The Spinothalamic Tract:
An Examination of the Cells of Origin of the Dorsolateral and Ventral Spinothalamic Pathways in Cats

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ABSTRACT

The locations of spinothalamic neurons and the funicular trajectories of their axons were studied in cats by retrograde transport of horseradish peroxidase (HRP). Five animals were used as controls to determine the cervical and lumbar laminar distributions of neurons contributing to the spinothalamic tract. An additional eight animals were used to determine the funicular trajectories of the spinothalamic axons of lumbar neurons by utilizing a series of thoracic spinal cord lesions in conjunction with retrograde transport of HRP from the sensory thalamus. Three of these animals underwent midthoracic ventral quadrant lesions, four animals underwent midthoracic dorsolateral funiculus lesions, and one animal underwent total spinal cord transection sparing the dorsal columns. The locations of the cells containing the HRP reaction product were then determined after a 3- to 5-day survival time, and the patterns of labeled cell locations of the lesion groups were compared to the control group patterns. In the lesioned animals, the cervical spinothalamic cell locations were used as a control to confirm the uniformity of the injection sites, transport and tissue processing.

The major finding of this study is that there exist two distinct components of the spinothalamic tract. The dorsolateral spinothalamic tract (DSTT) is made up of axons originating in contralateral spinal cord lamina I and has negligible contribution from the deeper spinal cord laminae. The axons of lamina I cells cross segmentally and ascend exclusively in the dorsolateral funiculus (DLF). The DSTT comprises approximately 25% of the total spinothalamic input from the lumbar enlargement. The ventral spinothalamic tract (VSTT) is made up of axons originating in spinal cord laminae IV-V and VII-X. Very few lamina I cells contribute axons to the VSTT. This crossed pathway ascends in the ventrolateral and ventromedial portions of the spinal cord. No cells contributing to the spinothalamic tract were identified in spinal cord segments caudal to a dorsal column sparing lesion, indicating that there are no spinothalamic tract axons traveling in the dorsal columns.

These results expand the classical concept of information processing by the spinothalamic tract. The DSTT is made up of lamina I cell axons. All lamina I spinothalamic cells respond exclusively to noxious peripheral stimuli. Hence the DSTT is a major nociceptive-specific ascending spinal pathway, yet lies outside the confines normally assigned to the spinothalamic tract. In contrast, the spinothalamic tract axons ascending in the traditional ventral location originate predominantly from neurons of wide dynamic range as well as from low-threshold neurons. These findings have novel implications concerning the relative importance, for nociception, of lamina I and deeper laminar input to the thalamus.

Key words: thalamus, spinal pathways, horseradish peroxidase, dorsolateral funiculus, ventral funiculi
The spinothalamic tract, an important nociceptive system in mammals, has been thought to be confined to the ventral quadrant of the spinal cord since its original description by Edinger (1889). This viewpoint has been supported by the clinical success of ventrolateral cordotomy for pain relief (Horrax, '29; Voris, '57; Rosomoff et al., '65; White et al., '50), identification of thalamic terminal degeneration following ventral quadrant spinal cord section (Berkley, '80; Boivie, '71, '79), and anatomic and physiologic identification of ventral quadrant axons that project to the thalamus (Applebaum et al., '75; Willis et al., '79; Giesler et al., '81a). The cells of origin of the spinothalamic pathway are found in three different spinal cord locations: the apex of the dorsal horn (lamina I), the deep dorsal horn (laminae IV–VI), and the ventral horn (laminae VII, VIII, and X). The relative contributions of these three areas to the spinothalamic tract varies between species (Trevino et al., '72, '73; Albe-Fessard et al., '74; Trevino and Carstens, '75). The lamina I contribution to the spinothalamic tract (Foerster and Gagel, '32; Kuru, '38; Morin et al., '51; Dilly et al., '68; Trevino et al., '73; Willis et al., '74; Albe-Fessard et al., '75; Trevino and Carstens, '75; Giesler et al., '76; Willis et al., '78; Chung et al., '79; Giesler et al., '79; Kensualo et al., '79; Willis et al., '79; Hayes and Rustioni, '80; Craig and Burton, '81; Kevetter and Willis, '83, '84; Craig and Kniffki, '84; Hylden et al., '85; Granum, '86) is of particular significance, since lamina I contains a major spinal cord concentration of nociceptive-specific cells (Christensen and Perl, '70; Price and Mayer, '75; Kumazawa et al., '75; Kumazawa and Perl, '78; Cervero et al., '79; Fitzgerald and Wall, '80).

