The Location of Spinothalamic Axons within Spinal Cord White Matter in Cat and Squirrel Monkey

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Abstract The locations of spinothalamic (STT) fibers in the spinal cord white matter have been identified in cat and squirrel monkey by light-microscopic visualization of labeled fibers following multiple thalamic injections of wheatgerm agglutinin conjugated to horseradish peroxidase. Thalamic injections were combined with either a constricting dural tie or an intraspinal injection of colchicine to facilitate axonal labeling at more rostral spinal levels.

In the cat, the ventral-to-dorsal distribution of labeled STT fibers was bimodal. In the ventrolateral white matter, labeled axons were coarse in nature and were primarily concentrated peripherally. In the dorsolateral white matter, labeled STT axons consisted of fine-caliber fibers concentrated in the ventral portion of the dorsolateral funiculus and were equally distributed throughout the medial and lateral white matter.

In the squirrel monkey, the distribution of STT fibers was unimodal, extending from the ventral surface of the spinal white matter to the ventralmost portion of the dorsolateral funiculus. As in the cat, however, the ventrally located axons were large and coarse and were primarily located in the peripheral white matter, whereas the dorsalmost STT fibers were of fine caliber and were distributed equally in the medial and lateral white matter.

Key words ascending projections, lamina I, pain, dorsolateral funiculus

The spinothalamic tract (STT) consists of fibers that cross segmentally and ascend to the thalamus in the contralateral spinal white matter. Although the bulk of these fibers are located in the ventrolateral white matter, lesion studies have recently demonstrated that some of these STT fibers are located more dorsally within the dorsolateral funiculus (DLF). In cat (Jones et al., 1985, 1987), these dorsally located STT fibers originate almost exclusively from spinal lamina I neurons and terminate in widespread regions of the medial and lateral thalamus (Craig et al., 1989; Stevens et al., 1989). In the primate, the dorsally located axons originate from lamina I and portions of laminae IV and V (Apkarian and Hodge, 1989b,c). In both cat and primate, this STT pathway located in the DLF has been termed the "dorsolateral spinothalamic tract" (DSTT), while the ventrally located STT fibers have been termed the "ventrolateral spinothalamic tract" (VSTT). Results of lesion studies, however, have raised several questions about the precise location within the spinal cord white matter for the axons of each pathway. These lesion studies have suggested that the fibers of the DSTT may not be located in the dorsalmost aspect of the DLF, but only in the ventral portion of the DLF (Apkarian and Hodge, 1989b,c; Stevens et al., 1989). If this is so, can the DSTT rightly be termed a separate pathway from the VSTT? Or are these pathways actually one pathway, with the axons of cells from more dorsal laminae remaining dorsally segregated in the contralateral white matter? In addition, it has been suggested from one retrograde labeling experiment (Stevens et al., 1989) that at least a portion of lamina I cell axons must be located within the medial DLF. The purpose of the present study was to directly visualize and localize STT axons in the spinal cord white matter in cat and squirrel monkey, using retrograde labeling with wheatgerm agglutinin conjugated to horseradish peroxidase (WGA:HRP) combined with either a constricting dural tie or intraspinal colchicine injections at the low thoracic/

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high lumbar spinal cord levels to enhance intra-axonal labeling.

MATERIALS AND METHODS

All animals were anesthetized with Nembutal (35–45 mg/kg, i.v.). Squirrel monkeys required prior sedation with ketamine. Under sterile operating conditions, a craniectomy was performed; then, using the atlases of Berman and Jones (1982) for the cat and Emmers and Akert (1963) for the squirrel monkey, multiple injections of 4% WGA:HRP were made unilaterally throughout the medial and lateral thalamus to total 1.0–2.8 μl of tracer. The intended injection targets in each experiment included the ventral posterior lateral (VPL) and the ventrolateral (VL) nuclei, the centrolateral nucleus, the central medial nucleus, and the nucleus submedius (Emmers and Akert, 1963; Berman and Jones, 1982). Following these injections, one of two procedures was performed to produce an accumulation of WGA:HRP within axons, so as to provide better visualization of fine-caliber STT fibers. In two cats and one squirrel monkey, a constricting tie was made within the spinal cord (L_1 and L_3 in cat; T_5 in monkey). The tie was applied during the same surgical procedure used for the thalamic injection. This was performed by cutting the dura and retying it over the dorsal surface. In the remaining animals (one cat and two squirrel monkeys), enhanced labeling was produced in transporting axons by injection of a 1% solution of colchicine in 2% dimethyl sulfoxide made into the spinal white matter (Hylden et al., 1986) (L_1 in cat; T_5 and L_4 in monkey), ipsilateral to the thalamic injection. Multiple injections were made into the ventrolateral funiculus and the DLF to total 3–4 μl of injected solution. In these colchicine experiments, colchicine injections were made in a separate surgical procedure performed 1 day prior to sacrifice. Total postthalamic injection survival time was 5–6 days.

