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Ascending Projections From the Area Around the Spinal Cord Central Canal: A *Phaseolus vulgaris* Leucoagglutinin Study in Rats

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Abstract

A single small iontophoretic injection of *Phaseolus vulgaris* leucoagglutinin labels projections from the area surrounding the spinal cord central canal at midthoracic (T6–T9) or lumbosacral (L6–S1) segments of the spinal cord. The projections from the midthoracic or lumbosacral level of the medial spinal cord are found: 1) ascending ipsilaterally in the dorsal column near the dorsal intermediate septum or the midline of the gracile fasciculus, respectively; 2) terminating primarily in the dorsal, lateral rim of the gracile nucleus and the medial rim of the cuneate nucleus or the dorsomedial rim of the gracile nucleus, respectively; and 3) ascending bilaterally with slight contralateral predominance in the ventrolateral quadrant of the spinal cord and terminating in the ventral and medial medullary reticular formation. Other less dense projections are to the pons, midbrain, thalamus, hypothalamus, and other forebrain structures. Projections arising from the lumbosacral level are also found in Barrington's nucleus. The results of the present study support previous retrograde tract tracing and physiological studies from our group demonstrating that the neurons in the area adjacent to the central canal of the midthoracic or lumbosacral level of the spinal cord send long ascending projections to the dorsal column nucleus that are important in the transmission of second-order afferent information for visceral nociception. Thus, the axonal projections through both the dorsal and the ventrolateral white matter from the CC region terminate in many regions of the brain providing spinal input for sensory integration, autonomic regulation, motor and emotional responses, and limbic activation.

Indexing terms:

anterograde axonal transport; dorsal column; nociception; visceral pain; sensorimotor integration; autonomic regulation

Several major ascending pathways originating from the spinal gray matter and terminating in the brain have been suggested to be involved in the transmission of visceral and somatic nociceptive information (for review see Willis and Coggeshall, 1991; Willis and Westlund, 1997). These ascending pathways include the spinothalamic tract (STT; Giesler et al., 1981; Milne et al., 1981; Foreman et al., 1984; Rucker et al., 1984; Ammons, 1987, 1989, 1990;

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Foreman, 1989; Hobbs et al., 1992), spinohypothalamic tract (SHT; Katter et al., 1996a,b), spinosolitary tract (SST; Menétrey and Basbaum, 1987), spinoreticular tract (SRT; Haber et al., 1982; Villanueva et al., 1988), spinoparabrachial tract (Cechetto et al., 1985; Bernard et al., 1994; Slugg and Light, 1994), and pathways projecting from the spinal cord to forebrain structures, such as the amygdala and the septal nuclei (Burstein and Giesler, 1989; Burstein and Potrebic, 1993). It is clear from these studies that there are a variety of routes by which visceral and somatic nociceptive information is relayed by the spinal cord.

Another major ascending pathway, the dorsal column (DC) pathway, has been thought of primarily in terms of transmission of tactile discriminative sensation. The DC consists of two types of ascending fibers. The first type, which can be referred to as the direct DC pathway, is composed of collaterals of primary afferent fibers whose cell bodies are located in the dorsal root ganglia. These ascending projections include both myelinated and unmyelinated primary afferent fibers that synapse in the dorsal column nuclei (DCN), including the gracile and cuneate nuclei, of the lower medulla (Kuo and De Groat, 1985; Patterson et al., 1990; Garrett et al., 1992; for review see Willis and Coggeshall, 1991). The second type, referred to as the postsynaptic dorsal column (PSDC) pathway, includes the axons of spinal neurons traveling in the dorsal funiculus to terminate synaptically in the DCN (Petit, 1972; Rustioni, 1974, 1976; Angaut-Petit, 1975a; Rustioni and Kaufman, 1977; Giesler et al., 1984; Giesler and Cliffer, 1985; Cliffer and Giesler, 1989; Cliffer and Willis, 1994). The spinal cord distribution of PSDC neurons has been studied in monkeys (Rustioni, 1976; Bennett et al., 1983; Cliffer and Willis, 1994), cats (Petit, 1972; Rustioni, 1974; Angaut-Petit, 1975a,b; Rustioni and Kaufman, 1977; Bennett et al., 1983), and rats (Giesler et al., 1984; Giesler and Cliffer, 1985; Cliffer and Giesler, 1989). A previous study in rats has shown that most of the PSDC neurons are localized in laminae III and IV of the dorsal horn, and a few cells are found in the area around the central canal (Giesler et al., 1984). The involvement of rat PSDC neurons in the transmission of nociception has remained controversial for some time (Giesler and Cliffer, 1985), although PSDC neurons are reported to respond to noxious cutaneous and heat stimuli in cats (Uddenberg, 1966, 1968; Brown and Fyffe, 1981; Brown et al., 1983).

There is growing evidence, however, showing that the DC pathway may contribute to central transmission of visceral nociceptive information (Amassian, 1951a,b, 1952; Aidar et al., 1952; Rigamonti and Hancock, 1974, 1978; Berkley and Hubscher, 1995; Al-Chaer et al., 1996a,b; Hirshberg et al., 1996; Houghton et al., 1997b; Nauta et al., 1997; Feng et al., 1998). Clinically, it has been reported that intractable pelvic cancer pain can be relieved by a limited midline myelotomy extending from the dorsal surface of the spinal cord to the level of the dorsal gray commissure (Hirshberg et al., 1996; Nauta et al., 1997). Experimentally, the recent behavioral, anatomic, and physiologic studies from our laboratories have suggested that the population of PSDC neurons in the area around the central canal that sends ascending projections to the DCN is responsible for the transmission of visceral nociceptive information. Behavioral studies demonstrated that DC lesions could reduce the nociceptive behaviors induced in rats with pancreatitis or by duodenal distention (Houghton et al., 1997a; Feng et al., 1998). Similarly, mechanical DC lesions or chemical lesions of the DCN dramatically reduced responses of thalamic neurons resulting from duodenal distention (Feng et al., 1998), from chemical stimulation of pancreatic afferent neurons in rats

(Houghton et al., 1997b; Wang et al., 1998), and from colorectal distention (Al-Chaer et al., 1996a, 1997a). Anatomically, retrograde tracing studies in rats demonstrated that axons traveling in the midline of the DC to the gracile nucleus originate from the PSDC neurons in the area around the central canal (CC) of the spinal cord (Hirshberg et al., 1996; Christensen et al., 1996; Westlund et al., 1996). Electrophysiological studies in rats have shown that the PSDC neurons in the area around the CC at the L6–S1 level of the spinal cord can be excited by colorectal distention or an injection of a chemical irritant, mustard oil, into the colon (Al-Chaer et al., 1996b, 1997b). These clinical and experimental studies provide evidence in support of the hypothesis that PSDC cells around the CC whose axons ascend in the DC pathway play an important role in visceral nociceptive processing.

Neurons in the area surrounding the CC in rats have previously been shown to give rise to fibers of the STT (Giesler et al., 1979; Kevetter and Willis, 1982, 1983; Granum, 1986; Burstein et al., 1990b), SRT (Kevetter et al., 1982; Kevetter and Willis, 1982, 1983; Menétrey et al., 1983; Nahin et al., 1983, 1986; Peschanski and Besson, 1984; Nahin and Micevych, 1986; see also Ruigrock and Cella, 1995), spinomesencephalic tract (SMT; Menétrey et al., 1982; Mantyh, 1982; Liu, 1983; Wiberg et al., 1987; Yezierski and Mendez, 1991), SST (Menétrey and Basbaum, 1987; Menétrey and de Pommery, 1991), SHT (Burstein et al., 1987, 1990a; Menétrey and de Pommery, 1991), spinoparabrachial tract (Menétrey and de Pommery, 1991; Kitamura et al., 1993), and spinoamygdaloid tract (Menétrey and de Pommery, 1991; Burstein and Potrebic, 1993).

Several tract tracing studies have shown that the region around the CC receives terminations from somatic and visceral afferents (Morgan et al., 1981, 1986; Kuo et al., 1983; Nadelhaft et al., 1983; Cervero and Connell, 1984; Kuo and De Groat, 1985; Roppolo et al., 1985; Neuhuber et al., 1986). This includes some Aδ mechanical nociceptors and unmyelinated visceral afferent fibers (Light and Perl, 1979; Sugiura et al., 1989). Neurons around the CC area respond to somatic and visceral nociceptive stimulation (Cervero, 1983; Nahin et al., 1983; Honda, 1985; Honda and Perl, 1985; Tattersall et al., 1986a,b; Ness and Gebhart, 1987, 1989; Berkley et al., 1993; Al-Chaer et al., 1996b). In addition, it is well known that the region around the CC also contains clusters of preganglionic autonomic neurons (Loewy and Spyer, 1990). It should be noted here, importantly, that the cells and afferent fiber innervation in this region are localized not only within the lamina X area but also in the medial aspect of lamina VII (and the dorsal gray commissure at lumbosacral segments), based on the results of previous studies (Honda, 1985; Honda and Perl, 1985; Tattersall et al., 1986a; Ness and Gebhart, 1987; Al-Chaer et al., 1996b; Hirshberg et al., 1996; Christensen et al., 1996; Westlund et al., 1996).

Previous anterograde tracing studies with injections centered in the midline gray matter surrounding the CC of the lumbosacral cord have shown that ascending axons travel in the midline of the DC and terminate in the gracile nucleus (Hirshberg et al., 1996; Westlund et al., 1996). However, the full extent of ascending projections and terminations from the neurons in the CC area is described in detail in the present study. In addition, in the present study, distinctive differences and similarities are noted for projections from the thoracic cord, which receives a substantial projection of nociceptive information from the thoracic viscera, for example, the pancreas and duodenum.

To characterize the projections further, a single small iontophoretic microinjection of an anterograde tracer, *Phaseolus vulgaris* leucoagglutinin (PHA-L), was made into the CC area at either the midthoracic or the lumbosacral level of the spinal cord in a series of rats. PHA-L was chosen as the anterograde tracer in this study because previous reports have shown that PHA-L has several advantages over wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP; Gerfen and Sawchenko, 1984; Cliffer and Giesler, 1988): 1) PHA-L is taken up mainly by cell bodies and not significantly by passing fibers, 2) it is easy to make small injections of PHA-L, 3) labeled terminal varicosities and axons are clearly identified, and 4) PHA-L is unlikely to be transported across synapses. Preliminary results have been reported in abstract form (Wang et al., 1997).

MATERIALS AND METHODS

A total of 38 male adult Sprague-Dawley rats weighing 250–340 g were used in this study. Experiments were approved by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston in accordance with NIH guidelines.