Since the majority of lamina I cells that project rostrally are located in the contralateral dorsolateral funiculus (Zemlan et al., '78; Molenaar and Kuypers, '78; McMahon and Wall, '83, '85; Apkarian et al., '85), some authors have questioned the importance of their contribution to the spinothalamic system (Molenaar and Kuypers, '78; McMahon and Wall, '83). Preliminary experiments conducted in our laboratory have clearly shown, however, that in cats, some of the lamina I cell axons that ascend in the dorsolateral funiculus terminate in the thalamus (Jones et al., '85). Therefore there are, in cats, two separate spinothalamic pathways: a ventral spinothalamic tract (VSTT), including both the ventrolateral and ventromedial pathways, and a dorsolateral spinothalamic tract (DSTT) made up primarily of lamina I cells axons. On the basis of our preliminary experiments (Jones et al., '85) it was unclear whether the axons of all contralaterally projecting spinothalamic lamina I cells ascend in the dorsolateral funiculus (DLP) or whether there is a distribution of these axons between the ventral and dorsal spinothalamic pathways.

The purpose of this study was to investigate the spinothalamic tract of cats. In particular, the relative contributions of the lumbar spinal cord laminae to the DSTT and to the VSTT were determined and compared to the total population of neurons whose axons make up the lumbar portion of the spinothalamic tract. Retrograde transport of horseradish peroxidase (HRP) from the thalamus to the cervical and lumbar spinal cords was combined with a variety of mid-lumbar spinal cord lesions to investigate this question.

METHODS

Twenty-two adult domestic cats were used for these experiments. Nembutal anesthesia (55 mg/kg), sterile surgical technique, and antibiotic prophylaxis were used in all procedures. HRP conjugated to wheatgerm agglutinin (WGA-HRP, n = 20) or HRP-Sigma VI (n = 2) was used as the neural tracer.

A total of 1 μl of 2% WGA-HRP in 0.2 M mannose and 0.9% saline or 1 μl of 50% HRP-Sigma VI in 0.9% saline was injected stereotactically into the somatosensory thalamus. In accordance with known spinothalamic termination sites (Boivie, '71; Applebaum et al., '79; Berkley, '80; Craig and Burton, '85; Apkarian et al., '87), three to five injections were made in each animal to include the ventral posterior lateral nucleus (VPL), the ventral lateral nucleus (VL), the central lateral nucleus (CL), the posterior group (PO) and the nucleus submedius (SM). The stereotactic coordinates for these injections were determined from standard atlases (Jasper and Ajmone-Marsan, '54; Berman and Jones, '82). The WGA-HP was injected with a 1-μl Hamilton syringe at a rate of 0.02 μl/min beginning 5 min after initial needle placement. The needle was not removed for 5–10 min after completing the injection to reduce the spread of HRP along the needle track.

Some animals, at the time of injection, also underwent thoracic laminectomy (T11-T12), through which spinal cord lesions were made by microsurgical technique. The lesions included ventral quadrant (VQ) section ipsilateral to the thalamic injection, dorsolateral funiculus section ipsilateral to the thalamic injection, and a spinal cord transection sparing the dorsal columns.

After a 3- to 5-day survival time, animals were anesthetized with Nembutal (40 mg/kg), pretreated with intravenous heparin, and perfused transcardially with 0.9% saline (1.0 liter) and then by 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 (2.0 liters at room temperature for 30 min). This was followed by 1.0 liter of 5% sucrose in the same buffer at 4°C. The tissue was removed and refrigerated overnight in a 5% buffered sucrose solution. The diencephalon was sectioned coronally (60–80 μm) from the level of the anterior commissure to the inferior colliculus to include rostral midbrain structures. Representative transverse sections were collected from the brainstem and the first cervical segment to include the dorsal column nuclei and the lateral cervical nucleus. Transverse sections were collected from the lumbar (L5-S1) and cervical (C6-T1) enlargements. The cervical enlargement sections were collected to determine the consistency of the pattern of transport from animal to animal.

Tissue sections were processed for HRP histochemistry by using tetramethyl benzidine as the chromagen (Mesulam, '79) and lightly counterstained with 1% neutral red. The thalamic injection sites and the extent of the spinal cord lesions were reconstructed by means of Nissl-stained (5% cresyl violet) sections.

Cells within the spinal gray matter that demonstrated the distinct stippling characteristic of HRP label were counted and their locations were mapped from the transverse sections by brightfield and darkfield microscopy. Cell locations were characterized according to the laminar scheme of Rexed ('54).

RESULTS

Thalamic injection sites

Twenty-two cats underwent multiple thalamic HRP injections, some in combination with various thoracic spinal white matter lesions. Since many spinal cord neurons project directly to the midbrain (Trevino, '76; Willis et al., '79;
Fig. 1. Representative drawings of the extents of thalamic injection sites at the level of VPL and PO in cats with no spinal cord lesions (Controls 1, 2, 3, 4, and 5). The needle tracts are represented by blackened area. The dark portion of the injection site, as seen after TMB processing, is stippled. The drawings and nomenclature were adapted from Jasper ('54).