The animals were perfused by dual cannulation of the descending aorta in ascending and descending directions. Perfusates consisted of 1 liter of normal saline, followed by a solution of 1% paraformaldehyde–1.25% glutaraldehyde in phosphate buffer (pH 7.4; 2.5 liters) and a final washout with a 5% glycerine buffer solution. After perfusion, the tissue was removed into this same buffer solution for overnight storage at 4°C. Sections (80 μm) were cut obliquely to produce long profiles of labeled axons and thus to aid visualization. Sections were collected into phosphate buffer and reacted by the tetramethylbenzidine method of Mesulam (1978). Data were collected using either brightfield or darkfield microscopy from the spinal segments immediately rostral to the dural tie or colchicine injection. Labeled axons were plotted on spinal cord tracings, and histograms were made to illustrate the percentage of the total ipsilaterally labeled STT fibers within horizonal bins progressing from the dorsal root entry zone to the ventral surface of the spinal cord. Data were also analyzed using radial bins originating at the central canal; however, since the results were the same, only data from the horizonal bin analysis are presented.

RESULTS

In all animals, the injection sites extended throughout the medial and lateral thalamic nuclei. Medially, the injections occasionally extended across the midline; laterally, the injections often included the internal capsule. In no experiments did injections extend ventrally into the hypothalamus or caudally into the midbrain, where spinal projections to and from these regions would contribute to the fiber labeling observed (Burstein et al., 1987; Yezierski, 1988; Holstege and Cowie, 1989).

Two different techniques were employed in an attempt to increase intra-axonal labeling and thereby to aid in visualization of fine-caliber fibers. The dural tie technique was attempted first in both cat ($n = 2$) and squirrel monkey ($n = 1$). This procedure did seem to result in better labeling in fine-caliber DLF axons; however, most were still very difficult to visualize even with high-power objectives. Subsequently, intraspinal injections of colchicine was tried in each species ($n = 1$ for cat and $n = 2$ for squirrel monkey). In each case, the colchicine experiments produced superior axonal labeling, although the pattern of labeling was consistant between paradigms.

In all cat experiments (dural tie and colchicine), the dorsal–ventral distribution of labeled STT fibers was bimodal (Figs. 1A–1C). Labeled STT axons in the ventral white matter were concentrated in the periphery. These ventrally located STT fibers were coarse in nature and easily visualized. Labeled STT fibers in the DLF were located from the level of the central canal to the dorsal root entry zone, although labeled fibers were less often seen in the dorsalmost portion of the DLF. Labeled STT fibers within the DLF, unlike those in the VLF, were located throughout the medial and lateral white matter extending from the periphery to the region immediately adjacent to the dorsal horn grey matter. In addition, labeled fibers in the DLF were distinctly smaller in size than were more ventrally located fibers. Labeled fibers in the DLF were often visible only with high-power observation, and many approached the limit of resolution of the light microscope. Absolute measurements of the relative fiber sizes could not be made with the light microscope.

