Ascending projections from spinal cord and brainstem to periaqueductal gray and thalamus
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Chapter 6

Less than 15% of the spinothalamic fibers originate from neurons in lamina I in cat

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ABSTRACT
Lamina I neurons sending their axons into the spinothalamic tract are thought to play a crucial role in nociception, but many spinothalamic fibers do not originate from lamina I neurons. In cat, no consensus exist about what percentage of the spinothalamic tract cells is located in lamina I. After wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) injections that covered large parts of the thalamus, retrogradely labeled cells were plotted and counted in all segments of the spinal cord. Results show that, averaged over all spinal segments, the percentage of labeled lamina I neurons was 4.9-14.2%. These results demonstrate that, in contrast to what is concluded in several previous studies, lamina I in the cat provides only a limited part of the total spinal input to the thalamus.

INTRODUCTION
The spinothalamic tract is best known for its role in the transmission of information concerning nociception, temperature and crude touch from spinal cord to thalamus. The pathway is usually associated with projections from Rexed’s lamina I, but no consensus exists about what portion of all spinothalamic fibers originates in lamina I neurons. Studies in rat, cat and monkey (rat: Kemplay and Webster, 1986; Burstein et al., 1990b; cat: Trevino and Carstens, 1975; Carstens and Trevino, 1978a; Carstens and Trevino, 1978b; Comans and Snow, 1981; Wiberg and Blomqvist, 1984b; monkey: Trevino and Carstens, 1975; Willis et al., 1979; Apkarian and Hodge, 1989a) have reported that the vast majority of the spinothalamic cells is not located in lamina I. On the other hand, according to Craig et al. (1989b), in cat about half of the spinothalamic tract originates in lamina I. This view has been adopted in subsequent papers (Craig, 1989a; Zhang et al., 1996) as well as in recent reviews (Craig, 2003c). These discrepancies might be caused by differences in thalamic injection sites and/or the selection of the studied spinal segments, especially since the contribution of lamina I cells to the spinothalamic tract differs between spinal segments. Only in rat all segments of the spinal cord were studied after large injections that covered almost all parts of the thalamus (Kemplay and Webster, 1986; Burstein et al., 1990b). Until now, in cat only one or a few segments of the spinal cord were studied (Trevino and Carstens, 1975; Carstens and Trevino, 1978a; Carstens and Trevino, 1978b; Comans and Snow,
Chapter 6

1981; Wiberg and Blomqvist, 1984b; Craig et al., 1989a; Craig et al., 1989b). The aim of the present study is to determine the relative contribution of lamina I cells to the spinothalamic tract, on the basis of retrogradely labeled cells in all segments of the cat spinal cord (C1-Coc2) after large injections in the thalamus.

MATERIALS AND METHODS

Four female cats were used. For surgery, the animals were initially anesthetized with intramuscular ketamine (Nimatek, 0.1 ml/kg) and xylazine (Sedamun, 0.1 ml/kg), and subsequently ventilated with a mixture of O\(_2\), N\(_2\)O (1:2) and 1-2% halothane, while ECG and body temperature were monitored. In each case, large injections of WGA-HRP in saline were made in the thalamus using a Hamilton syringe. Injections were made stereotaxically, using the atlas of Berman and Jones (1982) and with each insertion of the syringe multiple injections were made. Table 1 shows details of the injection sites, volumes of injected tracer and the survival time per case. In two cases survival times up to 96 hours were applied to ensure proper labeling of the neurons in the lower spinal cord.

