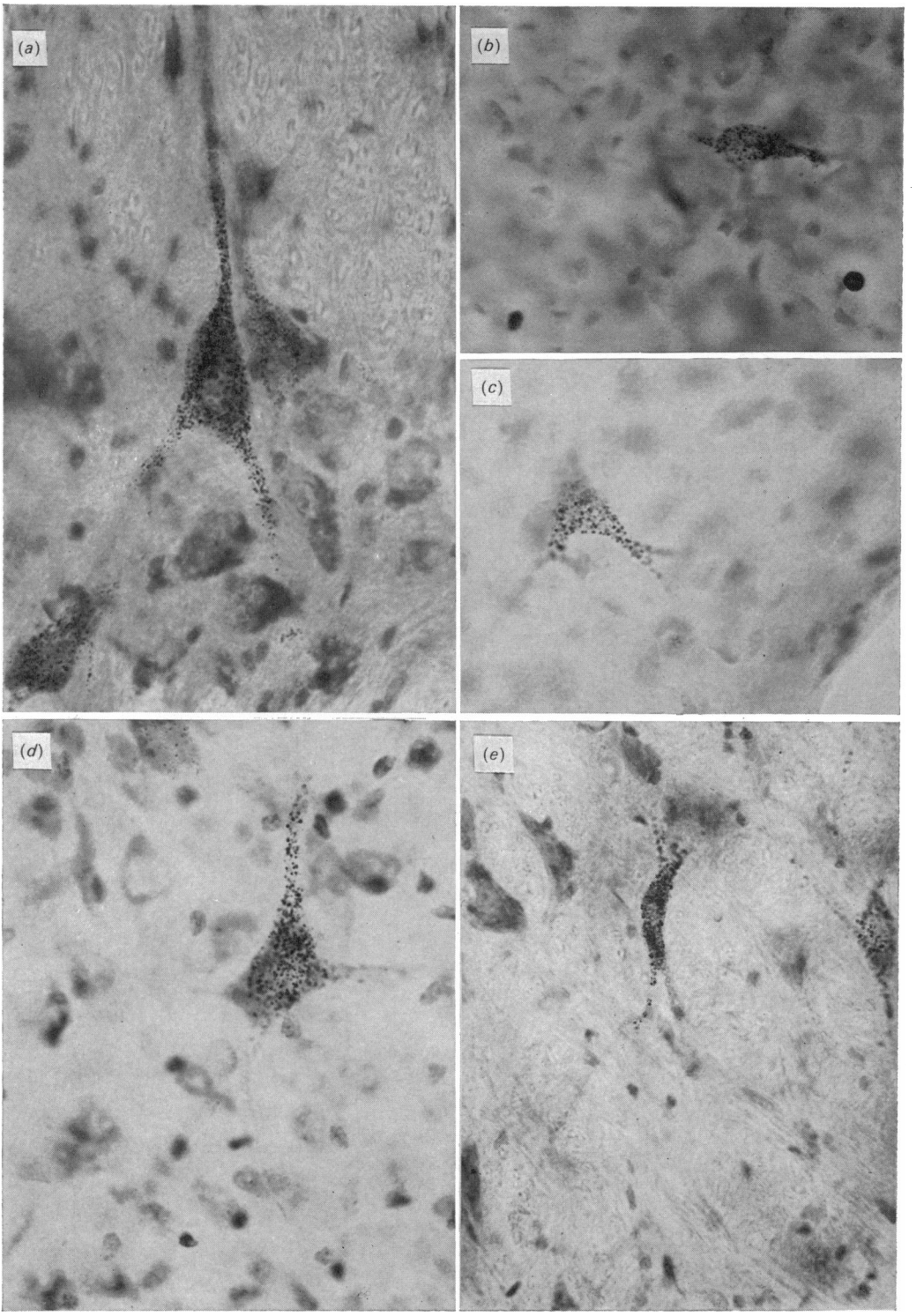


Fig. 7. Photomicrographs of HRP labelled cells in (*a*) raphe magnus, (*b*) and (*c*) the paraventricular hypothalamic nucleus (in (*c*) note third ventricle at bottom right), (*d*) nucleus subcoeruleus and (*e*) nucleus gigantocellularis. Approximately × 1310.