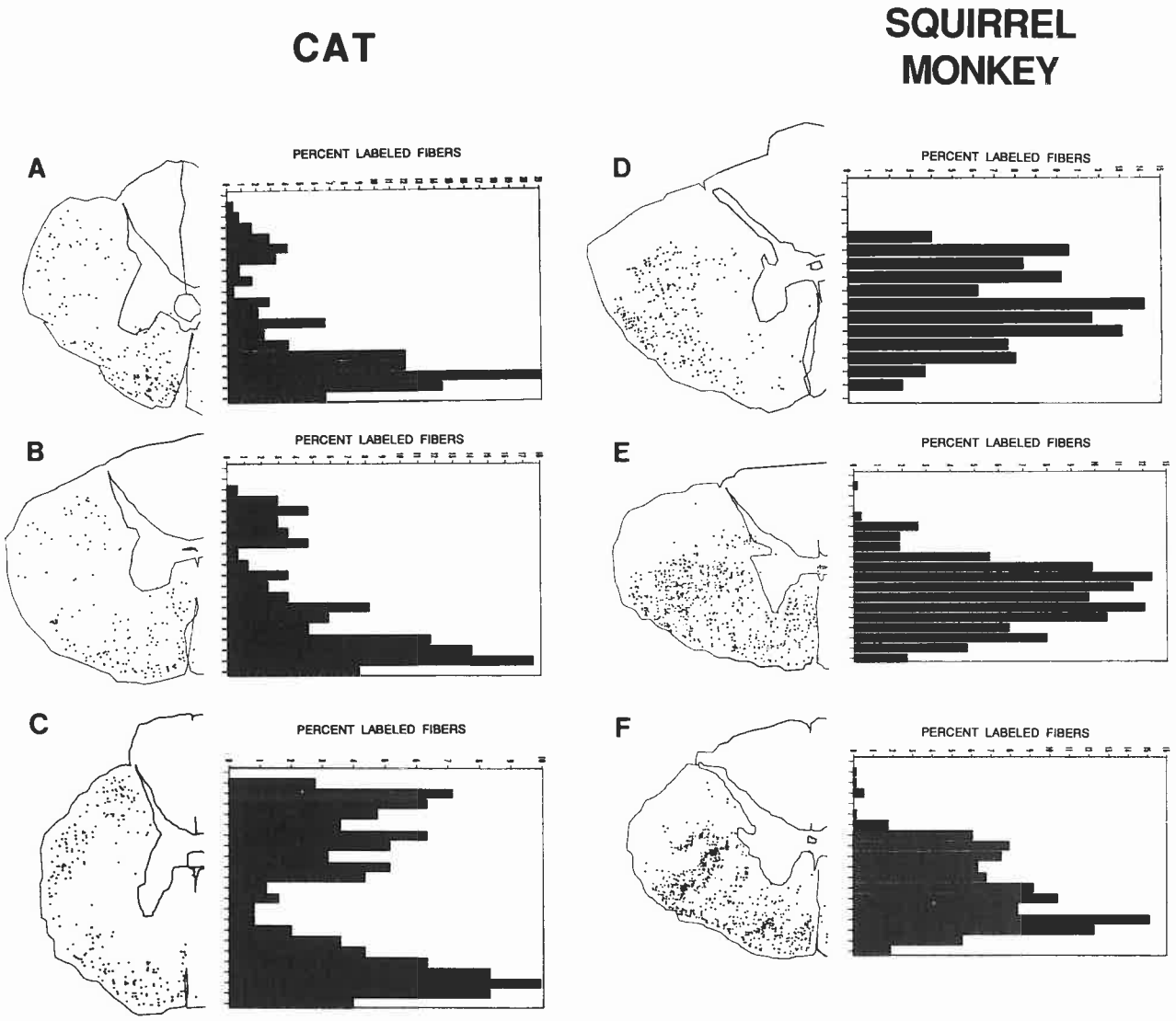

FIGURE 1. Fiber plots of sample spinal cord sections from three cat experiments and three squirrel monkey experiments, with accompanying histograms illustrating the percentage of contralaterally labeled fibers in horizonal bins from the dorsal root entry zone to the ventral surface of the spinal cord. A, B, and D show results from dural tie experiments; C, E, and F show results from colchicine experiments. All cat experiments demonstrated a bimodal distribution of STT axons. All squirrel monkey experiments demonstrated a unimodal distribution. In both species, axons in the ventral white matter were concentrated more peripherally, whereas dorsally they were more evenly distributed through the medial and lateral white matter.

In all squirrel monkey experiments (dural tie and colchicine), the distribution of STT fibers was unimodal, extending from the ventral medial sulcus to the ventral portion of the DLF (Figs. 1D–1F). As in the cat experiments, however, the ventrally located STT fibers in the squirrel monkey were coarse in nature, whereas the more dorsally situated fibers were very fine, often approaching the limit of resolution of the light microscope (Fig. 2). Also, as in the cat, ventral STT fibers appeared to be concentrated peripherally, whereas the dorsalmost fibers were located medially as well as peripherally.