Surgery and injections of PHA-L

Rats were deeply anesthetized with sodium pentobarbital (Nembutal; 50 mg/kg, i.p.). The midthoracic or lumbosacral cord was exposed by a laminectomy, and the dura was opened. A single iontophoretic microinjection of PHA-L (2.5% in 0.05 M phosphate buffer, pH 8.0; Vector, Burlingame, CA) was made into the CC area at the midthoracic level (1.0–1.1 mm depth) or lumbosacral level (0.8 mm depth) of the spinal cord with a glass micropipette (tip diameter 15–20 μm). In some sacral animals, injections were angled into the CC area to avoid the medial dorsal spinal vessels and the DC white matter. There were no differences in terminal labeling with either method of approach to the CC. Discontinuous positive pulses of DC current (3–5 μA, 7 seconds on and off) were applied for 20 minutes. The wound was sutured, and the rats were given antibiotics (Bicillin, Wyeth Laboratories Inc.; 40,000 units i.m. total).

Immunohistochemistry

After a 4–6-week survival period, the rats were killed with an overdose of sodium pentobarbital and perfused transcardially with 150 ml of warm (37°C) heparinized saline, followed by 1,000 ml of cold (4°C) 4% paraformaldehyde and 150 ml of 30% sucrose. The brain and spinal cord were removed carefully and immersed in 30% sucrose/phosphate buffer overnight for cryoprotection. Tissue blocks were cut in 40-μm-thick coronal serial sections on a freezing microtome, and the sections were collected in ice-cold phosphate buffer containing 0.1% sodium azide (NaN_3; Sigma, St. Louis, MO). After rinsing in 2% normal rabbit serum (NRS) containing 0.3% Triton X-100 at room temperature for 30 minutes, a one-in-five series of tissue sections was processed immunohistochemically with goat anti-PHA-L antibody (1:10,000; Vector)/2% NRS containing 0.3% Triton X-100 at 4°C for 36–48 hours. After rinsing in 0.1 M phosphate-buffered saline (PBS) for 20 minutes, the sections were incubated with biotinylated rabbit anti-goat IgG (1:400; Vector)/2% NRS containing 0.3% Triton X-100 at room temperature for 1 hour. Then the sections were rinsed in 0.1 M PBS for 20 minutes before incubating in avidin-biotin-horseradish peroxidase

(HRP) complex solution (ABC Kit, Vector; recommended dilution ×2 in PBS) at room temperature for 1 hour. After rinsing in 0.1 M PBS for 20 minutes, the sections were reacted with 1) 0.025% DAB/phosphate buffer containing 0.0025% H_2O_2 or 2) 0.015% DAB/0.1 M acetate buffer (pH 6.0) containing 0.24% nickel ammonium sulfate and 0.0015% H_2O_2, for 5–10 minutes. Then the sections were mounted onto gelatin-coated glass slides, air dried, cleared with xylene, and coverslipped with DPX. Another one-in-five series of tissue sections was stained with cresyl violet or neutral red.

The sections were observed under conventional bright-field optics using a Nikon microphot-FXA light microscope (NCB11, HE, and ND2 filters). Photomicrographs were made with AGFAPAN APX25 professional film (batch developed in TMX RS) and printed on Kodak F4 Kodabrome II RC paper. Injection sites and labeled axons and varicosities were drawn with the aid of a camera lucida attachment. The sections were superimposed on adjacent sections stained with cresyl violet or neutral red, and the nuclei were cytoarchitectonically identified according to the stereotaxic rat brain atlas of Paxinos and Watson (1986). In addition, the nomenclature of the subnuclei of the parabrachial (PB) nucleus was adopted from the description of Fulwiler and Saper (1984). The reconstructions of injection sites were plotted on a template of the midthoracic and lumbosacral levels of the spinal cord (Fig. 1).

RESULTS

The present results are based on 10 selected experiments in which the single PHA-L injection site was localized in the CC area of the midthoracic or lumbosacral level of the spinal cord (n = 5 for each level; Fig. 1). These injection sites consisted of a densely stained core containing labeled cell bodies and an injection halo where diffuse extracellular staining was observed. PHA-L is thought to be preferentially taken up by the cells within the injection core but not by cells in the injection halo (Gerfen and Sawchenko, 1984). In addition, control injections were made in eight animals into the midline of the spinal cord at thoracic and lumbosacral sites that included the dorsal column (n = 3), medial zone of laminae I–V (n = 3), or lamina VII lateral to the ventral white commissure of spinal cord (n = 2; Fig. 2).

Injection sites

Midthoracic level of the spinal cord.—The midthoracic injection group description includes the results of five experiments in which the injection sites were localized in the CC area at the midthoracic level (Fig. 1, left column). In two experiments (experiments 45 and 121, injection in T7 and T9, respectively), the injection sites were restricted to the CC area. Two experiments (experiments 59 and 110, injection in T7 and T8, respectively) had injection sites in the CC area and the ventral aspect of the dorsal funiculus containing the corticospinal tract. In experiment 111, the injection site (T7) was localized not only in the CC area but also in the midline of the dorsal funiculus, including both the DC and the corticospinal tract. Experiment 110 was chosen as a representative case for camera lucida drawings and will be described in detail, because the injection site covered most of the CC

area at the T8 level of the spinal cord (diameter 200–250 μm), without significant spread to the white matter.

Lumbosacral level of the spinal cord.—In five experiments, injection sites were localized in the CC area at the lumbosacral (SI) level, and the descriptions from these animals are included in the lumbosacral injection group (Fig. 1, right column). Two experiments (experiments 36 and 107) had small injection sites that targeted the CC area. Three experiments (experiments 27, 33, and 118) had larger injection sites localized in the midline of the lumbosacral level of spinal cord, including lamina X and the medial aspect of lamina VII. Experiment 118 was chosen as a representative case for camera lucida drawings and will be described in detail.

Control injections.—Control injections were made in eight animals (Fig. 2) to compare the pattern of distribution of axons from neurons in the CC area with those from adjacent regions in the medial spinal cord, e.g., the DC and the medial aspect of the laminae I–V and ventromedial aspect of lamina VII.

Pattern of ascending projections

As an overview of the results, the ascending axons from the neurons in the CC area are observed in a number of brain sites, including areas in the medulla, pons, mesencephalon, diencephalon, telencephalon, and cerebellum. Generally, a higher density of PHA-L-labeled fibers is seen at the medullary level than at other levels. The density and number of labeled axons in the diencephalon and other forebrain structures are much smaller. In comparing the pattern of ascending projections from the thoracic and lumbosacral groups of PHA-L-injected animals, a similar termination pattern was noted. However, labeled fibers in Barrington's nucleus are found only in projections of the lumbosacral group, and the primary termination sites in the DC nuclei from thoracic and lumbosacral levels are different. Thoracic projections to the cerebellum are observed resulting from some uptake into the adjacent Clarke's column.

The pattern of distribution of labeled terminals and axons was mapped successively starting from sections just rostral to the injection sites, at the C1 level of the spinal cord, and the brainstem through the level of the diencephalon (experiment 110, midthoracic group; experiment 118, lumbosacral group in Fig. 3). In addition, the location of terminal labeling in telencephalic regions will be described in this report. The comparative density of PHA-L-labeled fibers in selected experiments following midthoracic and lumbosacral injections is shown in Table 1.

Spinal cord.—Beginning at a level several sections rostral to the injection site, the labeled axons are seen ascending at the lateral edge of the DC bilaterally as well as in the ventrolateral funiculus and the medial aspect of the ventral funiculus bilaterally. A few axons are found in the lateral funiculus of the spinal cord near the spinal gray matter (Fig. 3.1). In the lumbosacral injection group, the labeled axons then migrate to assume a position near the midline of the DC as well as in the ventrolateral funiculus at the midthoracic level of the spinal cord. A few fibers are observed in the ventral and lateral funiculus of the spinal

cord. At midcervical levels the DC projection of the thoracic injection group migrates to a position adjacent to the dorsal intermediate septum.

At the C1 level of the spinal cord (Fig. 3.2), the labeled axons are seen coursing longitudinally in the DC and the ventrolateral white matter of the spinal cord. The PHA-L-labeled axons originating from the midthoracic level of the spinal cord are seen traveling primarily in the superficial part of the DC near the dorsal intermediate septum, whereas those from the lumbosacral level have migrated to ascend in the superficial part of the medial gracile fasciculus. Ascending axons in the DC arising from both the midthoracic and the lumbosacral levels of the spinal cord have bilateral projections with an ipsilateral predominance. The labeling in the ventrolateral and lateral funiculus of the spinal cord is observed bilaterally (Fig. 3.2). Propriospinal fibers are observed originating from axons traveling in the ventrolateral quadrant of the spinal cord coursing dorsomedially out of the white matter and terminating in the spinal gray matter, mainly in the area adjacent to the central canal and in the ventral horn. Propriospinal fibers leaving the DC terminate in the gray matter of the dorsal horn.

Medulla.—The fibers traveling in the DC terminate in the dorsal column nuclei (DCN) with a strong ipsilateral predominance. There is a topography of the terminations from neurons in the CC area at midthoracic and lumbosacral levels of the spinal cord in the present study. Generally, the terminal labeling is concentrated in the caudal half of the DCN. In the region of the DCN rostral to the area postrema, the density and number of fibers become less.

In three of five cases with midthoracic injections, the fibers and terminals are localized primarily in the dorsal and lateral rims of the gracile nucleus (Gr) and medial portions of the cuneate nucleus (Cu; Figs. 3.3–3.6 [left column], 4B,D). In one case, the fibers are only found in the lateral rim of the Gr, whereas, in another case, the labeling is seen exclusively in medial portion of the Cu. In the example illustrated in Figure 3 (experiment 110), however, terminations from PSDC cells in the midthoracic level of the spinal cord are also found in the dorsomedial aspect of the Gr, even though labeled fibers are seen traveling near the dorsal intermediate septum of the DC at the C1 level. Long axons with short arborizations and many terminal varicosities are seen coursing from the dorsolateral to the ventromedial aspect of the Gr (Fig. 4B). In experiment 111 with an injection site that includes the CC area and the entire midline of the DC, the labeled fibers are observed in the lateral and medial rims of the Gr and the medial aspect of the Cu. The labeling in the lateral rim of the Gr and medial portion of the Cu consists mainly of short axonal arborizations with many terminal and en passant varicosities. However, the labeling coursing along the medial rim of the Gr contains less intensely stained short axons with few varicosities.

In all cases with injections at the lumbosacral level, terminations are observed primarily in the medial rim of the Gr (Figs. 3.3–3.6 [right column], 4E). In two cases, labeling is also seen in the ventromedial aspect of the Gr (Fig. 3.3, 3.4 [right column]). A low to medium density of terminal labeling is found in the Gr. In two cases, however, a low density of fibers is also found in the Cu.

PHA-L-labeled fibers continuing from the ventrolateral and ventral funiculi of the spinal cord are found terminating throughout the full rostrocaudal extent of the ventral medulla. The labeling is localized in the lateral reticular nucleus (LRt) and the lateral paragigantocellular reticular nucleus (LPGi); the caudal part of the nucleus of the solitary tract (NTS); and the medullary reticular formation, including the subnucleus reticularis dorsalis (SRD); the subnucleus reticularis ventralis (SRV; Villanueva et al., 1991), the gigantocellular reticular nucleus (Gi), the raphe magnus nucleus (RMg), and the raphe obscurus nucleus (ROb). In some cases, a sparse density of labeling is also seen in the trigeminal nucleus caudalis. The labeled axons and terminals in the ventral and medial medulla from midthoracic injections and those from lumbosacral injections are shown in Figure 3.3–3.9.