became clearly discernible (Fig. 6*d*). Fibres were seen to cross the midline either to terminate in the contralateral dorsal horn or to enter the contralateral DL column. The second group of fibres traversed lamina VII and gained acess to the contralateral side by coursing ventral to the central canal. This projection was observed throughout the contralateral laminae VIII and IX and nucleus commissuralis (nucleus dorsomedialis). At the level of $S_2$ the two major sites of preterminal degeneration described above appeared more clearly (Fig. 6*e*). Following cervical transection of the DL column little degeneration was observed in the dorsal horn or DL column contralaterally.

## DISCUSSION

### *Technical considerations*

Litchy (1973), from a study of excised artificially bathed frog sartorius nerve, suggested that direct uptake of HRP by cut nerves was possible. Since then HRP uptake by injured nerves, and subsequent retrograde labelling of cell bodies, has been observed in the monkey dorsal motor nucleus of the vagus (dmnX) following transection of the nerve (DeVito, Clausing & Smith, 1974), in the mouse facial nucleus following transection of the nerve (Kristensson & Olsson, 1974), in the chicken isthmo-optic nucleus following injury to the retina (Halperin & LaVail, 1975), in the rat dorsal root ganglion following transection of the sciatic nerve (Furstman, Saporta & Kruger, 1975), in monkey retinal ganglion cells following nerve injury in the lateral geniculate (Bunt *et al.* 1975) and in the rat piriform cortex and olfactory tubercle following injury to the stria medullaris or rostral habenula (Herkenham & Nauta, 1977).

In a study comparing modes of HRP delivery, DeVito *et al.* (1974) found that cutting the vagus nerve and applying HRP produced more labelled cells in the dmnX than when HRP was applied to terminal areas. A study of HRP transport in injured nerves *viz-à-viz* transport via intact nerve endings led to the conclusion that, initially, injured axons accumulate less HRP than intact axons, probably because of a transient inability of the injured axons to take up protein marker (Halperin & LaVail, 1975). However, after this initial stage, the transport and uptake of HRP is accelerated. As early as 6–7 hours after injury and HRP application traumatized neurons contain more HRP than intact neurons.

One important question regarding the relation of labelled cells to the transection site is whether *intact* axons located on either side of the transection site took up HRP. In a study employing iontophoretic application of HRP, and examining afferents to the habenular nucleus, Nauta *et al.* (1974) did not find evidence for uptake by intact fibres. In the present study also it appeared that intact fibres did not take up protein marker. For example, in a transection that was limited ventrally to the dorsal half of the AL column (case P24), few, if any, lateral vestibular cells contained reaction product, while a complete AL transection resulted in significant labelling of the lv (case P21). This suggests that intact vestibulospinal fibres in the ventral half of the AL columns (where gliosis and degenerating axons were not observed in case P24) did not take up and transport HRP. Similarly, dorsally located rubrospinal fibres did not appear to take up HRP unless they were transected (compare cases P21 and P24). This correlation of the location of labelled cells with the transection site also suggests that uptake of HRP by terminals near the area of application accounted for only a small percentage of the labelled cells observed.

It thus appears that injured axons did take up HRP, while intact passing axons

did not, negating one possible source of false *positive* results. The possibility of false *negative* results should be seriously entertained if the system under study is highly collateralized. For example, cells giving rise to the reticulospinal tract appear to have many axon collaterals (Peacock & Wolstencroft, 1976). If HRP is applied to a small percentage of the severed collaterals (e.g. in coccygeal or sacral segments, of the spinal cord), HRP reaction product in the cell bodies may not be visible.

### *Cells of origin of descending lateral column tract fibres*

#### *Hypothalamus*

In the present study labelled cells were observed in the paraventricular nucleus of the hypothalamus, mostly ipsilateral to the transection. Beattie, Brow & Long (1930) placed lesions in the hypothalamus and traced degeneration as far posteriorly as the sacral segments of the cord. Later studies did not confirm this projection and their results were dismissed. The present study, and those of Kuypers & Maisky (1975), Hancock (1976) and Saper, Loewy, Swanson & Cowan (1976) support Beattie's original suggestion of direct hypothalamospinal connexions.