DISCUSSION

In the present study, WGA:HRP-labeled STT axons were directly visualized and counted in spinal cord white matter. There was discordance, however, between the numbers of labeled STT fibers counted in this study and the number of labeled STT neurons reported in

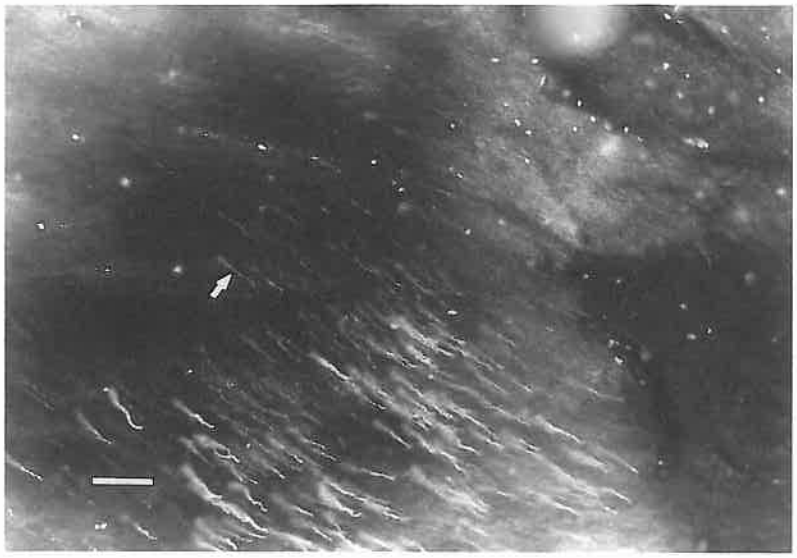

FIGURE 2. Photomicrograph of WGA:HRP-labeled STT axons in the squirrel monkey. Coarse axons are located more ventrally, whereas the dorsalmost axons are of fine caliber (arrow), approaching the limit of resolution. Calibration bar: 100 μm.

previous studies. According to an earlier report of ours (Apkarian and Hodge, 1989a) on the squirrel monkey, the numbers of contralateral STT neurons in the lumbosacral spinal cord are approximately double the numbers of labeled axons counted in the present study. There are several possible reasons for this discrepancy. The smallest labeled axons were very difficult to visualize; therefore, many of these or smaller-caliber STT fibers may have been missed in this count. Also, reacted sections were relatively thick (80 μm) and cut at an oblique angle to obtain long profiles of axons, which aided in visualization. However, because of this procedure, accurate axonal counts in the heavily labeled region of the ventrolateral funiculus became impossible. In addition, these large numbers of coarse, intertwined axons in the ventrolateral funiculus could mask the presence of finer-caliber fibers located in the same region. Therefore, the fiber counts presented in this study were not considered absolute, but were rather used as indicators of a pattern of label for each pathway.

The present report supports the view that the STT may be considered as two separate pathways: a dorsolateral pathway consisting of fine-caliber axons originating primarily from lamina I, and a ventral pathway of more coarse fibers originating from deeper laminae. Earlier reports have repeatedly described the STT as two separate pathways (Stookey, 1929; Gardner and Cuneo, 1945; Kuru, 1949; Kerr, 1975): a more dorsal (lateral) pathway, the DSTT, conducting pain and temperature sensation; and a ventral pathway, the VSTT, conducting touch and pressure sensation. These notions are consistent with recent findings, since the lamina I component of the STT, now known to be dorsally segregated in the contralateral white matter, is described as conducting pain and temperature sensation to the thalamus (Craig and Kniffki, 1985). Additional evidence has also supported the view that the STT may be considered as two separate pathways. In the cat, the lamina I axons are not only dorsally segregated, but also separated in the contralateral spinal white matter (Jones et al., 1985, and the present paper). In the primate, the fine-caliber, dorsal STT axons, although not separated from the ventral pathway, do remain dorsally segregated. In addition, the DSTT and VSTT differ not only in their cells of origin (the DSTT primarily from lamina I and the VSTT from cells in deeper laminae—Jones et al., 1985, 1987; Apkarian and Hodge, 1989b,c; Stevens et al., 1989), but also in their respective peripheral inputs (Christensen and Perl, 1970; Willis et al., 1974), their spectra of axonal sizes (Kuru, 1949; Applebaum et al., 1975; Craig and Kniffki, 1985), and their thalamic terminations (Craig and Burton, 1981; Apkarian and Hodge, 1989c). All of these lines of evidence provide ample support for the description of the STT as consisting of separate dorsal and ventral components.

