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Funicular location of ascending axons of lamina I cells in the cat spinal cord

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The laminar distribution of spinal cord neurons projecting suprasegmentally through different funiculi was determined in the cat using horseradish peroxidase (HRP) injections combined with selective spinal cord lesions. The lesions were designed to limit the caudal transport of HRP to either the ventral funiculi or the dorsolateral funiculus. HRP injections in the ventromedial or ventrolateral funiculi resulted in labeling primarily within laminae IV-VIII and a virtual lack of labeling within lamina I. When the dorsolateral funiculus was injected, 20-25% of all labeled cells were located in lamina I, bilaterally. These results demonstrate that the ascending lamina I projections are through the dorsolateral funiculus.

Neurons located within lamina I of the spinal cord dorsal horn have been shown, by retrograde transport studies, to project directly to the contralateral thalamus, together with a smaller projection to the ipsilateral thalamus[5,9,13,27]. Also, physiological studies have demonstrated that this spinothalamic. lamina I projection is nociceptive specific[10,28]. Experiments done by Kumazawa et al.[15] have indicated that a significant number of lamina I cells can be activated antidromically by stimulating the contralateral ventrolateral funiculus (VLF), implying that they ascend through the contralateral VLF and hence are part of the classically described spinothalamic tract. Recently, however, McMahon et al.[17] demonstrated in the rat, that the lamina I cells with ascending projections were most often antidromically activated by dorsolateral funiculus (DLF) stimulation as opposed to VLF stimulation. This discrepancy raises the question concerning the major funicular location of ascending fibers of lamina I cells. Due to the importance of the VLF in the perception of pain and the importance of lamina I in the discrimination of nociceptive information[7,10,14,28], it becomes important to determine the funicular location of the lamina I ascending pathway. The purpose of this study was to determine the funicular location of ascending projections of lamina I neurons in the cat.

Nine cats weighing 2.5-3.5 kg were used in this study. The animals were anesthetized with Nembutal (35 mg/kg) and a mid-thoracic laminectomy was performed under sterile conditions. Subsequent to a lesion at the T7-T8 level, horseradish peroxidase (HRP) injections (Type VI; Sigma) were made at T5-T6 level of the spinal cord by placing an HRP-soaked gelfoam pellet within the white matter. Pellets were placed at least 10 segments rostral to the lumbar enlargement and attempts were made to limit pellet placement to the spinal white matter in order to minimize labeling of propriospinal neurons. Following a 3-day survival time the animals were reanesthetized and perfused through the descending aorta with 1 liter of saline followed by a solution of 1% paraformaldehyde-1.25% glutaraldehyde in 0.1 M phosphate buffer. After 30 min of fixation, the fixative was washed out with the same buffer solution to which sucrose was added to a final concentration of 10% (w/v). The thoracic and lumbar spinal cords were removed and placed in 10% sucrose-phosphate buffer. The tissue was then cut in serial 60 μm sections and reacted with tetramethyl benzidine to visualize the retrogradely transported HRP[19]. Labeled cells were mapped in the lumbar enlargement

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(L5–S1) and lesion and injection sites verified.

In one control experiment, HRP was placed in the dorsolateral funiculus (DLF) following a caudal DLF lesion in the thoracic cord. The paucity of labeling in the caudal spinal cord (occasional lamina X cells) was accepted as verification of lack of HRP leakage to adjacent funiculi. In addition, since in all other experiments the numbers of labeled cells, throughout the rostro-caudal extent of the lumbar enlargement, remained relatively constant, it was assumed that the number of labeled propriospinal neurons was minimal.

Following an HRP injection in the VLF (experiments H78 and H81) or in the ventromedial funiculus (VMF) (experiments H80 and H82) combined with lesions designed to destroy the DLF, unilaterally or bilaterally, as illustrated in Fig. 1B, C, labeled cells were found primarily in the contralateral lamina VIII and medial lamina VII, constituting 60–70% of all labeled cells in the lumbar enlargement. In experiment H81 in which the VLF was injected, 783 labeled cells were identified (62 sections) of which 467 were located within contralateral laminae VII and VIII. In experiment H82 in which the VMF was injected, 531 cells were identified (108 sections) of which 306 were located within contralateral laminae VII and VIII. When the VMF or VLF was injected with HRP, a small population of cells was also found located in the contralateral laminae IV–VI, as was a distinct group of cells located in the contralateral, lateral lamina VII. The main difference between HRP injections in the VMF as opposed to HRP injections in the VLF was that a larger number of cells was found ipsilateral to the injection in the latter (Fig. 1B). The most striking feature of the experiments with injections in the VMF or VLF was the almost complete lack of labeled cells within lamina I. In the experiments in which the VLF was injected, H81 demonstrated 3 labeled lamina I cells out of 783 total labeled cells (62 sections) and H78 demonstrated one labeled lamina I cell out of 545 total labeled cells (69 sections). In the experiments in which the VMF was injected, H82 demonstrated no labeled lamina I cells out of 531 total labeled cells (108 sections) and H80 demonstrated 4 labeled lamina I cells out of 166 labeled cells (80 sections).