#### *Red nucleus*

It has been generally reported that the rubrospinal tract arises from the large cells of the magnocellular red nucleus; however, Pompeiano & Brodal (1957) reported that, in the cat, cells of all sizes showed chromatolysis following transection of the tract. Following HRP application in the present study, at most one third of the labelled cells were large or giant cells typical of the magnocellular red nucleus. The remainder, although located in the magnocellular portion, were medium sized. Additionally, a large number of unlabelled cells were observed. This may be because of limitations of the HRP technique, but more probably because all red nucleus cells do not send axons to the spinal cord, but rather to the cerebellum (Brodal & Gogstad, 1954) or brain stem (Waldberg, 1958; Courville, 1966; Poirier & Bouvier, 1966).

In the present study labelled cells were observed exclusively contralateral to the side of HRP application in all animals. The crossed nature of red nucleus fibres has been noted in many species (Collier & Buzzard, 1901; Cooper & Sherrington, 1940; Pompeiano & Brodal, 1957; Petras, 1967; Edwards, 1972; Kuypers & Maisky, 1975).

A substantial number of labelled red nucleus cells was observed following HRP application as far posterior as $T_{10}$. Pompeiano & Brodal (1957) observed retrograde changes following transection as far caudal as $L_1$. Similarly, other authors have reported rubrospinal fibres extending to the posterior portion of the spinal cord (Collier & Buzzard, 1901; Orioli & Mettler, 1956; Petras, 1967; Brown, 1974; Kuypers & Maisky, 1975).

By applying HRP to lesions in various portions of the lateral columns, a relatively circumscribed location could be assigned to the rubrospinal fibres. It appears that these fibres descend in the DL columns, for lesions restricted to the AL columns result in few, if any, labelled red nucleus cells (compare animal P21 to P24). This observation is in agreement with other studies in the rat (Brown, 1974) and cat (Petras, 1967).

*Central grey and interstitial nucleus of Cajal*

Following a $C_2$ transection and HRP application, a few labelled cells were observed in the central grey and the interstitial nucleus of Cajal, ipsilaterally. These cells occurred at the mesencephalic level of the central grey. The present results agree with those of Carpenter, Harbison & Peter (1970) in the monkey, and of Kuypers & Maisky (1975) in the cat.

*Superior colliculus*

Labelled cells were observed in the deep layers of the contralateral superior colliculus following transection and HRP application at $C_2$. In the cat, Kuypers & Maisky (1975), using the HRP technique, observed a similar contralateral projection to cervical levels. Rasmussen (1936) traced fibres from the superior colliculus to the lower cervical cord, but not further. Verhaart & Van Beusekom (1958), Altman & Carpenter (1961) and Petras (1967) obtained similar results.

*Locus coeruleus and subcoeruleus*

Labelled cells were observed in nucleus subcoeruleus and locus coeruleus ipsilaterally. This projection to the spinal cord has been observed in the cat and the monkey with both the HRP (Kuypers & Maisky, 1975; Hancock & Fougerousse, 1976; Castiglion, Gallaway & Coulter, 1977) and autoradiographic techniques (Pickel, Segal & Bloom, 1974). Also, following lc lesions there is a reduction in dopamine-$\beta$-hydroxylase activity in the spinal cord (Ross & Reis, 1974).

*Recticular formation*

*Nucleus reticularis pontis oralis.* Transection of the ventral or middle portion of the lateral columns resulted in labelled cells throughout rpoo, bilaterally, and nucleus subcoeruleus, ipsilaterally. Using the Marchi method, Papez (1926) reported a bilateral projection from rpoo in the cat spinal cord. The bilateral nature of this projection has been noted by others in the cat (Kuypers & Maisky, 1975) and primate (Bodian, 1946). A transection limited to the DL columns resulted in few labelled cells in the pontine rf, except for a relatively distinct small group of cells at the lateral margin of rpoo. This distinct cellular group is approximately in the same position as the $A_7$ cell group of Dahlstrom & Fuxe (1964).