Fig. 2. Drawings of thalamic injection sites in four cats with thoracic VQ lesions ipsilateral to the injection site (VQ lesions 1, 2, 3, and 4).

Abbreviations

CL centrolateral n.
LGN lateral geniculate n.
MD medial dorsal n.
PO posterior n., lateral division
POm posterior n., medial division
PUL pulvinar
SM n. submedius
VL ventral lateral n.
VPL ventral posterior lateral n.
VPM ventral posterior medial n.

Mantyh, '82; Wiberg and Blomqvist, '84; McMahon and Wall, '85), only those animals in which the injection did not spread to the midbrain were included in the data analysis. In addition, the targeted thalamic nuclei (VPL, PO, CL, SM), which are those with known STT terminations, had to be well filled. Thirteen of these animals had HRP injections that included the targeted sensory thalamic nuclei and did not impinge upon midbrain structures. Only these 13 animals were used for data analysis. The extents of the HRP injection sites are shown in Figures 1–3 for all 13 cats. Each drawing in these figures is a compilation of multiple diencephalic sections through the injected region, illustrating the maximum extent of the HRP injection. A photomicrograph of a sample section from which these drawings were obtained is shown in Figure 4. The injection sites were subdivided into a central dark, concentrated portion that surrounded the point of injection and an adjacent region of lighter label. Figures 1–3 show the dark portion of the injection at the level of the body of VPL and at the level of PO. In these animals the dark portion of the injection covered most of the somatosensory thalamus and was considered to be the primary region of HRP uptake (Kim and
Dorsal to the cortex along the needle track. The injection site occasionally included a portion of the contralateral intralaminar nuclei.

**Unlesioned animals**

Cats with unilateral thalamic injections and no spinal cord lesion served to identify the lumbar and cervical cells of origin of the total spinothalamic tract (VSTT and DSTT). WGA-HRP was used in three experiments (Controls 1, 2, and 3), whereas HRP-Sigma VI was used in two experiments (Controls 4 and 5). The distribution of label was examined in the cervical and lumbar enlargements. Labeled cells, found contralateral to the thalamic injection site, were located mainly in lamina I, laminae IV and V, the medial aspect of lamina VII, and lamina VIII. Occasionally a few HRP-labeled neurons were found within the substantia gelatinosa. Ipsilateral to the injection site a small number of cells were located in lateral lamina I just medial to the dorsal root entry zone and in the medial aspect of lamina VII. The percentage of labeled ipsilateral cells varied from 5% to 10% of the total labeled cells in control experiments. The locations and density of label in the lumbar enlargement of one experiment (Control 1) is shown in Figure 6. In all control experiments the numbers of labeled cells in the cervical enlargement were similar to those in the lumbar enlargement. A larger percentage of the labeled cells were found in laminae IV-V in the cervical spinal cord than in the lumbar spinal cord (mean of 46% vs. mean of 21%; Table 2). In HRP-Sigma VI control experiments the number of HRP-labeled cells located within the nucleus proprius of the lumbar enlargement was smaller than the number found in WGA-HRP experiments. However, this difference was statistically insignificant (Mann-Whitney U test).

The percentage of labeled lamina I cells in the lumbar enlargement varied from 14% to 30% of the total labeled cells. The density of the lamina I projection can be observed in horizontal sections (Fig. 9B). Labeled lamina I neurons often occurred in clusters of three to six cells just medial to the dorsal root entry zone (Fig. 9A). Many labeled lamina I neurons were oriented longitudinally (Fig. 9C). The HRP-labeled cells in the deeper laminae were polygonal or flattened and appeared larger than most lamina I cells (Willis et al., '79; Meyers and Snow, '82b). The total numbers of HRP-labeled cells in the lumbar enlargement and in the cervical enlargement were similar for each animal. However, there was a larger concentration of labeled neurons in laminae VII and VIII of the lumbar enlargement than in the cervical enlargement (Table 2).

Control 1 (Table 2), had two to four times as many labeled cells per section as the other control experiments. Hence, the injection coordinates, survival time (5 days), and neural tracer (WGA-HRP) used in Control 1 were used in subsequent experiments, in which thoracic spinal lesions were made.