In general, labeled fibers are seen coursing bilaterally through the rostrocaudal extent of the ventral and medial medulla. PHA-L-labeled axons are seen extending dorsomedially from the ventral medulla across the reticular formation, including the SRD and SRV, to the region of the NTS (Figs. 3.3–3.9, 4C), or ascending longitudinally through the ventral medulla. In addition, ascending PHA-L-labeled axons localized in the ventral medulla are seen traversing the tissue section toward the midline. In the caudal medulla, a large number of labeled varicose fibers are concentrated in the area of the medial and dorsal aspect of the LRt and within the LRt (Fig. 3.3–3.8). In general, the projections to the LRt are concentrated dorsally from the midthoracic cord and ventrally from the lumbosacral cord.

At the level between the rostral end of LRt and the caudal portion of LPGi, labeled fibers are concentrated in the LRt and LPGi and ascend longitudinally (Fig. 3.7–3.9). Fibers bearing varicosities are also seen coursing dorsomedially from ventral medulla to the medial medullary reticular formation. At the level between the caudal aspect of the LPGi and the facial nucleus, labeled axons and terminals are concentrated in the LPGi bilaterally (Fig. 3.9, 3.10). PHA-L-labeled fibers are also observed coursing from the LPGi to the medial reticular formation, including the Gi and the ROb.

At the rostral medullary level, near the caudal aspect of the facial nucleus, the density of labeling becomes less intense (Fig. 3.10). In most cases, labeled fibers are seen coursing bilaterally from the ventral medulla to the medial reticular formation between the medial aspect of the facial nucleus and terminating in the RMg, ROb, and Gi. In all cases but one, a medium density of labeling in the Gi and a low density of labeled fibers in RMg and ROb are observed. The labeling in the Gi and raphe nuclei consists of axons bearing many terminal varicosities.

Pons.—In the pons, the PHA-L-labeled fibers from neurons in the CC area are localized in the pontine reticular formation; the ventrolateral tegmentum; the dorsolateral tegmentum, including the locus coeruleus (LC) and the subcoerulear region (SubC); and the parabrachial nucleus (PB), including the medial PB subnucleus (PBm), the internal lateral PB subnucleus (PBil), the superior lateral PB subnucleus (PBsl), the central lateral PB subnucleus (PBcl), the external lateral PB subnucleus (PBel), and the Kölliker-Fuse nucleus (KF). The labeling at this level is plotted in Figure 3.12–3.14 and Figure 6. The labeling is seen bilaterally at this level.

At the pontomedullary junction, a medium density of labeled fibers is observed bilaterally in the A5 region adjacent to the exit of the facial nerve, in the dorsal and lateral aspects of the lateral superior olivary nucleus (LSO; Figs. 3.12, 3.13, 5B). The labeled fibers in this region consist primarily of axons with many arborizations.

The labeled fibers in the ventrolateral tegmentum (medial to the facial nerve) continue from the rostral ventral medulla (RVM) into the caudal pons. Generally, a small number of labeled fibers are seen coursing from the dorsal and medial aspects of LSO medially and terminating in the pontine reticular formation. In addition, a medium density of labeling, in most cases, is observed coursing dorsolaterally, medial to the motor trigeminal nucleus (Mo5), and terminating diffusely in the SubC region (Fig. 3.13). Axons with several collaterals bearing varicosities are seen in the SubC region.

Fibers in the SubC region continue coursing dorsally toward the LC. In two of the five cases with midthoracic injections and all of the lumbosacral cases, a sparse to low density of the labeling is observed bilaterally in the LC. In most cases, fine and short axons bearing varicosities in the SubC are seen coursing dorsolaterally to ventral and medial aspects of the LC. In addition, labeling inside the LC is observed arising from collaterals of fibers coursing along the fourth ventricle (Fig. 5A). There is no apparent topographic organization for the innervation of the LC; fibers with boutons can be seen throughout the rostrocaudal extent of the LC for both thoracic and lumbosacral injections. At any particular level, only one or two varicose fibers can be seen within the LC (Fig. 5A). However, the labeling is focused in the caudal half of the LC. Terminal labeling is also seen in the pericoerulear region ventral and medial to the LC, including in Barrington's nucleus. In four cases with lumbosacral injections, a low density of labeled fibers is observed in Barrington's nucleus, whereas no fibers are found in this structure with midthoracic injections. In two cases, labeling is also seen in the mesencephalic trigeminal nucleus at this level (Me5).

In addition, ascending axons traveling in the ventrolateral tegmentum are also observed coursing dorsally with the lateral lemniscus and passing through the ventrolateral aspect of PB and KF. A medium to low density of labeled fibers and terminals is seen in the KF (Figs. 6, 7D). Most of these terminals are concentrated in the caudal half of the KF. The labeling in PB is concentrated in the middle and rostral one-third of the lateral PB subnuclei. Very few fibers are seen in the caudal aspect of PB. The labeled fibers in the lateral PB subnuclei are seen bilaterally, with a slight ipsilateral predominance. Most of the labeling is concentrated in the PBil (Figs. 6, 7B,C). Generally, the labeled fibers in the PB are long, thin axons with numerous terminal boutons. In addition, less density of fibers is seen in the PBcl, PBdl, PBsl, PBel, and KF. A sparse to low density of labeled fibers is found in the PBm.

Mesencephalon.—Labeled fibers are seen in the caudal lateral and ventrolateral portions of the periaqueductal gray (PAG), the cuneiform nucleus (CnF), the deep mesencephalic region (DpMe), the red nucleus, the anterior pretectal nucleus, the pars reticulata of the substantia nigra (SNR), the superior colliculus (SC), and the intercollicular region of the midbrain (InC; see Table 1; Fig. 3.15, 3.16). Basically, the labeling in the mesencephalon is bilateral with strong contralateral predominance.

The labeling in the mesencephalon (Fig. 3.15, 3.16) continues from the axons traveling in the rostral aspect of lateral PB. These axons are seen oriented from ventrolateral to dorsomedial. Low to high densities of PHA-L-labeled fibers and terminals are localized in the ventrolateral PAG. The terminals are concentrated in the caudal aspect of the ventrolateral and lateral PAG. In most cases, the labeling in the InC is of low density and scattered. In an example shown from an animal with a lumbosacral PHA-L injection (experiment 107), basket-like terminations of labeled fibers are found around cells of the Me5 adjacent to the PAG region (Fig. 8A).

Thalamus.—The density and number of labeled axons in the area rostral to the mesencephalon is small (see Table 1). The areas in the thalamus that contain labeled fibers include the posterior thalamic nuclear group, the midline thalamic nuclei (including the paraventricular thalamic nucleus [PV; Fig. 8B], the intermediodorsal nucleus, and the reuniens nucleus), the intralaminar nuclei (including the parafascicular nucleus [Fig. 3.17], the posterior intralaminar nucleus [PIL], the central medial nucleus, and the central lateral nucleus [Fig. 3.18]), the peripeduncular nucleus (PP), the parvicellular part of subparafascicular thalamic nucleus (SPFPC; Fig. 3.16), the mediodorsal nucleus, the ventral lateral nucleus, the ventral medial nucleus, and the lateral habenular nucleus (see Table 1). In general, PHA-L labeling in the thalamus is almost entirely contralateral. In most thalamic structures, very few or a low density of fibers with a small number of boutons are observed.

A sparse to low density of labeling is observed in the SPFPC in all of the midthoracic cases and one of lumbosacral cases, and a medium to high density of labeling is seen in this structure in four cases with lumbosacral injections (Fig. 3.16). Thus, a higher density of labeling is observed in sections from animals with lumbosacral injections. In these four cases, long, varicosity-bearing, and terminal arborizations are seen throughout the entire extent of SPFPC. In the caudal aspect of SPFPC, the labeled fibers are observed coursing medially, above the region of medial lemniscus in thalamus, and to the rostral portion of SPFPC where it abuts the ventral part of the caudal pole of the ventral posterior thalamic nucleus, parvicellular part (VPPC). The labeling is focused in the caudal half of SPFPC. In most cases (four midthoracic cases and two lumbosacral cases), a sparse to low density of PHA-L labeling is observed lateral to SPFPC in the PIL and PP, but a medium to high density of labeled fibers is seen in two cases with a lumbosacral injection.

Hypothalamus.—Fibers are also localized in the hypothalamus, including the supramammillary nucleus, the posterior hypothalamic area (PH), the lateral hypothalamic area (LH; Fig. 3.16–3.19), the dorsal hypothalamic area, the zona incerta (ZI; Fig. 3.16 [left column]), the paraventricular hypothalamic nucleus, the supraoptic decussation (sox; Fig. 8C and Fig. 9A), and the medial and lateral preoptic areas (see Table 1). Generally, the labeling in the hypothalamus is bilateral with slight contralateral predominance in most cases, but two cases (one with a midthoracic and one with a lumbosacral injection) have a strong ipsilateral labeling. In addition, a higher density of labeled fibers is seen in the LH and PH than in any other areas in the hypothalamus.

The labeling in the ZI in most cases is sparse, except in one heavily labeled case each from a midthoracic injection (experiment 110) and from a lumbosacral injection (experiment 27). In

the lumbosacral experiment 27, PHA-L-labeled fibers are primarily localized in the caudal pole of ZI. These fibers consist of many fine axons with long arborizations and terminals coursing ventromedially from lateral ZI, between the medial lemniscus and the substantia nigra, to medial ZI (Fig. 3.16).

Telencephalon.—Although the labeled fibers in the telencephalon are very scattered, PHA-L-labeled fibers are observed in the globus pallidus, the central nucleus of the amygdala (Fig. 9), the substantia innominata, and the basal nucleus of Meynert (Fig. 9A; see Table 1). The labeling in the telencephalon is generally bilateral.

Interestingly, in an experiment with a lumbosacral injection (experiment 118), labeled fibers are seen coursing in the external capsule to terminate in expanded deep layers of the caudal granular insular cortex located just outside the external capsule (2.56 mm caudal to bregma; Fig. 10). Although a few boutons are seen in layer VI of the granular insular cortex, more are found in a small, discrete region of deeper layer expansion underlying the granular insular cortex that is not labeled in any atlas available to us. Terminal boutons are observed in this discrete region scattered among multipolar neurons. The cytoarchitecture of the region matches Cajal's description for the claustrum (DeFelipe and Jones, 1988), which is located 1.8 mm more rostrally (at 1.4 mm caudal to bregma) in the rat, so this site may represent a caudal extension of the claustrum in the rat.

Cerebellum.—Terminal labeling in the cerebellum is present in all cases of midthoracic injections but in none of the cases with lumbosacral injections. The fibers in the cerebellum are distributed bilaterally. In the present study, the cerebellar labeling is not analyzed in detail because the labeled projections to this structure may be attributed to uptake by neurons of Clarke's column.