*Nucleus reticularis pontis caudalis.* Following transection of the lateral columns and subsequent HRP application, few, if any, labelled cells were observed in rpoc. A small reticulospinal projection from rpoc through the lateral columns was suggested in the older literature (Papez, 1926). However, using the modified Gudden method, Torvik & Brodal (1957) found few changed cells in rpoc following transection of the lateral columns. Nucleus pontis caudalis appears to project predominantly through the ventral columns. This predominantly ventral column projection of rpoc has been verified using the silver impregnation method by Nyberg-Hansen (1965) and Petras (1967). The present results are consistent with these more recent reports.

*Nucleus reticularis gigantocellularis.* The largest group of labelled cells anywhere in the brain was observed in the ventral ngc. The location of labelled cells seen in the present study on the rat agrees with other studies in the cat using the Marchi (Papez, 1926), silver impregnation (Nyberg-Hansen, 1965; Petras, 1967) and Gudden (Torvik & Brodal, 1957) methods. The HRP technique also gives a similar location

of reticulospinal cells in the rat (Burton & Loewy, 1977) and cat (Kuypers & Maisky, 1975).

*Location of reticulospinal fibres in the spinal cord.* Individual reticular nuclei appeared to project through discrete portions of the cord. The number of labelled rpoo cells was correlated with the ventral extent of the transection. This is in good agreement with data in the cat where fibres from rpoo appear to traverse the ventral columns, with only a moderate number of fibres located in the ventromedial portion of the AL columns (Nyberg-Hansen, 1965). Similarly, fibres from rpoc, in the cat, occurred even further medially than fibres from rpoo (Petras, 1967), and perhaps this would account for the absence of labelled cells in rpoc in the present study. Irrespective of the location of the transection in the lateral columns, many labelled ngc cells occurred, suggesting that ngc fibres project diffusely throughout the lateral columns. Electrophysiological evidence suggests that retriculospinal fibres from ngc traverse the ventral and lateral columns, which is in good agreement with anatomical data from the cat (Nyberg-Hansen, 1965; Petras, 1967; Peacock & Wolstencroft, 1976) and from the results of the present study.

*Vestibular complex*

*Lateral vestibular nucleus.* The predominantly uncrossed nature of the vestibulospinal tract observed in the present study is in agreement with older observations using the Marchi or Gudden methods (Russel, 1897; Lloyd, 1899; Collier & Buzzard, 1901; Fraser, 1901; Gray, 1926; Foerster & Gagel, 1932; Buchanan, 1937; Ferraro, Pacella & Barrera, 1940; Cooper & Sherrington, 1940; Pompeiano & Brodal, 1957). Nyberg-Hansen & Mascitti (1964) followed degenerating fibres ipsilaterally through the cord following lv lesion, while Kuypers & Maisky (1975), using the HRP technique, also found that the lv projects to the cord ipsilaterally.

Following HRP application as far posterior as $T_{10}$, labelled cells were observed in the lv in the present studies. That the lv projects to the most posterior segments of the cord has been reported in the rabbit (Hashimoto, 1928), the cat (Pompeiano & Brodal, 1957; Massapust, 1957; Petras, 1967), the dog (Russel, 1897), the monkey (Kuypers, Fleming & Fairnholt, 1962) and man (Collier & Buzzard, 1901).

Vestibulospinal fibres appeared to course through the ventral portion of the spinal cord in the present experiments. This is in agreement with Petras (1967), who found, in the cat, that vestibular fibres were predominantly in the ventral columns.

*Descending vestibular nucleus.* Labelled cells were observed from the posterior portion of the dv caudally through the anterior portion of nucleus cuneatus following HRP application at $C_2$. Following HRP application at $T_{10}$, labelled cells were limited to the anterior level of nucleus cuneatus.