For perfusion the animals were initially anesthetized with intramuscular ketamine (Nimatek, 0.1ml/kg) and xylazine (Sedamun, 0.1ml/kg), followed by an overdose of intraperitoneal 6% pentobarbital sodium. They were perfused transcardially with 1.5 liters of 0.9% saline, immediately followed by 1.5 liters of 0.1M phosphate buffer (pH 7.4), containing 4% sucrose, 1% paraformaldehyde and 2% glutaraldehyde. After perfusion, the brains and spinal cords were removed, post-fixed for two hours and stored overnight in 25% sucrose in phosphate buffer at 4°C. Subsequently, the brain and all spinal segments were cut into 40µm transverse frozen sections, of which every fourth section was incubated according to the tetramethyl benzidine (TMB) method. An extra series of sections of the diencephalon with overlying cortex was incubated with diaminobenzidine (DAB) in order to define the injection site. To determine the location and number of the labeled profiles in the spinal cord, in each case all retrogradely labeled neurons in all processed sections of all spinal segments were plotted and counted. Labeled neurons in the dorsal column nuclei (DCN) and lateral cervical nucleus (LCN) in the upper cervical cord were not included in the counts.

RESULTS

Figure 1 shows schematic drawings of the injection sites. In all cases, many labeled neurons were found throughout the length of the spinal cord, located in laminae I, III-VIII and X. In cases 2517 and 2519 the longer survival times did not result in more labeled cells in the lumbosacral cord, but many faintly labeled cells were found in laminae III and IV almost exclusively on the contralateral side of the cervical and, to a lesser extent, lumbar enlargements. These cells were not observed in the cases with 65-66 hrs survival time. It is assumed that the weakly labeled cells in laminae III and IV are the result of transsynaptic labeling via heavily labeled cells in LCN and DCN, because these nuclei receive many projections from specifically laminae III and IV (Rustioni and Kaufman, 1977; Craig, 1978). The lightly labeled cells in laminae III and IV were, therefore, not included in the counts of spinothalamic cells.
Lamina I-thalamus projecting neurons

Table 1 gives the total numbers of labeled neurons found in the C1-Coc2 segments of the spinal cord. On average, 70% of all labeled neurons and 80% of the labeled lamina I cells was located contralaterally. Labeled lamina I neurons were most numerous (701) in case 2547, with an injection site that covered the intralaminar nuclei of the left thalamus and the medial thalamus on both sides. In case 2517, in which the lateral and intralaminar parts of the thalamus were injected, but not the most medial parts, the lowest number (233) of labeled lamina I neurons was found. Percentages of labeled lamina I cells of the total number of labeled cells ranged from 4.9% in case 2517 to 14.2% in case 2547. On the contralateral side percentages of lamina I cells of 6.0-17.8% were found, and on the ipsilateral side from 2.3%-7.1% (Table 1).

![Schematic drawings of the injection sites. The core of each injection site is shown in dark gray, the halo in light gray. Needle tracks are indicated in black.](image)

Table 1. Detailed information on injected volumes, injection coordinates, survival time and numbers of labeled neurons found in a 1:4 series of sections per case. The percentages of lamina I neurons are given between parentheses. Coordinates according to the atlas of Berman and Jones (1982). AP, anterior-posterior; DV, dorsal-ventral; LM, lateral-medial; lam, lamina.