Control injections.—In general, the pattern of ascending projections in control groups (Table 2) is quite different from the projections labeled by injections made in the CC area. In one control animal (experiment 125), the injection site is centered among the axons in the midline of the DC at the T8 level of the spinal cord. No cell bodies in the gray matter are labeled. Labeled fibers are seen exclusively traveling in the DC, and a low density of lightly labeled fibers with very few boutons is localized in the DCN. The quality of the stain within the fibers is different from that in the gray matter injections. In experiments 124 and 128, in which injection sites involved the medial portion of laminae III and IV, a very high density of labeled fibers is observed in the DCN, and a sparse to low density of fibers is seen in other regions of brainstem. Experiments 25 and 129, with injection sites in the medial zone of lamina V, show a low to medium density of fibers in the DCN and a sparse to low density of fibers in some structures in brainstem and diencephalon, and no fibers are found in the forebrain. A very high density of labeled fibers and terminals is observed in the DCN when the injection site is centered in laminae II–IV of the spinal cord dorsal horn (experiment 51). However, fewer fibers are seen in other brainstem structures. In experiments 31 and 32, in which the injection lies at the ventral border of laminae VII but is centered in the dorsomedial portion of lamina VIII (lateral to the ventral white commissure), no fibers are found in the DCN. Although a sparse to medium density of fibers is seen in some areas of the brain, most structures receiving a projection from the CC region have no labeled fibers.

DISCUSSION

The present study provides evidence that the neurons in the CC area project to a number of specific sites in the brain, sites thought to be involved in nociceptive transmission, sensorimotor integration, autonomic regulation, motor control, emotional expression, and limbic activation. In particular, characterizations of the ascending projections and terminations of PSDC neurons in the area close to the central canal at midthoracic or lumbosacral levels are reported here.

Technical considerations

PHA-L is a reliable tract tracing method when iontophoretically applied and produces the highly restricted injection sites required for accurate mapping of projections of small neuronal populations. Previous investigators (Lee et al., 1988; Shu and Peterson, 1988) have reported that PHA-L can be transported retrogradely. However, retrograde transport of PHA-L was not evident in the present study; no PHA-L-labeled cell bodies were found anywhere in the brain. In addition, because no labeled cell bodies were found away from the injection sites, transsynaptic labeling is unlikely with PHA-L in this study. These observations are consistent with previous PHA-L studies (Cliffer and Giesler, 1989; Cliffer et al., 1991; Craig, 1991b).

Owing to the movements of the thoracic spinal cord caused by breathing, in some cases, injection sites included not only the CC area but also the midline of the DC. Previous investigators have reported that PHA-L is not effectively taken up and transported by fibers of passage (Gerfen and Sawchenko, 1984; Grove et al., 1986). However, anterograde labeling through axons of passage has been previously reported in the rat by others (Cliffer and Giesler, 1988; Rhoades et al., 1989; Schofield, 1990). The transport of PHA-L by the axons of the DC is not an important problem in this study insofar as control injections made into the DC show that 1) only lightly labeled fibers with very few boutons were found in the DCN after PHA-L injections directly into the DC (experiments 124, 125, and 128), 2) the quality of labeling from fibers of passage in the DC clearly differs from labeling arising from neurons in the CC area, 3) the DC has been shown to be somatotopically organized (Giesler et al., 1984; Cliffer and Giesler, 1989). The locations of terminations from axons in the DC, many of which arise from lower spinal levels, are quite different from those arising from neurons in the CC area in the thoracic cord. Thus, labeling through axons of passage in the DC is minor and is clearly distinguishable from that resulting from uptake by neurons in the CC area. Furthermore, to avoid the dorsal spinal vessels, the lumbosacral injections were angled into the CC area through gray matter, avoiding the DC altogether. A retrograde tracing study (Christensen et al., 1996) has confirmed the existence of a spinal projection from neurons in the CC area to the DCN. Moreover, data from the DC control injections are in agreement with previous anterograde transport studies (Cliffer and Giesler, 1988; see also discussion in Cliffer and Giesler, 1989).

Previously, an anterograde tracing study (Craig, 1995) has shown that projections and terminations from lamina I neurons are observed 1) along the entire rostrocaudal extent of the ventrolateral medulla (VLM), especially between the trigeminal nucleus caudalis and the lateral reticular nucleus (LRt) in the caudal VLM and 2) labeling in the thalamus was almost

entirely contralateral. In this study, labeling in the brain could potentially have arisen from uptake by ascending fibers from STT neurons crossing in the spinal gray commissure. However, this possibility also appears unlikely insofar as very few labeled fibers and terminals are found in the VLM and the projection to the thalamus from neurons in the CC area is almost completely contralateral in the present study. Likewise, no labeled cells were seen in the dorsal horn away from the injection site. If crossing axons had been labeled, the projection to thalamus should have been bilateral. Labeling in the DCN might also have been from collateral branches of primary afferent fibers in the spinal gray matter. This is also unlikely insofar as no labeled axons or cell bodies were seen in the dorsal root ganglia.

Neurons in the spinal gray matter other than in the CC area or fibers of passage in the DC might have been labeled when electrodes were inserted into the CC area. This possibility is also unlikely; PHA-L has been shown to be transported preferentially in the anterograde direction when introduced iontophoretically (Gerfen and Sawchenko, 1984).

Anatomical considerations

The present study demonstrates that neurons in the CC area project to many specific areas in higher brain centers through the dorsal and ventrolateral funiculi of the spinal cord. The projection sites include a number of specific areas, which are believed to be involved in integration of sensory information, homeostatic regulation, motor and emotional control, and limbic activation. Many of these regions have reciprocal projections back to the spinal cord.

The area around the CC.—Anatomical studies have shown primary afferent fibers from the major splanchnic nerve (Cervero and Connell, 1984; Kuo and de Groat, 1985; Neuhuber et al., 1986), the intercostal nerve (Cervero and Connell, 1984; Neuhuber et al., 1986), the inferior cardiac nerve (Kuo et al., 1984), the left renal nerve (Kuo et al., 1983), the pelvic nerve (Morgan et al., 1981; Nadelhaft et al., 1983), and the hypogastric nerve (Neuhuber, 1982; Morgan et al., 1986) terminating near this area. Morphologically, a heterogeneous population of neurons has been found in this area (Nahin et al., 1983; Honda and Perl, 1985). For the rat, using a retrograde tract tracing method, Nahin et al. (1983) reported that fusiform, pyramidal, and stellate types of neurons were identified in the area surrounding the spinal cord central canal. However, no evident correlations between functional categories and morphological features were found (Honda and Perl, 1985). In addition, the CC area has been shown to receive inputs from neurons of the rostral medulla associated with descending modulation of nociception (Martin et al., 1981; Holstege and Kuypers, 1982; Light, 1985). A large body of immunohistochemical studies has demonstrated that several compounds, for example, serotonin (Steinbusch, 1981), substance P (Gibson et al., 1981; Sasek et al., 1984; Leah et al., 1988), enkephalin (Hökfelt et al., 1977; Gibson et al., 1981; Leah et al., 1988; Nahin, 1988), dynorphin (Sasek et al., 1984; Leah et al., 1988; Nahin, 1988), cholecystokinin (Gibson et al., 1981; Sasek et al., 1984; Leah et al., 1988), neuropeptide Y (Leah et al., 1988), bombesin (Leah et al., 1988), somatostatin (Leah et al., 1988), and vasoactive intestinal polypeptide (Gibson et al., 1981; Sasek et al., 1984; Nahin, 1988), are localized in the area around the CC. Many of these substances contribute to neural transmission or descending modulation of nociceptive information.

Although the ascending projections from the lateral portion of lamina VII were not observed in this study, according to previous anatomical studies, there is a parallel ascending projection from the lateral aspect of lamina VII. Autonomic preganglionic nuclei processing sensory information and regulating autonomic function send axons peripherally providing autonomic control, but cells in these regions have also been shown to project to supraspinal levels such as PAG and hypothalamus (Burstein et al., 1990a,b; Vanderhorst et al., 1996). Comparisons suggest that there are fewer projection neurons localized in the lateral aspect of lamina VII of the spinal cord than in the area near the CC (Kevetter et al., 1982; Menétrey et al., 1982; Burstein et al., 1990a). In addition, only a sparse density of PHA-L-labeled fibers and terminals from the lateral portions of lamina VII is observed in the PAG, PB, and KF (Bernard et al., 1995).

Projections through the dorsal funiculus.—The results of the current study demonstrate that axons originating from PSDC neurons in the CC area travel ipsilaterally in the DC and project heavily to the DCN. These findings confirm previous anatomical (Hirshberg et al., 1996; Westlund et al., 1996) and electrophysiological (Al-Chaer et al., 1996b) studies demonstrating that there are a large number of PSDC neurons in the area adjacent to the CC that relay visceral nociceptive information to the DCN. These findings contrast with those of Giesler et al. (1984), who demonstrated fewer PSDC neurons in the area around the CC in the cervical and lumbar enlargements of the spinal cord following injections of HRP into the DCN. In the present study, however, tracer was injected at levels of the spinal cord heavily innervated by visceral afferents. To label this population of PSDC neurons better, other retrograde tracers, such as Fluoro-Gold, have been reported to be more sensitive than HRP (Burstein et al., 1990b). Results obtained in our laboratories (Christensen et al., 1996) have confirmed using Fluoro-Gold that large numbers of PSDC neurons are localized in the area around the CC at lumbosacral levels of the spinal cord.

As was mentioned above, fibers originating from neurons in the CC area at the midthoracic and lumbosacral levels of the spinal cord travel in the DC near the dorsal intermediate septum and midline, respectively. These findings indicate that ascending projections from PSDC neurons near the CC are somatoviscerotopically organized at the spinal cord level. The results are similar to those previously described for PSDC neurons in the spinal cord dorsal horn (Giesler et al., 1984; Cliffer and Giesler, 1989).

With regard to the terminals in the DCN, in some cases the results from this study show that projections from midthoracic segments of the spinal cord terminate ipsilaterally not only in the ventral aspect of Gr and the medial aspect of Cu but also in the dorsal aspect of Gr, whereas those from the lumbosacral level are localized in the dorsomedial and ventral aspects of Gr. Thus, together, projections from the CC area of the spinal cord form a shell around the Gr. These findings are consistent with those of an anterograde tracing study (Cliffer and Giesler, 1989) in which labeling was also seen in the ventral part of the Gr following injection of PHA-L into the dorsal horn of the cervical, thoracic, or lumbar cord.

Projections through the ventrolateral funiculi.—The present study demonstrates that neurons in the CC area project to many specific areas in the brainstem and forebrain through the ventrolateral funiculi of the spinal cord. Particularly, structures in the brainstem receive

most of the projections and terminations from neurons in the CC area. The projections and terminations are observed primarily in the caudal portion of the NTS, the ventral and medial pontine and medullary reticular formation (including the RMg, Rob, LRt, LPGi, and Gi), the noradrenergic cell regions (including the LC, SubC, A5, and KF), the PB, the caudal ventrolateral and lateral portions of the PAG, and the Cnf. These projections and terminations are generally bilateral in the medulla; however, the PAG receives predominantly contralateral projections. Forebrain structures also receive minor projections from neurons in the CC area. The regions innervated include the thalamus, zona incerta (ZI), hypothalamus, granular insular cortex, basal nucleus of Meynert, central nucleus of the amygdala (Ce), globus pallidus, and substantia innominata.