While many studies have not observed a projection from the descending vestibular nucleus to the spinal cord, a few indicate that some projection does exist. Hashimoto (1928), Yoshida (1924) and Schuren (1912) reported retrograde cellular changes following spinal cord transection. Buchanan (1937) observed degenerating fibres as far posterior as the cervical cord, while Rasmussen (1932) could trace them to the thoracic cord. In a study that is consistent with the present findings and other studies using the HRP technique, Allen (1927) reported that cells located at the posterior extent of the dv, and lateral to the solitary tract, project to the cord.

*Raphe nuclei*

In the present study labelled cells were observed in nucleus raphe magnus following transection and HRP application to the ventral, middle or dorsal portions of the lateral columns, suggesting that these descending fibres are distributed throughout the lateral columns. Following HRP application at $C_2$ an appreciable number of labelled cells were also seen in nucleus raphe pallidus. Studies in the cat, using the Gudden method (Brodal, Taber & Waldberg, 1960), autoradiography (Bobillier *et al.* 1976), and the HRP technique (Kuypers & Maisky, 1975), also indicate nucleus raphe magnus as the major raphe nucleus giving rise to spinal projections.

*Spinal cord – pattern of degeneration*

By comparing patterns of preterminal degeneration following transection both to our HRP findings and to the results of previous studies which lesioned discrete descending systems, the nuclear origins of some of the degenerating fibres can be suggested. Laminae V, VI, VII and VIII appear to receive fibres from the vestibular complex (Nyberg-Hansen & Mascitti, 1964) and the pontine reticular formation (Torvik & Brodal, 1957; Petras, 1967). Contralateral preterminal degeneration probably resulted from fibres of the pontine, and, to a lesser extent, medullary reticular formation, which project bilaterally, as the vestibular complex appears to project only ipsilaterally (Petras, 1967). Additionally, propriospinal fibres, whose cell bodies lie in the zona intermedia, pass to the ventral portion of the grey matter (Réthelyi & Szentágothai, 1969).

Fibres coursing through the DL columns include some from the red nucleus, the reticular formation (mainly ngc), the pontine cell group at the border of the lateral lemniscus, and, to a lesser extent, the nucleus subcoeruleus. The preterminal degeneration observed below the level of transection in the lateral basilar region of the dorsal horn and in the lateral part of the zona intermedia was probably due to transected rubrospinal fibres. The projection of these fibres to laminae V, VI, VII and possibly VIII is in agreement with the findings of Hinman & Carpenter (1959), Nyberg-Hansen & Brodal (1964) and Petras (1967). The two major projections to the contralateral side from the transected DL column are: through the dorsal grey commissure to the dorsal horn and Lissauer's tract; and ventral to the central canal to lamina VIII and the medial motor nuclei. Most of this degeneration may be due to the interruption of propriospinal fibres, as both projections were less intense following a $C_2$ transection. HRP labelled cells were observed through the length of the spinal cord in the dorsal horn, and it appears that most of the input to the dorsal horns, especially laminae II and III and to a lesser extent IV, is not due to long descending systems (Petras, 1967), but to medium and long range spinospinal fibres (Szentágothai, 1951, 1964; Réthelyi & Szentágothai, 1969).

*Functional considerations*

The descending systems examined in the present study influence the control of both sensory and motor functions of the spinal cord. The modulation of posture and movement by the vestibulo- and rubrospinal tracts is well documented (Hongo, Jankowska & Lundberg, 1969). Also, complex reflexes such as mating reflexes are mediated by supraspinal structures. Mating capabilities are drastically decreased in female rats following AL column transections, while dorsal, ventral or DL column transections have little effect (Kow *et al.* 1977). The present study provides a neuro-

anatomical basis for interpreting such behavioural data. For example, vestibulo- or reticulospinal fibres coursing through the AL columns may mediate mating behaviour. This suggestion is supported by studies demonstrating that bilateral lesions of the lateral vestibular nucleus or nucleus gigantocellularis disrupt mating reflexes (Modianos & Pfaff, 1976), as well as by studies showing that electrical stimulation of the lateral vestibular nucleus facilitates female rodent mating reflexes (Modianos & Pfaff, 1975, 1977).