When HRP was injected in the DLF (experiments H76 and H79), combined with lesions of the ventral quadrant (Fig. 1A), 30–40% of all contralaterally labeled cells and 10–15% of all ipsilaterally labeled cells were located in lamina I. Altogether, in experiment H76, of 1822 total labeled cells (79 sections), 337 were located within lamina I, and in experiment H79, of 347 total labeled cells (82 sections), 76 were located within lamina I. The remaining HRP-labeled cells were located primarily within the contralateral lamina VIII (15% of total labeled cells) and the ipsilateral laminae IV–VI (40–45% of total labeled cells).

There was a large variation in the total number of labeled neurons between experiments. This is likely a reflection of variations in the size of the HRP pellet, its placement and the extent of the corresponding lesion. However, the pattern of distribution of labeled neurons was constant within each paradigm which implies that the variation in absolute numbers is probably due to variations of labeling in a single funiculus.

These results show that when the ventral spinal white matter is injected with HRP, the largest population of labeled cells is located within the contralateral laminae IV–VIII. Cells of these deeper laminae are classically described as the cells of origin of the spinothalamic[5,26,27], spinoreticular[11,12,16,18], and spinotectal[25] tracts, which are known to project through the ventral funiculi. Each of these tracts has a small ipsilateral projection which has been described as located within the ventral white matter; however, the present study indicates that this ipsilateral projection travels outside of the ventromedial funiculus.

Injections of the dorsolateral funiculus result in a substantial ipsilateral labeling within laminae IV–VI. This population likely corresponds to the cells of origin of the spinocervical tract which has been shown to originate in the nucleus proprius and projects ipsilaterally through the DLF to terminate in the lateral cervical nucleus[3,8]. In addition, cells of origin of non-primary afferent fibers to the dorsal column nuclei are located within the nucleus proprius and project to the dorsal column nuclei through the ipsilateral DLF[23]. The DLF injections were accompanied by more caudal ventral lesions, but the dorsal columns were left intact. It is possible that some of the HRP spread into the dorsal column axons resulting in label of the neurons of the dorsal column postsynaptic pathway. However, this does not compro-