Many of the findings of the present study are consistent with results of previous anterograde and retrograde studies. Earlier studies of silver-stained degeneration (Lund and Webster, 1967; Mehler, 1969; Zemlan et al., 1978) or anterograde tract tracing studies with the use of WGA-HRP (Wiberg and Blomqvist, 1984; Wiberg et al., 1987; Yezierski, 1988) or PHA-L (Cliffer et al., 1991; Slugg and Light, 1994; Bernard et al., 1995; Feil and Herbert, 1995; Newman et al., 1996) reported that these areas received spinal inputs. Neurons in the area surrounding the spinal cord CC have been retrogradely labeled from the NTS, medullary and pontine reticular formation, PB, LC, PAG, thalamus, hypothalamus, and other forebrain structures (Giesler et al., 1979; Kevetter et al., 1982; Kevetter and Willis, 1982, 1983; Chaouch et al., 1983; Liu, 1983; Menétrey et al., 1982, 1983, 1992; Nahin et al., 1983, 1986; Peschanski and Besson, 1984; Nahin and Miceyvich, 1986; Menétrey and Basbaum, 1987; Leah et al., 1988; Hylden et al., 1989; Burstein et al., 1990a,b; Aston-Jones et al., 1991; Menétrey and de Pommery, 1991; Yezierski and Mendez, 1991; Burstein and Potrebic, 1993; Kitamura et al., 1993). In terms of efferent projections, the CC region contains not only neurons with projections to higher brain centers but also preganglionic neurons (Petras and Cummings, 1972; Hancock and Peveto, 1979). Previous literature reporting spinal projections to SPFPC, ZI, or Me5 could not be found.

Comparisons to the projections from lamina I neurons.—The pattern of projections from neurons in the CC area is different from the findings of previous anterograde transport studies of lamina I neurons of the spinal cord (Craig, 1991a,b, 1995), particularly at spinal and medullary levels. For example, the present study demonstrated that axons originating from neurons in the CC area travel in the dorsal column and in the ventrolateral funiculus of spinal cord bilaterally. Fiber terminations are concentrated in the ventral and medial reticular formation, including RMg, ROb, LRt, LPGi, and Gi. By contrast, projections from lamina I neurons are concentrated in the middle of the contralateral lateral funiculus of the spinal cord and terminate heavily in the ventral lateral portion of medullary reticular formation between the trigeminal nucleus caudalis and the LRt. In addition, the labeling from lamina I neurons decreases in the ventrolateral medulla (VLM) at the level between the LRt and facial nucleus and then intensifies in the rostral VLM, near the caudal aspect of the facial nucleus (Craig, 1995). However, this tendency is not observed in this study.

It is noteworthy that the present study demonstrates innervation of the raphe nuclei in the rostral medulla. Raphe nuclei, including both the raphe magnus nucleus and raphe obscurus

nucleus, receive direct afferent inputs from neurons in the CC area. Compared to the previous study describing lamina I projections (Craig, 1995), the present study demonstrates that a more prominent projection to the raphe nuclei and ventromedial reticular formation arises from neurons in the CC area relative to the sparse projection from lamina I neurons. The lamina I projection terminates most heavily in the ventrolateral reticular formation. Although the CC region is a key nociceptive and autonomic processing region, a significant direct spinal input to raphe nuclei has not been described previously (cf. Abols and Basbaum, 1981). Previously, it has been shown that the raphe nuclei receive projections primarily from supraspinal structures, such as the PAG, hypothalamus, and reticular formation (Holstege, 1987; Hermann et al., 1997).

The present study shows that the fiber projections to catecholamine cell regions from neurons in the CC area are generally bilateral. The labeling is concentrated in the caudal half of the LC and KF. These findings are similar to the projections from lamina I neurons (Craig, 1992, 1995; Westlund and Craig, 1996). However, the pattern of projections from lamina I neurons to the LC, SubC, and KF appears different from that from neurons in the CC area. 1) Axons originating from spinal lamina I neurons terminate in the LC and SubC bilaterally with a strong contralateral predominance. However, in the present study, a strong contralateral predominance is not observed. 2) Axons of lamina I neurons enter the LC from the ventrolateral or dorsolateral pontine tegmentum and the continuation of fibers from the LC terminate in the SubC. However, in the present study, the projections from neurons in the CC area continue rostrally from the RVM and terminate in the SubC regions. Labeled fibers in the SubC regions continue dorsally into the LC and the pericoerulear region from the medial side.

The labeled fibers in the PAG are primarily localized in the caudal portions of the ventral and ventrolateral PAG in the current study. The pattern of distribution of terminations originating from the neurons in the CC area is similar to that from lamina I neurons in the cat and monkey (Craig, 1995). Unlike the case with the observations by Craig (1995), in which a clear topographic organization existed in the lateral column of PAG of cats and monkeys, no topographic organization is observed in this area in the present study for the rat. However, the possibility of the presence of such a topographic organization in the projections from the neurons in the CC area could not be ruled out, because restricted injections were made in a relatively small population of experimental animals.

For the thalamus, the present study revealed labeled fibers in the parvicellular part of subparafascicular nucleus (SPFPC), the peripeduncular thalamic nucleus (PP), and the posterior intralaminar nucleus (PIL). Unlike the case with the termination from lamina I neurons (Craig, 1991b), no projections from neurons in the CC area to the thalamus were found in the ventrobasal complex and submedius nucleus of the thalamus.

Functional considerations

The CC ascending projections appear to provide afferent information from the spinal cord to many areas in the brain involved in processing and transmission of sensory information, autonomic and homeostatic regulation, and emotional control. In the following sections, the functional characteristics of neurons near the CC area and CC projections will be described.

Neurons in the area near the CC.—Neurons in the gray matter surrounding the CC receive inputs from cutaneous Aδ mechanical nociceptors and unmyelinated visceral afferent fibers that are activated by noxious stimuli (Light and Perl, 1979; Sugiura et al., 1989). Neurons in the area adjacent to the CC have been shown to respond to both visceral and somatic input (Honda, 1985; Honda and Perl, 1985; Ness and Gebhart, 1987, 1989; Berkley et al., 1993; Al-Chaer et al., 1996b). Neurons in this region may respond to innocuous stimuli, to noxious stimuli, and to both in rats and cats. In contrast, Nahin et al. (1983) reported that all of the neurons identified in this area responded exclusively to noxious stimuli. Al-Chaer et al. (1996b) demonstrated that a population of PSDC neurons located in and around the CC area projects to the DCN and that these PSDC neurons respond to innocuous and noxious cutaneous mechanical stimuli and to mechanical and chemical noxious visceral stimuli.

Integration of sensory information.—The results of the present study show that neurons in the CC area project to higher brain centers involved in processing and transmission of sensory information (including nociception). Importantly, this study provides conclusive anatomical evidence in support of previous electrophysiological studies by (Al-Chaer et al., 1996a,b), which demonstrated that a population of PSDC neurons localized near the central canal is excited by noxious colonic visceral stimulation. The visceral nociceptive information is conveyed by way of PSDC neurons in the medial area of the lumbosacral spinal cord level near the CC to the Gr, which in turn activates neurons in the VPL nucleus of thalamus. Transmission of pelvic visceral nociceptive information can be nearly eliminated by a midline lesion of the DC at the T10 level in rats (Al-Chaer et al., 1996a,b) or at the T8 or T10 level in humans (Hirshberg et al., 1996; Nauta et al., 1997). The conclusion of previous studies from our laboratories is that the DC pathway involving PSDC neurons is more important for pelvic visceral nociceptive transmission than are pathways in the ventrolateral funiculus of the spinal cord, such as the spinothalamic tract. In contrast, pathways in the ventrolateral funiculus are more important for transmitting somatic nociceptive information. In addition, DC lesions in the upper cervical spinal cord extending beyond the dorsal intermediate septum can reverse the home-cage behavior following the induction of pancreatitis in rats (Houghton et al., 1997a) and can block the behavioral responses and nociceptive transmission from duodenal distention (Feng et al., 1998) and chemical stimulation of pancreatic afferent fibers (Houghton et al., 1997b; Wang et al., 1998). Unlike the study by Al-Chaer et al. (1996b), which demonstrated neurons in the dorsomedial part of Gr that could be excited by colonic nociceptive stimuli, the present study shows that fibers from lumbosacral spinal cord project not only to the dorsomedial aspect but also to the ventral portion of the Gr. Therefore, these findings suggest that the visceral input to the DCN, from projection neurons in the CC area, may be involved in a variety of functions.

In previous electrophysiological studies, nociceptive responses have been recorded in the posterior thalamic group (for review see Albe-Fessard et al., 1985; Poggio and Mountcastle, 1960; Carstens and Yokota, 1980; Guilbaud et al., 1980; Peschanski et al., 1981), the intralaminar thalamic nuclei (Berkley et al., 1995), the medial thalamic nuclei (Dostrovsky and Guilbaud, 1990), the SPFPC (Dong et al., 1978), and the lateral habenular nucleus

(Benabid and Jeaugey, 1989). Most of the neurons in the intralaminar nuclei respond exclusively to noxious somatic and visceral stimuli (Peschanski et al., 1981; Berkley et al., 1995). In addition, neurons in the intralaminar thalamic nuclei respond exclusively to noxious, but not to weak, mechanical stimuli (Perl, 1984). For conscious human subjects, it has been reported that electrical stimulation of the intralaminar thalamic nuclei causes feelings of burning pain (Perl, 1984; Lenz and Dougherty, 1997).

Previous investigators (for references see Burstein et al., 1990a) have shown that neurons in the hypothalamus respond to a variety of somatosensory (including mechanical, thermal, and visceral) stimuli. Neurons in the dorsal horn of the lumbosacral spinal cord have been shown to respond to both noxious somatic and visceral stimuli (Burstein et al., 1991; Katter et al., 1996b). However, the SHT neurons near the CC have not been well characterized.

Projections from the CC area to the LRt, rostral ventral and medial reticular formation (including the RMg, Rob, and Gi), A5 region, LC, KF, and PAG are also observed in the present study. Previous studies have shown that electrical or chemical stimulation of these areas can produce antinociceptive effects (Willis et al., 1977; Basbaum and Fields, 1978; Haber et al., 1980; Tattersall et al., 1986b; Hodge et al., 1986; Yaksh, 1986; Janss et al., 1987; Jones and Gebhart, 1988; Zhao and Duggan 1988; Burnett and Gebhart, 1991; Miller and Proudfit, 1991; Proudfit, 1992; Yeomans et al., 1992; Yeomans and Proudfit, 1992) through known descending projections (Westlund and Coulter, 1980; Kuypers and Martin, 1982).