Descending systems can also modulate sensory input. For example, electrical stimulation of various areas of the brain can produce analgesia (Liebeskind & Paul, 1977). Stimulation-produced analgesia (SPA) may block pain sensation through connexions to the spinal cord, for SPA can totally arrest the firing rate of Class 2 nociceptive cells located in the dorsal horn (Oliveras, Besson, Guilbaud & Liebeskind, 1974). Further, SPA can be blocked by bilateral lesions of the DL columns (Liebeskind & Paul, 1977). Thus combined neuroanatomical and behavioural studies can elucidate structural–functional relationships in this field.

## SUMMARY

The location of the cells of origin and the projection areas of descending fibre tracts of the spinal cord lateral columns were examined in rats. Unilateral micro-transections of subpopulations of lateral column fibres, at $C_2$ or $T_{10}$, with subsequent application of horseradish peroxidase to the severed axons, allowed identification, by retrograde labelling, of those cell groups projecting to the spinal cord through the lateral columns. Additionally, the pattern of fibre and preterminal degeneration below the level of transection was examined using the Fink–Heimer silver impregnation technique.

The largest number of labelled cells was observed in the ventral portion of nucleus gigantocellularis, projecting ipsilaterally through both the anterolateral (AL) and dorsolateral (DL) columns. Labelled cells were observed in the dorsal portion of the lateral vestibular nucleus (lv) following a $T_{10}$ transection, and throughout the nucleus following a $C_2$ transection. Protein marker was observed in the large Deiters' cells of the lv, ipsilaterally. Also following an AL, but not a DL, column transection, retrograde labelled cells occurred throughout nucleus reticularis pontis oralis (rpoo), bilaterally. At the border of rpoo and the lateral lemniscus, a discrete group of labelled cells was observed bilaterally following a DL column transection. This group of reticulospinal cells was located in a position similar to that of the $A_7$ cell group reported in histofluorescence studies.

The most extensive group of labelled cells following a DL column transection occurred in the magnocellular portion of the contralateral red nucleus. Although labelled cells were observed in the red nucleus following either a $C_2$ or $T_{10}$ DL column transection, labelled cells were more numerous and extended further rostrally and dorsally, following a high cervical transection. Labelled cells in nucleus raphe magnus were also more numerous following a DL column transection.

Additional groups of labelled cells were seen following both an AL or DL column transection. These groups included nucleus subcoeruleus ipsilaterally, and nucleus reticularis ventralis and the nucleus of the tract of spinal V, bilaterally. Labelled cells were observed as far forwards as the hypothalamus, occurring predominantly in the paraventricular nucleus, ipsilaterally. A few labelled cells were observed in the lateral hypothalamus.

Some cell groups were labelled only after a $C_2$ transection. These included the interstitial nucleus of Cajal, ipsilaterally, the descending vestibular nucleus and the deep layers of the superior colliculus, contralaterally, and the central grey matter and nucleus raphe pallidus.

Fibre and preterminal degeneration resulting from unilateral AL or DL column transection was examined. Following an AL column transection degeneration was most intense in the ipsilateral laminae V, VI and VII. Following a DL column transection, degeneration was most intense ipsilaterally in laminae V, VI and VII and bilaterally in lamina VIII at anterior levels of the lumbar enlargement. At these levels, degeneration was also observed in the contralateral dorsal DL column adjacent to the substantia gelatinosa. Posteriorly, the dorsal DL column degeneration became more intense contralaterally, and small numbers of degenerating fibres coursed from the transected DL column through the grey matter to the lateral and ventral columns bilaterally. Following a DL column transection degeneration was observed in the grey matter at posterior lumbar and sacral levels contralaterally in laminae II, III, IV, VIII and IX.