**Ventral quadrant lesions**

Four cats (VQ lesion 1, 2, 3, and 4) had adequate thalamic WGA-HRP injections combined with ventral quadrant lesions ipsilateral to the injection side. These lesions were designed to block HRP transport via the ventral quadrant and thereby elucidate the cells of origin of the dorsolateral spinothalamic projection. The number and distribution of HRP labeled neurons in the cervical spinal cord of these animals were similar to the data found for the control
The number and location of labeled neurons found in the contralateral lumbar enlargement varied with the size and position of the thoracic VQ lesion. In one animal (VQ lesion 1) the thoracic lesion included all of the ventral quadrant and all but a small portion of the dorsal aspect of the dorsolateral funiculus (Fig. 5, VQ lesion 1). The number of labeled cells in the contralateral lumbar enlargement was small, indicating that the STT crosses below the level of the lesion. The labeled neurons in this animal were located in lamina I exclusively (Table 3, VQ lesion 1). When the VQ lesion spared a larger portion of the dorsolateral funiculus (Fig. 5, VQ lesions 2 and 4), a small number of labeled cells were found in the deeper laminae (Fig. 7A; Table 3, VQ lesions 2 and 4), yet more than 90% of the contralateral lumbar enlargement label was still located in lamina I. When the DLF and part of the ventral funiculus were spared (Fig. 5, VQ lesion 3), an even larger proportion of labeled cells were located in deeper laminae (Fig. 7B, Table 3, VQ lesion 3).

### Dorsolateral funiculus lesions

Three cats (DLF lesion 1, 2, and 3) had lesions of the thoracic dorsolateral funiculus made ipsilateral to the thalamic injections. These lesions (Fig. 5, DLF lesion) were designed to block HRP transport through the DLF and served to identify the cells of origin of the ventral spinothalamic projections. In these animals the cervical HRP label...
TABLE 2. Distribution of Contralaterally Labeled Cells in the Lumbar and Cervical Enlargements of Cats Following a Thalamic HRP Injection and No Spinal Lesion

<table>
<thead>
<tr>
<th>Cervical enlargement</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Mean %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-III</td>
<td>2.73(^1)</td>
<td>0.22</td>
<td>0.33</td>
<td>1.01</td>
<td>0.50</td>
<td>0.50</td>
<td>38(^2)</td>
</tr>
<tr>
<td>IV-VI</td>
<td>4.50</td>
<td>0.56</td>
<td>1.02</td>
<td>0.35</td>
<td>0.40</td>
<td>0.40</td>
<td>46</td>
</tr>
<tr>
<td>VII-X</td>
<td>1.00</td>
<td>0.24</td>
<td>0.43</td>
<td>0.11</td>
<td>0.15</td>
<td>0.15</td>
<td>16</td>
</tr>
<tr>
<td>Total cells per section</td>
<td>8.23</td>
<td>1.02</td>
<td>1.78</td>
<td>1.47</td>
<td>1.05</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>246</td>
<td>55</td>
<td>91</td>
<td>58</td>
<td>21</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Total sections</td>
<td>30</td>
<td>34</td>
<td>36</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

| Lumbar enlargement   |         |    |    |    |    |   |        |
| Laminae              |         |    |    |    |    |   |        |
| I-III                | 2.13    | 0.33 | 0.22 | 0.45 | 0.36 | 0.36 | 21     |
| IV-VI                | 2.06    | 0.69 | 0.35 | 0.31 | 0.17 | 0.17 | 21     |
| VII-X                | 2.90    | 1.33 | 1.06 | 1.06 | 1.13 | 1.13 | 58     |
| Total cells per section | 7.08   | 2.35 | 1.63 | 1.82 | 1.66 | 1.66 |        |
| Total cells          | 964     | 78  | 98  | 72  | 60  | 60  |        |
| Total sections       | 119     | 33  | 60  | 40  | 36  | 36  |        |

\(^1\)Average number of HRP-labeled cells per section.
\(^2\)Mean percentage of labeled cells of all control experiments.

was similar to that of control animals. In the lumbar enlargement, the main HRP-labeled cell populations were located in contralateral laminae IV-V and VII-VIII (Fig. 8, Table 4). The distribution, number, and morphology of lumbar HRP labeled neurons in laminae IV-V and laminae VII-VIII were similar to control characteristics. There were very few labeled lamina I cells found contralateral to the injection site, and HRP-labeled lamina I cells were present ipsilaterally only when the HRP injection site extended across the midline (Fig. 3, DLF lesion 2; Fig. 8).

Dorsal column-spared lesion

A thoracic spinal lesion that spared only the dorsal columns and a small portion of the dorsalmost aspect of the contralateral dorsolateral funiculus was made in one animal (Fig. 5, Dorsal Column Spared). The dorsal columns and their blood supply appeared intact. The injection site included medial and lateral thalamic nuclei with minimal spread across the midline (Fig. 3, Dorsal Column Sparing). The number and distribution of HRP-labeled cells in the cervical enlargement (3.95 cells per section) was similar to findings for other cervical controls. Caudal to the lesion, in the lumbar enlargement, there were no HRP-labeled cells, suggesting that spinothalamic axons do not course through the dorsal columns.