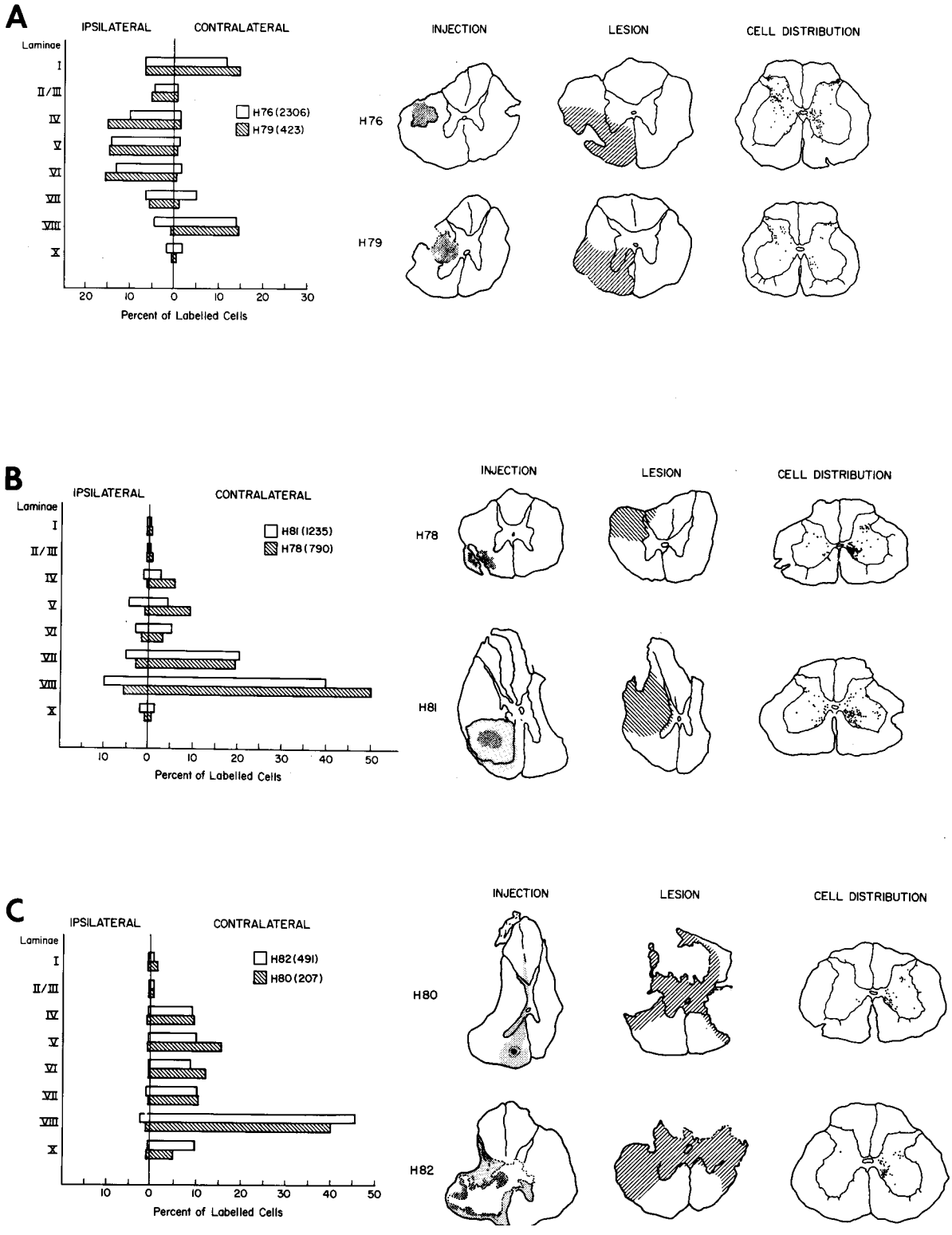

Fig. 1. The laminar distribution of HRP-labeled cells in the lumbar enlargement together with the injection and lesion sites for 6 cats. Bars represent percent of total labeled cells within each lamina ipsilateral and contralateral to the injection. The total number of labeled cells per 100 sections is indicated within parentheses for each animal. The mid-thoracic injection site and the extent of the corresponding low thoracic lesion is illustrated for each animal. The cell distribution plot shows the position of all HRP-labeled cells from 10 randomly selected sections through the L6–L7 segments of each cat. A: distribution of HRP-labeled cells in cats H76 and H79 following a dorsolateral injection of HRP combined with a more caudal ventrolateral lesion. B: distribution of HRP-labeled cells in cats H81 and H78 following a ventrolateral injection of HRP and a more caudal lesion of the dorsolateral funiculus. C: distribution of HRP-labeled cells in cats H82 and H80 following a ventromedial injection of HRP and a more caudal bilateral lesion which spares only the ventral white matter.

mise the main findings of this study since it has been shown that HRP injections in the dorsal column, with or without DLF lesions, result in labeled cells mainly within lamina IV with a smaller population within laminae V–VII, and no labeled cells within lamina I (ref. 2).

The most striking result from this investigation was the large number of lamina I cells labeled, both ipsilateral and contralateral to a DLF injection (20–25% of total labeled cells), and the virtual lack of labeling within lamina I following injections of the VMF or VLF (0–2%). These results represent an important departure from previous notions concerning lamina I anatomy and are surprising in view of previous physiological studies demonstrating that fibers of lamina I neurons project through the VLF[15,22].

A number of investigations have shown that cells of lamina I project directly to the contralateral thalamus[1,5,9,26–28] and tectal regions[25], and the fibers of this projection have been presumed to follow the classical spinothalamic tract. Although projections of lamina I fibers have been described within the DLF[4,6,20,21,24], these were thought to be propriospinal neurons. Yet, in one study[20], fibers of lamina I neurons projecting to supraspinal levels were reported to travel mainly in the contralateral DLF. In addition, Brown et al.[3] demonstrated that following HRP injections in the region of the lateral cervical nucleus, large numbers of lamina I cells were labeled, bilaterally, at the lumbar level and concluded that they were labeled by fibers of passage within the DLF. Their reported percentage of lumbar lamina I cells labeled from injections of the DLF at the cervical level closely agrees with the results of the current study following injections at the thoracic level. This indicates that the dorsolateral funicular course of the ascending fibers of lamina I cells is maintained throughout the spinal cord.

The results of the present study demonstrate that essentially all of the ascending lamina I projections are located within the DLF. Since it has been shown that lamina I cells project to the thalamus, reticular formation and tectal regions, one must conclude that these projections are via the DLF. This implies the presence of a distinct lamina I, dorsolateral, spinothalamic pathway which is currently under investigation in this laboratory. Further investigations are also needed to determine whether an ascending dorsolateral lamina I projection exists in other species.

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