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## ABBREVIATIONS

| | |
|---|---|
| *ah* | anterior hypothalamic nucleus |
| *amb* | nucleus ambiguus |
| *BIC* | brachium of the inferior colliculus |
| *ca* | central amygdaloid nucleus |
| *CC* | crus cerebri |
| *cmg* | central nucleus of the medial geniculate body |
| *cn* | cochlear nucleus |
| *Co* | nucleus commissuralis |
| *coa* | cortical amygdaloid nucleus |
| *CSC* | commissure of the superior colliculus |
| *CST* | corticospinal tract |
| *DGC* | dorsal grey commissure |
| *dio* | dorsal inferior olive |
| *dm* | dorsomedial hypothalamic nucleus |
| *dr* | dorsal raphe |
| *dv* | descending vestibular nucleus |
| *F* | fornix |
| *gp* | globus pallidus |
| *gr* | nucleus gracilis |
| *iC* | interstitial nucleus of Cajal |
| *IC* | internal capsule |
| *ICP* | inferior cerebellar peduncle |
| *IM* | nucleus intermedio-medialis |
| *io* | inferior olive |
| *ip* | interpeduncular nucleus |
| *lc* | locus coeruleus |
| *lcn* | lateral cuneate nucleus |
| *lh* | lateral hypothalamic nucleus |
| *LL* | lateral lemniscus |
| *lmn* | lateral motor nucleus |
| *lr* | lateral reticular nucleus |
| *lv* | lateral vestibular nucleus |
| *ma* | medial amygdaloid nucleus |
| *MCP* | middle cerebellar peduncle |
| *mes V* | mesencephalic nucleus of V |
| *MFB* | medial forebrain bundle |
| *mg* | medial geniculate body |
| *mio* | medial inferior olive |
| *ML* | medial lemniscus |
| *MLF* | medial longitudinal fasciculus |
| *mmg* | marginal nucleus of the medial geniculate body |
| *mmn* | medial motor nucleus |
| *mr* | medial raphe |
| *MTT* | mamillothalamic tract |
| *mv* | medial vestibular nucleus |
| *nc* | nucleus cuneatus |
| *ngc* | nucleus gigantocellularis |
| *nll* | nucleus of the lateral lemniscus |
| *npv* | ventral parabrachial nucleus |
| *nrp* | paramedian reticular nucleus |
| *nst* | nucleus of the solitary tract |
| *nTB* | nucleus of the trapazoid body |
| *ntV* | nucleus of the tract of spinal V |
| *nIV* | nucleus of the trochlear nerve |
| *nV* | motor nucleus of the trigeminal nerve |
| *nVII* | nucleus of the facial nerve |
| *nX* | nucleus of the vagus nerve |
| *nXII* | nucleus of the hypoglossal nerve |
| *OT* | optic tract |
| *po* | nuclei pontis |
| *pr* | nucleus prepositus |
| *pv* | periventricular nucleus |
| *pvm* | paraventricular nucleus, magnocellular portion |
| *pvp* | paraventricular nucleus, parvocellular portion |
| *r* | red nucleus |
| *re* | nucleus reuniens |
| *rf* | reticular formation |
| *rm* | raphe magnus |
| *ro* | raphe obscurus |
| *rp* | raphe pallidus |
| *rpc* | nucleus reticularis parvocellularis |
| *rpo* | raphe pontis |
| *rpoc* | nucleus reticularis pontis caudalis |
| *rpoo* | nucleus reticularis pontis oralis |
| *rtp* | nucleus reticularis tegmenti pontis |
| *sc* | superior colliculus |
| *SCP* | superior cerebellar peduncle |
| *snc* | substantia nigra, zona compacta |
| *snl* | substantia nigra, lateral portion |
| *snr* | substantia nigra, reticular portion |
| *so* | superior olive |

*ST* solitary tract
*sv* superior vestibular nucleus
*TB* trapezoid body
*TSV* tract of spinal V
*vm* ventromedial hypothalamic nucleus
*VTD* ventral tegmental decussation
*VWC* ventral white commissure
*Zi* zona incerta

Numerals on brain sections refer to cranial nerves:

V trigeminal nerve
VII facial nerve
VIII vestibular nerve

Numerals on spinal cord sections refer to the cytoarchitectonic layers:

I–IX laminae I–IX
X substantia grisea centralis (central grey matter)