Autonomic regulation.—This study provides evidence that projections from neurons in the CC area are localized in areas such as NTS, LRt, A5 region, LC, SubC, KF, PB, PAG, ZI, hypothalamus, Ce, and GI, which are responsible for autonomic (cardiovascular, respiratory, micturition, and sexual) regulation. These areas involving autonomic regulation have been discussed in other comprehensive articles (Fulwiler and Saper, 1984; Cechetto and Saper, 1990; Loewy and Spyer, 1990; Depaulis and Bandler, 1991; Proudfit, 1992; Craig, 1995). Neurons in these areas have been shown to respond to somatosensory (including noxious) stimuli (Kruger and Albe-Fessard, 1960; Eickhoff et al., 1978; Rose, 1979; Gelsema et al., 1989; Bernard and Besson, 1990; Bernard et al., 1994; Hubscher and Berkley, 1994; Bester et al., 1995; Willis et al., 1997). Fos protein can be induced in the NTS, LC, SubC, KF, lateral PB, PAG, and hypothalamus following noxious cutaneous (Bullitt, 1990; Keay et al., 1994; Buritova et al., 1998) or visceral chemical (Hammond et al., 1992; Bon et al., 1996, 1997) or mechanical stimuli (Lantéri-Minet et al., 1993; Traub et al., 1996a,b). In addition, electrical or chemical stimulation in the medullary reticular formation, NTS, A5 region, LC, PB, PAG, ZI, Ce, and hypothalamus produces changes in cardiovascular reflexes (Loewy et al., 1979a, 1986; Cox et al., 1987; Iwata et al., 1987; Lovick, 1985; Sved and Felsten, 1987; Randich et al., 1988; Spencer et al., 1988, 1989; Bonham and Jeske, 1989; Chamberlin and Saper, 1992) and respiration (Feldman and Ellenberger, 1988; Motekaitis et al., 1994). Furthermore, Strack et al. (1989) demonstrated that neurons in the A5 region, ventromedial medulla, caudal raphe nuclei, and PVH send descending projections to innervate the sympathetic preganglionic neurons in the intermediolateral cell column (IML) involved in cardiovascular control. Therefore, cardiovascular and respiratory response patterns might be influenced or elicited by somatic

and visceral input transmitted through ascending projections from neurons in the CC area of the thoracic spinal cord.

The brainstem projections from neurons near the CC area at lumbosacral levels of the spinal cord are likely related to autonomic regulation of pelvic organs. Brainstem control of eliminative and sexual functions has been reviewed (Morrison, 1987; de Groat and Steers, 1990). In the present study, it is noteworthy that Barrington's nucleus, an area involved in micturition control, receives ascending projections only from neurons in the CC area at lumbosacral levels of the spinal cord. Anterograde tracing studies in the rat (Loewy et al., 1979b), cat (Holstege et al., 1979, 1986), and monkey (Westlund and Coulter, 1980) have shown that neurons in Barrington's nucleus project directly to the sacral intermediolateral cell group, which contains the parasympathetic preganglionic neurons innervating the bladder. In addition, neurons in the CC area at lumbosacral levels project to the RMg, parapyramidal reticular formation, A5 and A7 region, LC, SubC, PAG, and hypothalamus. These findings are consistent with those from other retrograde transneuronal transport studies showing direct brainstem projections to parasympathetic preganglionic neurons in the sacral spinal cord. These transneuronal tracing studies with injections of pseudorabies virus into the rat urinary bladder and urethra and into the rat penis and clitoris have shown that these areas are involved in micturition reflexes and sexual functions (Nadelhaft et al., 1992; Marson et al., 1993; Marson, 1995; Vizzard et al., 1995).

Motor control and emotional expression.—The results of the present study also demonstrate projections from neurons in the CC area to areas involved in motor control, such as the LRt, R, and basal ganglia. The somatotopic relationships detailed previously in the literature for the LRt were evident in these studies (for review see Ruigrok and Cella, 1995). The LRt, R, and basal ganglia have been suggested to play a role in processing of sensory information related to motor functions (for reviews see Lidsky et al., 1985; Ruigrok and Cella, 1995). Therefore, the direct spinal projections to the basal ganglia, demonstrated in the present study, may provide a route for sensory input, including nociceptive information, to this region for modulation of motor functions.

Surprisingly, labeled fibers are seen forming baskets of terminations encasing cells in the mesencephalic trigeminal nucleus (Me5), the cell bodies of proprioceptors involved in reflexive jaw movements (Linden, 1978). The data presented here showing projections from neurons around the central canal area to the Me5 suggest that this direct projection may provide input from the spinal cord for jaw tensing, teeth baring, or exclamatory jaw opening.

Limbic activation.—The present study determined that the area around the CC sends direct projections to limbic structures, such as Ce, SI, and BM. The amygdala, particularly the Ce, is involved in the affective-emotional and behavioral responses to noxious events (Burstein and Potrebic, 1993). Previous studies have shown involvement of Ce in fear and emotional memory and behavior (for references see Bernard et al., 1996). Activity of neurons in the Ce can be recorded after noxious stimuli are applied (Bernard et al., 1990, 1992). Colorectal distention results in an increase in expression of Fos protein in this structure (Traub et al., 1996b). Decreased regional cerebral blood flow is observed in the amygdala region after noxious stimuli are applied in healthy humans (Derbyshire et al.,

1997). Thus, in combining the results of the current study with those of previous studies, it appears that the Ce plays an important role in affective-emotional and behavioral responses to noxious events.

CONCLUSIONS

The results of the present study clearly show that the ascending pathways originating from the neurons around the central canal project to a variety of specific structures involved in integration of sensory information, homeostatic regulation, motor control, emotional expression, and limbic activation. These findings are consistent with the hypothesis that projections of neurons around the central canal play a role in relaying sensory information, particularly visceral nociception, from the internal and external environment to the central nervous system. Regions innervated include sensory, emotional, motor, and autonomic integration sites as well as limbic regions. In particular, the new ascending pathway transmitting visceral nociceptive information via PSDC neurons in the CC area through the DC is clearly evident in this study. Thus, the present findings are strongly in support of recent clinical studies (Hirshberg et al., 1996; Nauta et al., 1997) and experimental studies (Al-Chaer et al., 1996b; Houghton et al., 1997; Feng et al., 1998) that have postulated the existence of such a pathway.

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Abbreviations

3V	third ventricle
4V	fourth ventricle
7	facial nucleus
7n	facial nerve
AH	anterior hypothalamic area
AP	area postrema
APT	anterior pretectal nucleus
Aq	cerebral aqueduct
AVVL	anterolateral thalamic nucleus, ventrolateral part
BM	basal nucleus of Meynert
Bar	Barrington’s nucleus
CC	area around the spinal cord central canal
Ce	central nucleus of amygdala

CL	centrolateral thalamic nucleus
CM	central medial thalamic nucleus
CnF	cuneiform nucleus
cp	cerebral peduncle
CPU	caudate putamen
Cu	cuneate nucleus
DA	dorsal hypothalamic area
DC	dorsal column
DCN	dorsal column nucleus
DpMe	deep mesencephalic nucleus
ec	external capsule
fr	fasciculus retroflexus
GI	granular insular cortex
Gi	gigantocullular reticular nucleus
GP	globus pallidus
Gr	gracile nucleus
Hb	habenular complex
Hypo	hypothalamus
icp	inferior cerebellar peduncle
InC	intercollicular nucleus
IO	inferior olivary nucleus
KF	Kölliker-Fuse nucleus
LC	locus coeruleus
LD	laterodorsal thalamic nucleus
LH	lateral hypothalamic area
LHb	lateral habenular nucleus
LPGi	lateral paragigantocellular nucleus
LPO	lateral preoptic area
LRt	lateral reticular nucleus

LSO	lateral superior olivary nucleus
MD	mediodorsal thalamic nucleus
ml	medial lemniscus
Mo5	motor trigeminal nucleus
MPA	medial preoptic area
NTS	nucleus of solitary tract
PAG	periaqueductal gray
PB	parabrachial nucleus
PBcl	central lateral parabrachial subnucleus
PBdl	dorsal lateral parabrachial subnucleus
PBel	external lateral parabrachial subnucleus
PBil	internal lateral parabrachial subnucleus
PBm	medial parabrachial subnucleus
PBsl	superior lateral parabrachial subnucleus
PBvl	ventral lateral parabrachial subnucleus
PF	parafascicular thalamic nucleus
PH	posterior hypothalamic area
PHA-L	*Phaseolus vulgaris* leucoagglutinin
PIL	posterior intralaminar thalamic nucleus
Pn	pontine reticular nucleus
Po	posterior thalamic nuclear group
PP	peripeduncular nucleus
PR	prerubral field
PrC	precommissural nucleus
PSDC	postsynaptic dorsal column
PV	paraventricular nucleus of thalamus
PVH	paraventricular hypothalamic nucleus
py	pyramidal tract
R	red nucleus

Re	reuniens thalamic nucleus
RI	rostral interstitial nucleus of medial longitudinal fasciculus
RMg	raphe magnus nucleus
Rob	raphe obscurus nucleus
RVM	rostral ventral medulla
SC	superior colliculus
scp	superior cerebellar peduncle
SHT	spinohypothalamic tract
SI	substantia innominata
SMT	spinomesencephalic tract
SNR	substantia nigra pars reticulata
sox	supraoptic decussation
Sp5	spinal trigeminal nucleus
sp5	spinal trigeminal tract
Sp5C	trigeminal nucleus caudalis
SPFPC	subparafascicular thalamic nucleus, parvicellular part
SRD	subnucleus reticularis dorsalis
SRT	spinoreticular tract
SRV	subnucleus reticularis ventralis
SST	spinosolitary tract
STT	spinothalamic tract
SubC	subcoerulear region
SUM	supramammillary nucleus
TC	tuber cinereum
VB	ventrobasal complex of thalamus
VL	ventrolateral thalamic nucleus
VLM	ventrolateral medulla
VM	ventromedial thalamic nucleus
VMH	ventromedial hypothalamic nucleus

VPL	ventral posterolateral thalamic nucleus
VPM	ventral posteromedial thalamic nucleus
VPPC	ventral posterior thalamic nucleus, parvicellular part
xscp	decussation of the superior cerebellar peduncle
ZI	zona incerta