Statistical comparison between groups

The number of labeled neurons per section in the dorsal horn of each animal was subdivided into three main groups: laminae I-III, laminae IV-VI, and laminae VII-X (see Tables 2-4). This grouping is intended to correspond to functionally distinct cell populations of the STT (see below for evidence and references), and is also designed to minimize variations due to erroneous placement of some labeled neurons into specific laminae. The number of labeled neurons in laminae II, III, IX, and X were very small and make a minimal contribution to the values shown in Tables 2-4. The ipsilateral STT was not studied statistically, since the number of labeled neurons was small and highly dependent on the degree of spread of the thalamic injection across the...
midline. Despite the dependence of the number of labeled cells in the contralateral cervical enlargement on the inclusion of PO in the injection site, the variations in injection sites were not associated with any change in the proportion of labeled cells found in laminae I-III in the contralateral cervical enlargement (Mann-Whitney U test, \( P < 0.45 \), Tables 2–4).

A nonparametric statistical comparison (Mann-Whitney U test) was made for each laminar grouping of the contralateral label in the cervical and lumbar enlargement between the control animals and the DLF- or VQ-lesioned cats. The results are summarized in Table 5. Because multiple comparisons were made with a single set of animals as the control group, the \( P \) values reported must be considered only approximations and are included as indicators of change rather than as precise probability measurements. This analysis showed no significant differences (\( P > 0.05 \)) in the number of HRP-labeled cells for each laminar grouping within the cervical dorsal horn between control and VQ- or DLF-lesioned animals despite the variation added by changes due to the density of PO injection. This suggests that the thalamic HRP injections and subsequent transport to the cervical dorsal horn were equivalent between the groups of animals. In the lumbar enlargement, there was a significant reduction in the number of labeled cells per section in the deeper laminae of VQ-lesioned animals compared to controls (laminae IV-VI, \( P < 0.04 \); laminae VII-X, \( P < 0.007 \)), and there was a significant reduction in the number of HRP-labeled cells in the superficial laminae of DLF-lesioned animals compared to controls (laminae I-III, \( P < 0.01 \)). These results strongly suggest that the differences of label seen in the lumbar dorsal horn are due to the placements of the lesions in the thoracic spinal white matter.
Fig. 7. Bar graphs of laminar distributions and plots of positions of HRP-labeled cells in two ventral quadrant-lesioned animals (VQ lesions 2 and 3). Ipsilateral and contralateral are in reference to the thalamic injection side. The ipsilateral VQ lesion at the midthoracic level is indicated in black for both animals. The cell plots are a compilation of 10 lumbar enlargement sections. Left is ipsilateral to the injection.

Relative sizes of the spinothalamic projections

In these experiments the maximum size of the total spinothalamic cell population was up to 7 cells per section in the lumbar enlargement and 8.5 cells per section in the cervical enlargement. The dorsolateral spinothalamic projection is approximately one fourth the size of this total spinothalamic projection. The ventral spinothalamic pathway is three fourths the size of the total spinothalamic tract. These estimates are based on a comparison of the number of labeled cells per section in lumbar cord in a control experiment (Table 2, Control 1) with the number in an animal with a ventral quadrant lesion (Table 3, VQ lesion 2; 1.9 cells per section), the number in an animal with a dorsolateral funiculus lesion (Table 4, DLF lesion 2; 6.0 cells per section). These three animals had similar injections, similar cervical labeling, and appropriate thoracic lesions. Since it is unclear whether the small number of lamina I axons ascending in the VSTT and the small number of axons of deeper laminae ascending in the DSTT represent the only output of the parent cells or represent collateral branches, it is possible that a single spinal cord neuron could contribute to both pathways.

DISCUSSION

The results of this study show the existence of predominantly crossed spinothalamic pathways that ascend through the DLF and VQ in cats. The cell populations that contribute to each pathway are distinct from one another. The dorsolateral projection originates predominantly in spinal cord lamina I in contrast to the ventral projections, which originate mainly from cell populations in laminae IV and V and in VII and VIII.