The present study describes fine-caliber STT axons located in the DLF contralateral to their cells of origin; however, these axons are not concentrated in the dorsalmost portion of the DLF. Earlier retrograde labeling studies in cat (Stevens et al., 1989) and monkey (Apkarian and Hodge, 1989b,c) utilizing thalamic tracer injections combined with selective spinal lesions have also suggested that the DLF lamina I STT axons are not concentrated in the dorsalmost portion of the DLF.

Another report in cat utilizing lamina I injections of anterogradely transported *Phaseolus vulgaris* leucoagglutinin (PHA-L; Craig, 1989) described a widespread distribution of lamina I axons in the contralateral white matter; however, the highest concentration of those axons was located in the lateral funiculus, with fewer identified in the dorsalmost white matter. Although these PHA-L experiments do not specifically identify the locations of STT lamina I axons, it may be assumed that the lamina I STT axons are a subset of that labeled population and that they too are not concentrated in the dorsalmost DLF white matter. Interestingly, Norrsell (1989a,b) recently described contralateral thermosensory deficits in cats with lesions in this same region, which he described as the ventral portion of the dorsal half of the lateral funiculus. Since lamina I neurons, the primary constituents of the DSTT, have been implicated in ascending thermoreception (Craig and Kniffki, 1985), these results offer evidence for a functional distinction between the DSTT and the VSTT.

One experiment from a previous study in our laboratory (Stevens et al., 1989) has suggested that at least a portion of the dorsally projecting lamina I STT axons must be located in the medial DLF, since a lesion of the DLF that spared the region immediately adjacent to the dorsal horn resulted in considerable lamina I labeling in more caudal spinal cord following a thalamic injection of a retrograde tracer. By direct visualization, the present study has shown that the more dorsally situated, fine-caliber STT axons are located medially as well as peripherally in the DLF. This differs from the peripherally concentrated STT fibers of the VLF.

In all animals evaluated in the present study, the most dorsally located STT fibers were small in size, often approaching the limits of resolution of the light microscope, whereas more ventrally located fibers were coarse in nature and easily visualized. From this study, however, it is impossible to state whether the smallest-caliber fibers visualized include unmyelinated axons or whether, despite label-enhancing procedures, the unmyelinated population remains undetected. Considering the vast number of unmyelinated axons present in the spinal cord white matter, especially within the DLF (Chung et al., 1985), the distributions of STT axons presented in the current study could be altered considerably if the bulk of these fine-caliber fibers remain undetected. Follow-up studies utilizing electron-microscopic evaluation will be necessary to resolve this issue.

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REFERENCES

APKARIAN, A. V., and C. J. HODGE, JR. (1989a) The primate spinothalamic pathways: I. A quantitative study of the cells of origin of the spinothalamic pathway. J. Comp. Neurol. *288*: 447–473.

APKARIAN, A. V., and C. J. HODGE, JR. (1989b) The primate spinothalamic pathways: II. The cells of origin of the dorsolateral and ventral spinothalamic pathways. J. Comp. Neurol. *288*: 474–492.

APKARIAN, A. V., and C. J. HODGE, JR. (1989c) The primate spinothalamic pathways: III. Thalamic terminations of the dorsolateral and ventral spinothalamic pathways. J. Comp. Neurl. *288*: 493–511.

APPLEBAUM, A. E., J. E. BEALL, R. D. FOREMAN, and W. D. WILLIS (1975) Organization and receptive fields of primate spinothalamic tract neurons. J. Neurophysiol. *38*: 572–586.

BERMAN, A. L., and E. G. JONES (1982) *The Thalamus and Basal Telencephalon of the Cat: A Cytoarchitectonic Atlas with Stereotaxic Coordinates*, University of Wisconsin Press, Madison.

BURSTEIN, R., K. D. CLIFFER, and G. J. GIESLER, JR. (1987) Direct somatosensory projections from the spinal cord to the hypothalamus and telencephalon. J. Neurosci. *7*: 4159–4164.