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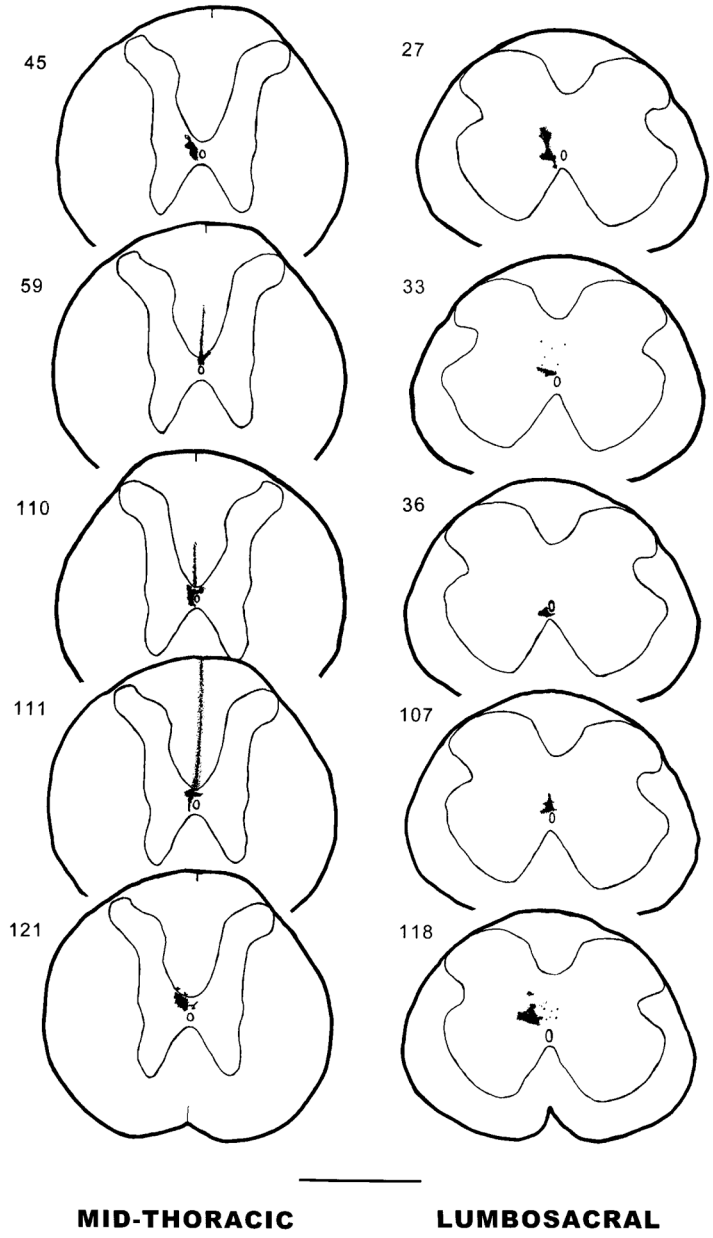


Fig. 1.

Camera lucida drawings of coronal sections of *Phaseolus vulgaris*-leucoagglutinin (PHA-L) injections into the area around the spinal cord central canal at midthoracic (T6–T9) or lumbosacral (L6–S1) levels of spinal cord. The numbers at left refer to the experimental animal number. Scale bar = 1 mm.

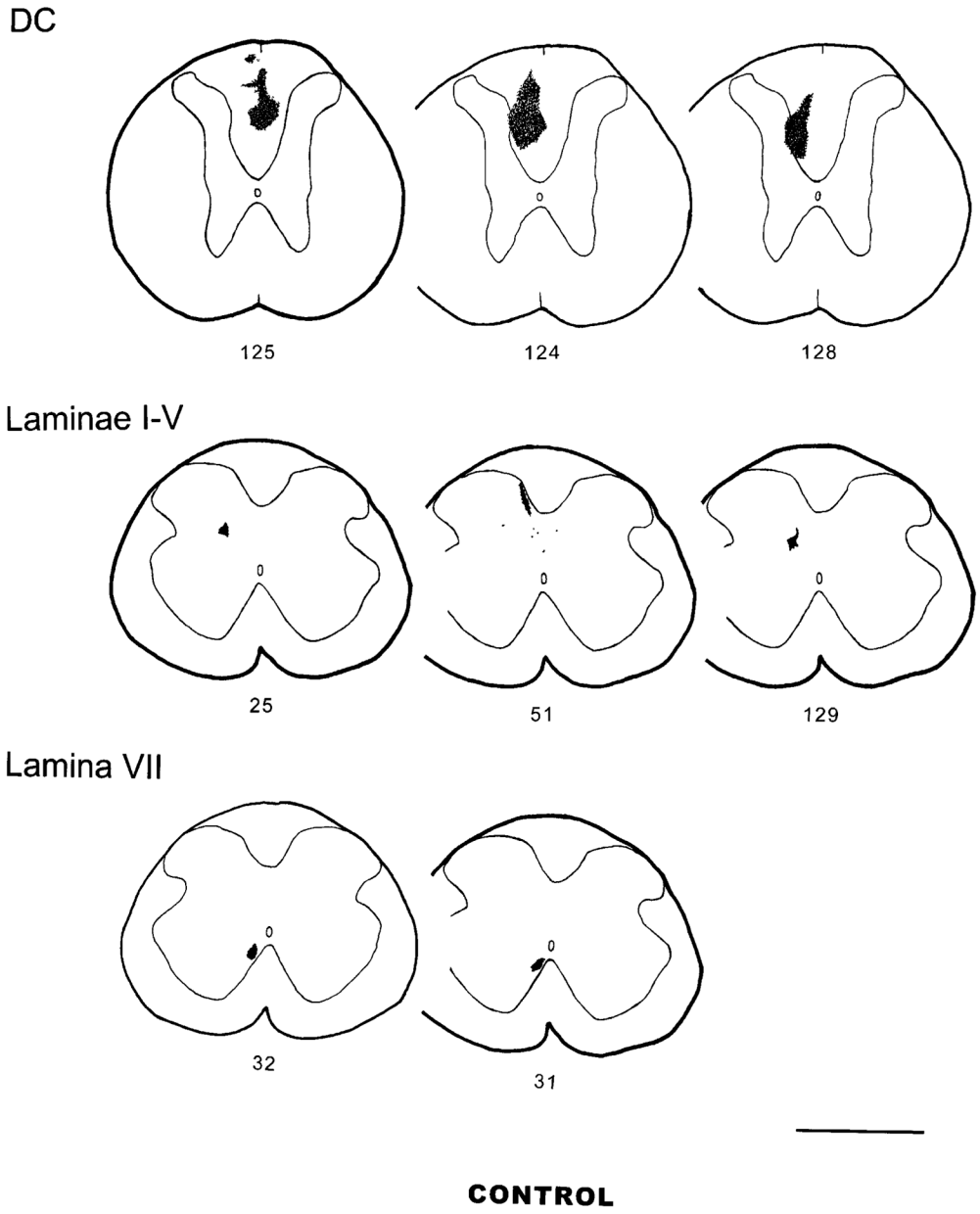
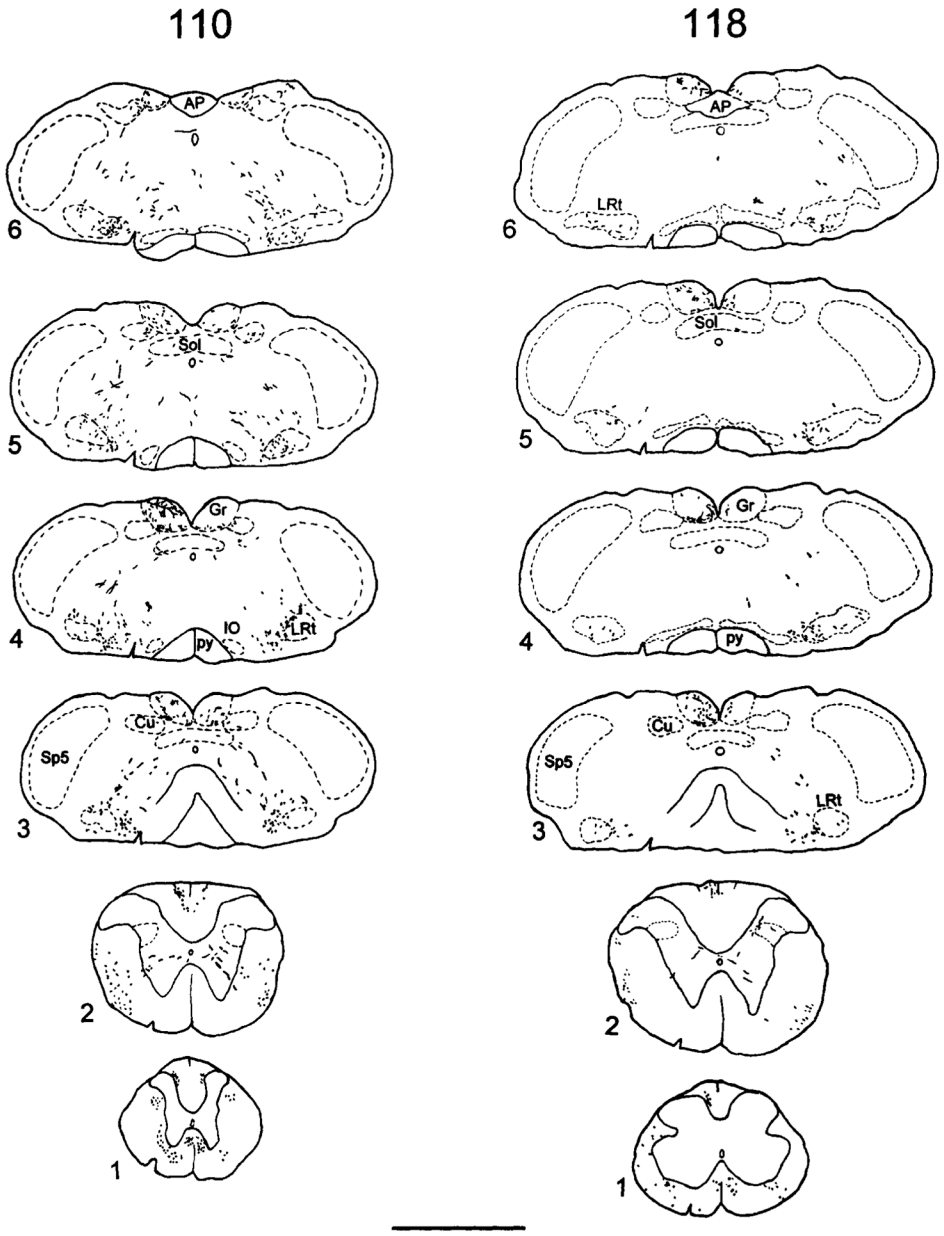
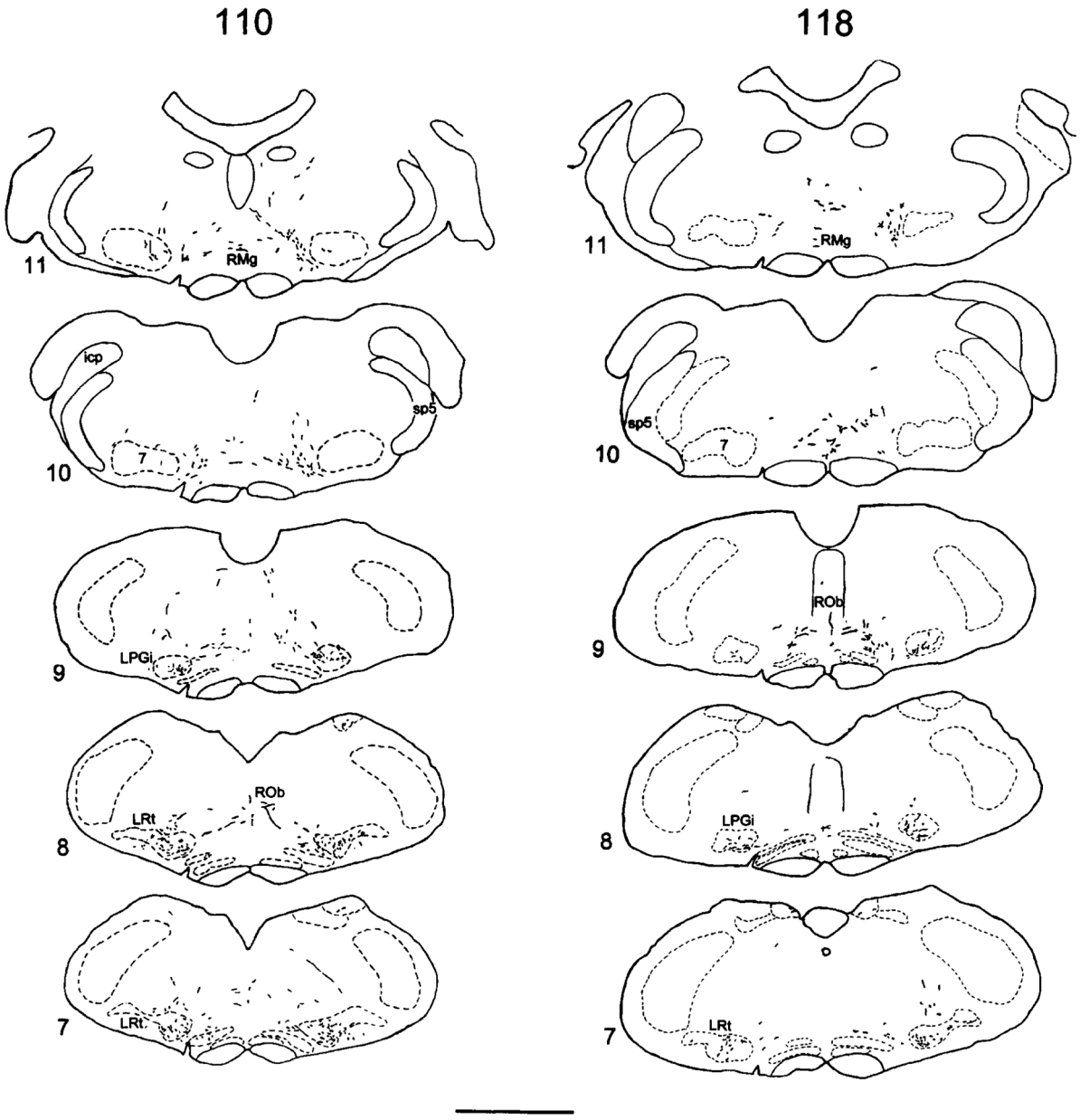
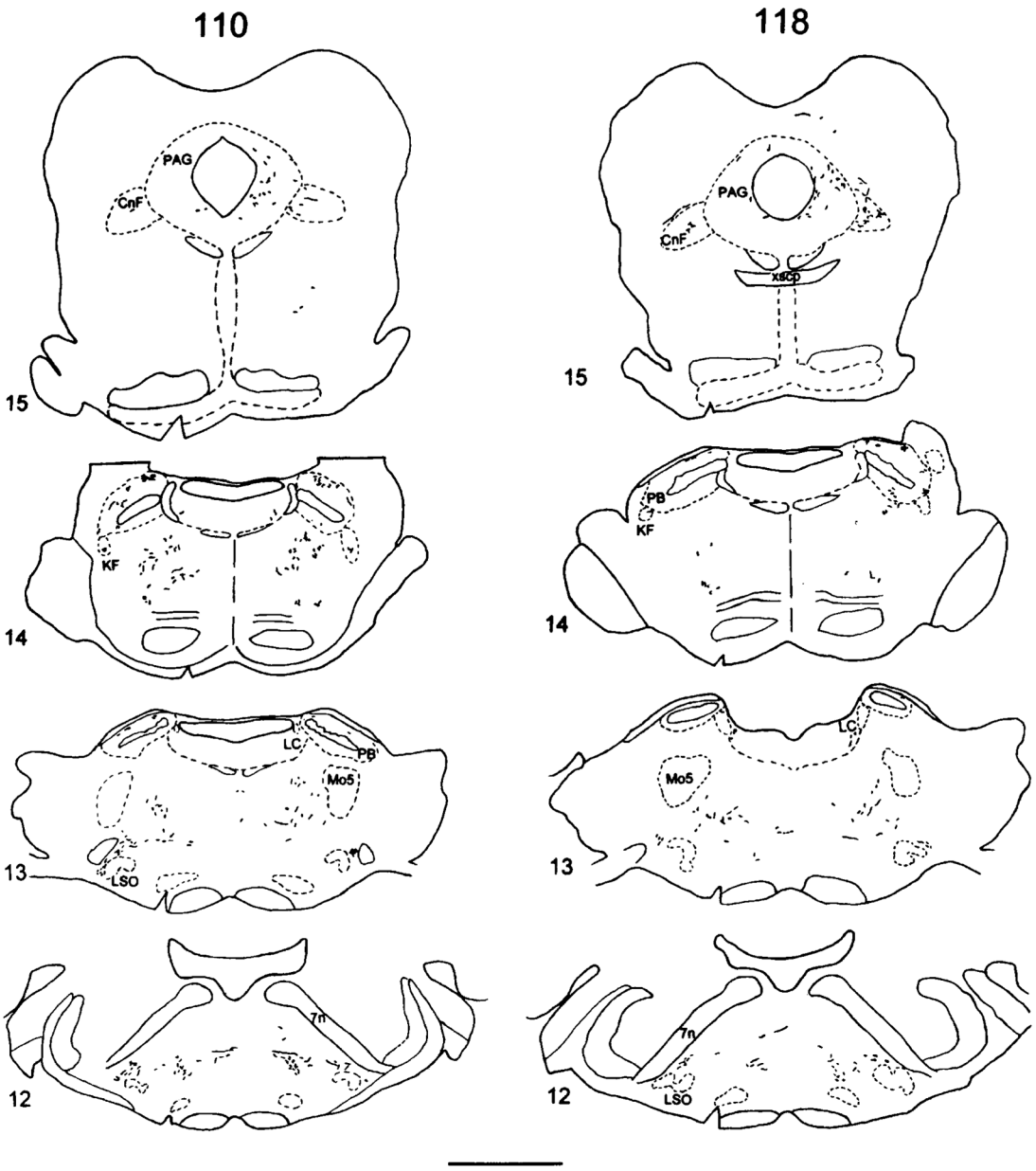