<table>
<thead>
<tr>
<th>Case</th>
<th>Total volume (µl)</th>
<th>Number of injections</th>
<th>Volume per injection (nl)</th>
<th>Coordinates</th>
<th>Survival time (hrs.)</th>
<th>=Total lam I-X</th>
<th>=Contralateral lam I-X</th>
<th>=Ipsilateral lam I-X</th>
</tr>
</thead>
<tbody>
<tr>
<td>2517</td>
<td>14.7</td>
<td>45</td>
<td>100-500</td>
<td>AP 6.0 - 12.0</td>
<td>96</td>
<td>4584 (4.9%)</td>
<td>3157 (6.0%)</td>
<td>189 (2.4%)</td>
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<td></td>
<td></td>
<td></td>
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<td>DV 0.5 - 6.0</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>LM 1.0 - 9.0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2519</td>
<td>6.0</td>
<td>15</td>
<td>250-500</td>
<td>AP 8.0 - 11.0</td>
<td>96</td>
<td>3798 (6.7%)</td>
<td>2774 (8.4%)</td>
<td>232 (2.3%)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>DV 1.5 - 8.5</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>LM 1.0 - 6.5</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2529</td>
<td>13.8</td>
<td>45</td>
<td>100-500</td>
<td>AP 6.0 - 12.0</td>
<td>65</td>
<td>6082 (10.3%)</td>
<td>4299 (12.1%)</td>
<td>521 (6.0%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>DV 0.5 - 6.0</td>
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<td></td>
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<td></td>
<td>LM 1.0 - 9.0</td>
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</tr>
<tr>
<td>2547</td>
<td>4.3</td>
<td>21</td>
<td>100-400</td>
<td>AP 7.0 - 12.0</td>
<td>66</td>
<td>4923 (14.2%)</td>
<td>3298 (17.8%)</td>
<td>1625 (7.1%)</td>
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<td></td>
<td>DV 0.8 - 5.0</td>
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<td></td>
<td></td>
<td></td>
<td>LM 0.0 - 3.8</td>
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</table>
In all cases large segmental differences existed, both in the absolute number of labeled cells per segment as well as in the percentages of lamina I cells (Fig. 2). In all cases the largest number of labeled neurons in laminae I-X was found in the upper cervical cord (C1-C3), and in the cervical and lumbar enlargements. Labeled lamina I neurons were most numerous in the enlargements, but in case 2547 many were also found in the upper cervical segments. While the percentage of lamina I cells never exceeded 14.2%, averaged over all segments, the percentage of lamina I neurons per segment was up to 44.5 on the contralateral side in the C8 segment in case 2547.

The 10.3% lamina I cells found in case 2529 might be most accurate, because in this case all parts of the thalamus were injected. The percentages found in cases 2517 and 2519 are probably too low, because the most medial thalamus, known to receive many afferents from lamina I neurons (Craig et al., 1989a), was not involved in the injection sites. Conversely, in case 2547, in which the most lateral thalamus escaped the injection site, considerably less labeled cells were observed in the laminae other than lamina I than in case 2529.

DISCUSSION

The present results are in agreement with many studies (Trevino and Carstens, 1975; Carstens and Trevino, 1978a; Carstens and Trevino, 1978b; Comans and Snow, 1981; Wiberg and Blomqvist, 1984b) that suggest that the majority of spinothalamic cells is not located in lamina I. Burstein et al. (1990b), using the fluorescent tracer FluoroGold, also studied all segments of the spinal cord after large thalamus injections, in rat. They found that only 11% of all spinothalamic input originates in the superficial dorsal horn, and 13.6% when the labeled neurons in the lateral spinal nucleus and lateral
cervical nucleus are not included in the counts. Our findings are at odds with the conclusions of Craig et al. (1989a). Their conclusions were based on studying only the C5-C7 and L5-S2 segments. Our results show that the study of only the segments of the enlargements results in wrong conclusions about the total spinothalamic input, because large segmental differences exist throughout the length of the spinal cord.

With respect to the use of different tracers, Craig et al. (1989a; 1989b) argued that especially for the labeling of lamina I cells, fluorescent tracers as Fast Blue (FB) and Diamidino Yellow (DY) are superior to (WGA)-HRP. Their data, however, show that only HRP not conjugated to WGA is a poor labeler of lamina I cells. In our study about the same number of lamina I neurons was labeled as in the study of Craig et al. with fluorescent tracers (1989a), but many more cells in other laminae than lamina I were labeled. Apparently, FB and DY fluorescent tracers are very poor labelers of non-lamina I cells, a problem also put forward by Craig et al. (1989b), themselves.

The present study has demonstrated that more than 85%, and probably close to 90%, of the spinothalamic cells is not located in lamina I, and therefore, not necessarily involved in the transmission of information about nociception and temperature. The exact function of these other cells remains to be determined.