Technical considerations

Several technical considerations are germane to the results of this study. Recent investigations (Gerfen et al., '82; Itaya and Van Hoesen, '82; Itoh et al., '84; Harrison et al., '84; Hultborn and Storai, '84) described transneuronal transport of WGA-HRP. However, transneuronal transport in the long tracts of the spinal cord has been described only in the anterograde direction (Peschanski and Ralston, '85). In the present study the dorsal column nuclei were regularly examined and cellular label was invariably found, but labeled fibers were very rarely found in the dorsal columns of the spinal cord, indicating that significant transsynaptic retrograde transport was not occurring in these long tracts (Jones et al., '85). In experiments using HRP-Sigma VI, which has not been demonstrated to undergo transsynaptic transport, the major spinothalamic cell populations were similar to those of WGA-HRP experiments. Recent studies in our laboratory, using retrogradely transported fluorescent dyes in conjunction with ventral quadrant lesions of the thoracic spinal cord, also demonstrated the existence of a lamina I DSTT whose cells of origin are similar in numbers and positions to these HRP experiments (Stevens et al., '85). Whereas transsynaptic transport cannot be completely excluded as a contributor to the results, it is doubt-
ful that it makes a significant contribution to cell labeling in these experiments. The uptake of HRP by fibers of passage in the corona radiata damaged by the injection needle does not present a problem in interpretation as far as spinal labeling is concerned, since the spinothalamic tract is the most rostral known efferent spinal projection. Therefore, limiting the diencephalic injection to the thalamus, avoiding injection spread to the midbrain, and using a low concentration of WGA-HRP (2%) allow the assumption to be made that all HRP-labeled spinal cord cells in this study have direct thalamic projections.

The variation in the number of labeled cells per section in the lumbar enlargement was large within a single experimental paradigm. This variation in the amount of HRP label could be due to differences between animals or to variability in injection sites, axonal uptake, and tissue processing (Willis et al., '79; Granum, '86). In these experiments, the density of the injection at the level of PO was related to the total number of cells labeled, but not to the relative laminar distribution of the labeled cells. Since the STT projection to PO is sparse in cats, (Boivie, '71, '79; Applebaum et al., '79; Berkley, '80; Craig and Burton, '85; Apkarian et al., '87) and since the PO injection does not change the laminar pattern of label, it is likely that the caudal injection of HRP in PO increases labeling in the spinal cord primarily by being taken up in the damaged STT fibers of passage rather than by the STT terminals in the PO region. Since there was not a statistically significant difference in the cervical laminar distributions between control and DLF- or VQ-lesioned animals, it seems unlikely that there was any systematic variation in the degree of HRP uptake, labeling, or tissue processing between these groups.

**Ascending projections to the thalamus**

The relative laminar distributions of STT neurons in the cervical enlargement of all 13 cats studied here are similar to those reported by Carstens and Trevino ('78) for large thalamic injections of HRP-Sigma VI. However, the total number of labeled neurons in the present study was two to three times larger than that reported by Carstens and Trevino ('78). There is no obvious reason for this variation in results, though there are clear technical differences between the studies. The locations and densities of STT cells

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**TABLE 4. Distribution of Contralaterally Labeled Cells in the Lumbar and Cervical Enlargements of Cats Following a Thalamic HRP Injection and Ipsilateral Thoracic Dorsolateral Funiculus Lesion**

<table>
<thead>
<tr>
<th>DLF lesion</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Mean %</th>
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<tr>
<td>Cervical enlargement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-III</td>
<td>1.09</td>
<td>2.28</td>
<td>0.43</td>
<td>29²</td>
</tr>
<tr>
<td>IV-VI</td>
<td>2.21</td>
<td>3.43</td>
<td>0.88</td>
<td>33</td>
</tr>
<tr>
<td>VII-X</td>
<td>0.81</td>
<td>0.93</td>
<td>0.32</td>
<td>18</td>
</tr>
<tr>
<td>Total cells per section</td>
<td>4.20</td>
<td>6.63</td>
<td>1.63</td>
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</tr>
<tr>
<td>Total cells</td>
<td>337</td>
<td>265</td>
<td>46</td>
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<tr>
<td>Total sections</td>
<td>80</td>
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<td></td>
</tr>
<tr>
<td>Lumbar enlargement</td>
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</tr>
<tr>
<td>Laminae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-III</td>
<td>0.15</td>
<td>0.12</td>
<td>0.01</td>
<td>6</td>
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<tr>
<td>IV-VI</td>
<td>0.35</td>
<td>1.98</td>
<td>0.06</td>
<td>29</td>
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<tr>
<td>VII-X</td>
<td>0.73</td>
<td>3.53</td>
<td>0.15</td>
<td>64</td>
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<tr>
<td>Total cells per section</td>
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<td>6.03</td>
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<td>Total cells</td>
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<tr>
<td>Total sections</td>
<td>72</td>
<td>80</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

¹Average number of HRP labeled cells per section.
²Mean percent of labeled cells of all DLF lesion experiments.
TABLE 5. Results of Mann-Whitney U Test Between Control and DLF- or VQ-Lesioned Animals

<table>
<thead>
<tr>
<th></th>
<th>Control vs. VQ lesion</th>
<th>Control vs. DLF lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical enlargement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-III</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IV-VI</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VII-X</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lumbar enlargement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laminae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-III</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IV-VI</td>
<td>P &lt; 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>VII-X</td>
<td>P &lt; 0.007</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = P > 0.05.