CHRISTENSEN, B. N., and E. R. PERL (1970) Spinal neurons specifically excited by noxious or thermal stimuli: Marginal one of the dorsal horn. J. Neurophysiol. *33*: 293–307.

CHUNG, K., J. SHARMA, and R. E. COGGESHALL (1985) Numbers of myelinated and unmyelinated axons in the dorsal, lateral, and ventral funiculi of the white matter of the S2 segment of cat spinal cord. J. Comp. Neurol. *234*: 117–121.

CRAIG, A. D., JR. (1989) Ascending lamina I axons in the cat are concentrated in the middle of the lateral funiculus. Soc. Neurosci. Abstr. *15*: 1190.

CRAIG, A. D., JR., and H. BURTON (1981) Spinal and medullary lamina I projection to nucleus submedius in medial thalamus: A possible pain center. J. Neurophysiol. *45*: 443–466.

CRAIG, A. D., JR., and K.-D. KNIFFKI (1985) Spinothalamic lumbosacral lamina I cells responsive to skin and muscle stimulation in the cat. J. Physiol. (Lond.) *365*: 197–221.

CRAIG, A. D., JR., A. J. LININGTON, and K.-D. KNIFFKI (1989) Cells of origin of spinothalamic tract projections to the medial and lateral thalamus in the cat. J. Comp. Neurol. *289*: 568–585.

EMMERS, R., and K. AKERT (1963) *A Stereotaxic Atlas of the Brain of the Squirrel Monkey* (Saimiri sciureus), University of Wisconsin Press, Madison.

GARDNER, E. and H. M. CUNEO (1945) Lateral spinothalamic tract and associated tracts in Man. Arch. Neurol. *53*: 423–430.

HOLSTEGE, G., and R. J. COWIE (1989) Projections from the rostral mesencephalic reticular formation to the spinal cord: An HRP and autoradiographical tracing study in the cat. Exp. Brain Res. *75*: 265–279.

HYLDEN, J. L. K., H. HAYASHI, and G. J. BENNETT (1986) Lamina I spinomesencephalic neurons in the cat ascend via the dorsolateral funiculi. Somatosens. Res. *4*: 31–41.

JONES, M. W., A. V. APKARIAN, R. T. STEVENS, and C. J. HODGE, JR. (1987) The spinothalamic tract: An examination of the cells of origin of the dorsolateral and ventral spinothalamic pathways in cats. J. Comp. Neurol. *260*: 349–361.

JONES, M. W., C. J. HODGE, JR., A. V. APKARIAN, and R. T. STEVENS (1985) A dorsolateral spinothalamic pathway in cat. Brain Res. *335*: 188–193.

KERR, F. W. L. (1975) The ventral spinothalamic tract and other ascending systems of the ventral funiculus of the spinal cord. J. Comp. Neurol. *159*: 335–356.

KURU, M. (1949) *Sensory Paths in the Spinal Cord and Brainstem in Man*, Sogensya, Japan.

MESULAM, M.-M. (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. *26*: 106–117.

NORRSELL, U. (1989a) Behavioural thermosensitivity after bilateral lesions of the lateral funiculi in the cervical spinal cord of the cat. Exp. Brain Res. *78*: 374–379.

NORRSELL, U. (1989b) Behavioural thermosensitivity after unilateral, partial lesions of the lateral funiculus in the cervical spinal cord of the cat. Exp. Brain Res. *78*: 369–373.

STEVENS, R. T., C. J. HODGE, JR., and A. V. APKARIAN (1989) Medial, intralaminar and lateral terminations of lumbar spinothalamic tract neurons: A fluorescent double-label study. Somatosens. Mot. Res. *6*: 285–308.

STOOKEY, B. (1929) Further light on the transmission of pain and temperature within the spinal cord: Human cordotomy to abolish pain sense without destroying temperature sense. J. Nerv. Ment. Dis. *69*: 552–557.

WILLIS, W. D., D. L. TREVINO, J. D. COULTER, and R. A. MAUNZ (1974) Responses of primate spinothalamic tract neurons to natural stimulation of hindlimb. J. Neurophysiol. *37*: 358–370.

YEZIERSKI, R. P. (1988) Spinomesencephalic tract: Projections from the lumbosacral spinal cord of the rat, cat, and monkey. J. Comp. Neurol. *267*: 131–146.