![Photomicrographs of HRP-labeled lamina I neurons contralateral to the thalamic injections.](image)

Fig. 9. Photomicrographs of HRP-labeled lamina I neurons contralateral to the thalamic injections. A: Triplet of HRP-labeled lamina I neurons located just medial to dorsal root entry zone in an animal with a ventral quadrant lesion placed ipsilateral to the thalamic injection (transverse section). B, C: Darkfield photomicrographs of horizontal sections of lumbar spinal cord through the marginal zone of the dorsal horn showing the rostrocaudal orientation of HRP-labeled lamina I cells. Bar = 100 μm.

in the lumbar enlargements of cats used as controls are similar to those found in HRP studies of the macaque (Trevino and Carstens, '75; Willis et al., '78a, '73; Hayes and Rustioni, '80), suggesting that the spinothalamic tracts in cats and monkeys are not as disparate as previously thought (Trevino and Carstens, '75; Carstens and Trevino, '78; Wil-

lis et al., '79). Based on the control experiments in this study, the number of STT neurons appears equally distributed between the cervical and lumbar enlargements, though there is a greater percentage of laminae IV-V cells contributing to the STT from the cervical enlargement and a greater percentage of laminae VII-X cells contributing to the STT in the lumbar enlargement. The distribution of HRP-labeled rat lumbar spinothalamic neurons in laminae IV and V and in VII and VIII is similar to that seen in cats and monkeys (Giesler et al., '79, '81b; Kevevett and Willis, '83, '84; cf. Granum, '86). However, there is a prominent projection from medial lamina VI in rats that is small in cats and is not present in monkeys (Menetrey et al., '84). In addition, the lamina I spinothalamic projection from the rat lumbar enlargement is small compared to cats and monkeys (Giesler et al., '79, '81b; Kevevett and Willis, '83; Menetrey et al., '84), and many of the reported rat spinothalamic lamina I neurons probably terminate in the rostral midbrain rather than the thalamus (McMahoon and Wall, '83, '85; Granum, '86; cf. Hylden et al., '85).

Previous studies using degeneration techniques have failed to identify a spinothalamic projection in the dorsolateral funiculus (Boivie, '70, '71; Nijensohn and Kerr, '75). Based on these studies, little thought has been given to the idea that a DSTT might exist as an additional spinal diencephalic pathway (Willis and Coggeshall, '78b). However, with the demonstration that the majority, if not all, of lamina I neurons that project rostrally send their axons through the dorsolateral funiculus in rats (Zemlan et al., '78; McMahon and Wall, '83), cats (Apkarian et al., '85) and monkeys (Molenar and Kuypers, '78), the possibility of the existence of a DSTT had to be reconsidered. The results of this study, as well as the results of our previous report (Jones et al., '85) clearly demonstrate the existence of a DSTT in cats made up primarily of lamina I cell axons. The DSTT comprises approximately 25% of the total spinothalamic projection from the lumbar enlargement. This value agrees closely with previous estimates of the contribution from lamina I to the spinothalamic tract (Carstens and Trevino, '78; Willis et al., '78a, '79).

The DSTT fibers are spread diffusely throughout the DLF of the lumbar spinal cord of cats contralateral to their cells of origin and ipsilateral to their thalamic termination sites (Jones et al., '85). Similarly, labeled fibers were observed in the DLF at the level of the cervical enlargement ipsilateral to the thalamic injection (unpublished observation). This indicates that axons within the DSTT cross segmentally, and that this pathway is present at lumbar, thoracic, and cervical levels.
The VSTT portion of the spinothalamic tract in cats has been demonstrated here to originate from cells in laminae IV-V and laminae VII-VIII. There are essentially no lamina I cell axons traveling in the VQ (Akparian et al., '85). No systematic evaluation was made of the differences in the locations of neurons contributing to the ventromedial and ventrolateral funiculi.

There is physiologic evidence that can be interpreted as support for the existence of a dorsolateral spinothalamic pathway. Electrical stimulation of the isolated DLF, in cats, results in evoked activity in the ipsilateral ventral basal complex and the posterior nuclear group of the thalamus (Calma, '65; Curry and Gordon, '72). The latency of this evoked activity was reported to be consistent with a direct spinothalamic projection (Calma, '65), even though a polysynaptic pathway could not be excluded. The latency of the evoked activity in the posterior group reported by Curry and Gordon ('72) after stimulation of the isolated DLF closely approximated that obtained after stimulation of the ipsilateral VQ and was abolished by a rostral lesion of the DLF. Behavioral studies (Kennard, '54) in cats have shown that sectioning of the DLF decreased the response to pin-prick stimulation below the level of the spinal cord lesion.

In monkeys it has been demonstrated (Vierck and Luck, '79) that DLF section done following a VQ section results in a transient increased tolerance to noxious electrical cutaneous stimuli applied contralaterally.

Preliminary retrograde transport studies in squirrel monkeys indicate the existence of a DSTT in this species (Jones et al., '85). Indirect evidence supports the existence of a crossed DSTT in man as well. Spinal lesions confined to the DLF in humans have resulted in chromatolytic neurons within the contralateral laminae I, IV, and V below the level of the lesion (Smith, '76). Correlation of clinical findings with the anatomic locations of spinal cord lesions in humans who underwent percutaneous cervical cordotomy revealed that discrete lesions of the DLF can result in contralateral analgesia (Moossy et al., '67; Sweet, '76).

Some investigators have presented physiologic findings that were considered to be consistent with a ventral projection of lamina I neurons in cats and monkeys (Kumazawa et al., '75; Price and Mayer, '75). Even though these studies purported to demonstrate a ventral lamina I projection, they were not controlled to exclude the possibility of activating axons of lamina I neurons within the DLF, especially since high-amplitude stimulation of the VQ was necessary to antidromically activate lamina I neurons.

**Functional implications**

The cells that are the origin of the VSTT are in laminae IV-V and VII-VIII of the spinal cord. These neurons respond to a wide range of cutaneous noxious and innocuous stimuli as well as to muscle and visceral stimuli (Trevisio et al., '72; Willis et al., '74; Fox et al., '80; Milne et al., '81; Meyers and Snow, '82a; Foreman et al., '77, '79, '84). The cells contributing to the VSTT project to medial and lateral thalamic nuclei (Carstens and Trevisio, '78; Applebaum et al., '79; Willis et al., '79; Giesler et al., '81a, '81b; Meyers and Snow, '82a; Keveetter and Willis, '83, '84; Craig and Burton, '85; Stevens et al., '85). Medially terminating VSTT axons originate primarily from cells in laminae VII-VIII (Giesler et al., '81a). Most of these cells are nociceptive-specific and have large, complex and at times bilateral receptive fields. Laterally terminating axons in monkeys originate from cells in laminae IV-V, are of wide dynamic range, and have small contralateral receptive fields. Therefore both nociceptive-specific and wide-dynamic-range cells seem to contribute axons to the VSTT.

The finding that there exists a unique population of neurons in spinal cord lamina I that respond to high-threshold mechanical stimulation and to noxious levels of cutaneous temperature has been an important step in unraveling the contradictions concerning how the spinal cord signals noxious peripheral stimuli (Christensen and Perl, '70). The demonstration that these lamina I cells contribute to the spinothalamic tract (Willis et al., '74) seemed to provide a clear explanation for the behavioral effects of anterolateral cordotomy. Even more impressive, in terms of identifying a labeled line for rostral transmission of information about noxious peripheral stimuli, have been the studies demonstrating that all lamina I cells that project to the thalamus and midbrain respond exclusively to noxious stimuli (Craig and Kniffki, '85; Hylden et al., '85). Like the spinothalamic cells originating in the deeper laminae of the spinal cord, those located in lamina I project to both medial and lateral portions of the sensory thalamus (Applebaum et al., '75, '79; Chung et al., '79; Giesler et al., '79, '81a, '81b; Keveetter and Willis, '83, '84; Craig and Kniffki, '85; Stevens et al., '85). Unique to the lamina I projection, however, is the termination in nucleus submedius of the thalamus (Craig and Burton, '81). The current study clearly demonstrates that in cats a major nociceptive-specific ascending spinal cord pathway, originating in lamina I, travels in the DLF rather than the VQ. It is unclear what the functional differences are between lamina I–DSTT and lamina V–VSTT nociceptive specific pathways.

There is some indication of the potential function of the DSTT. Chung et al. ('86) have shown that in monkeys, bilateral DLF section results in a decrease in the response of some thalamic neurons to noxious cutaneous stimulation. Preliminary work in cats has demonstrated a clear dependence of the responses of some thalamic neurons in VPL and PO to noxious cutaneous stimuli on ascending transmission through the DLF ipsilateral to the thalamic recording site (Hodge et al., '87). These findings are consistent with the previously described behavioral effects of DLF section in cats, monkeys, and man.

The clear separation of the lamina I ascending input to the thalamus from the remainder of the spinothalamic tract, in cats, allows experimental designs to test the relative importance of these two pathways (DSTT and VSTT), in acute and chronic preparations, in terms of both physiologic and behavioral responses to somatic sensory stimuli. The existence of a DSTT distinct from VSTT in humans would have profound clinical implications. This necessitates further studies in primates, which are currently under investigation in our laboratory.

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**LITERATURE CITED**