Fig. 2.
PHA-L control injection sites in the DC, laminae I–V, and VII (and VIII). Scale bar = 1 mm.







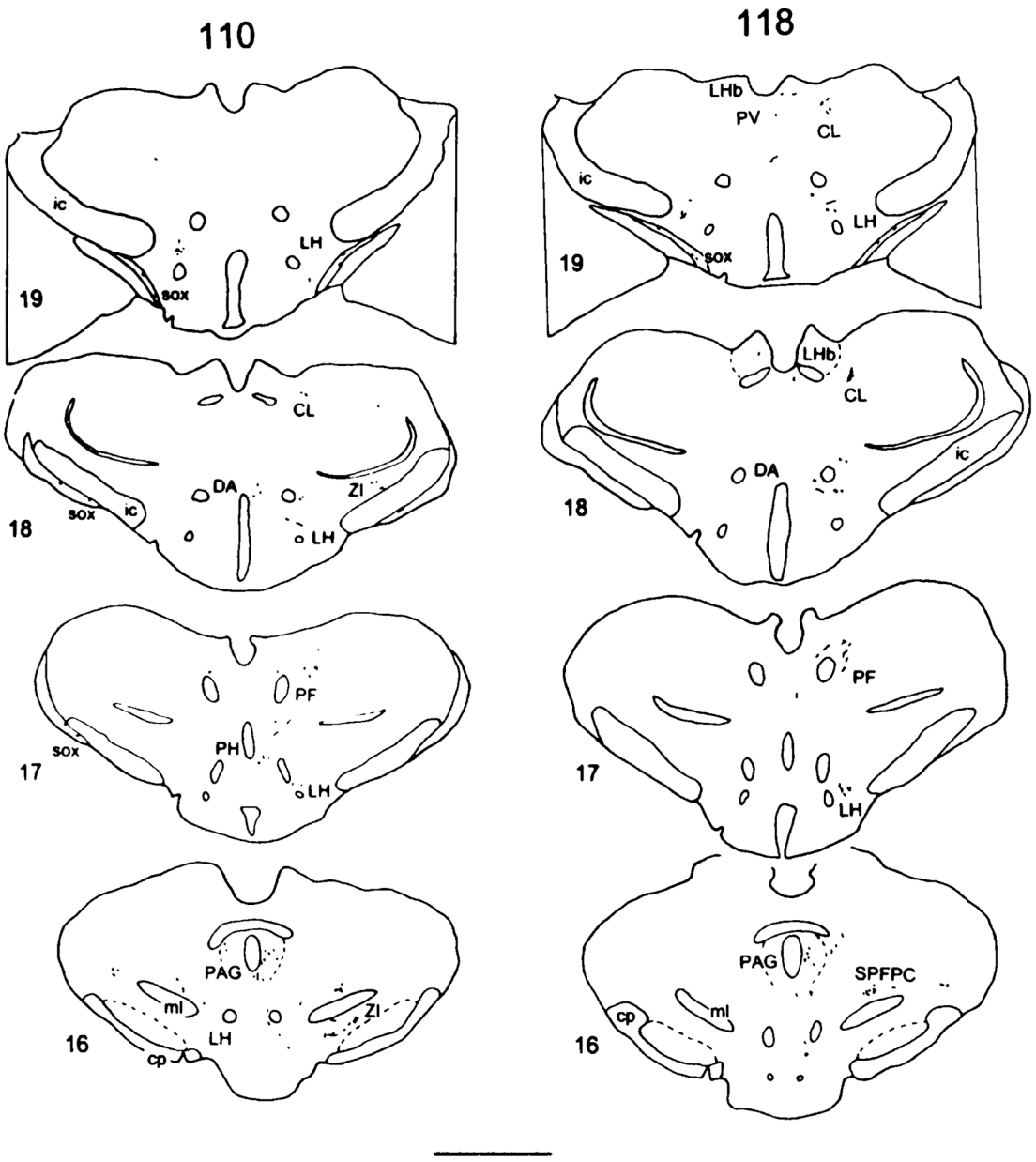


Fig. 3.

Camera lucida drawings of PHA-L-labeled fibers in a series of single transverse sections from experiment 110 in the midthoracic injection group and from experiment 118 in the lumbosacral injection group. Examples from a level several sections rostral to the injection site and sections from the C1 level of spinal cord through the level of the diencephalon. The sequence is numbered from caudal to rostral. The left side is ipsilateral to the injection site. Cytoarchitectonic delineations are based on adjacent Nissl-stained sections. The representative tissue sections from experimental animals 110 and 118 are referred to in the text by the numbers on their left. The sections are numbered sequentially from the spinal cord through the diencephalon. For abbreviations, see list. Scale bars = 2 mm.

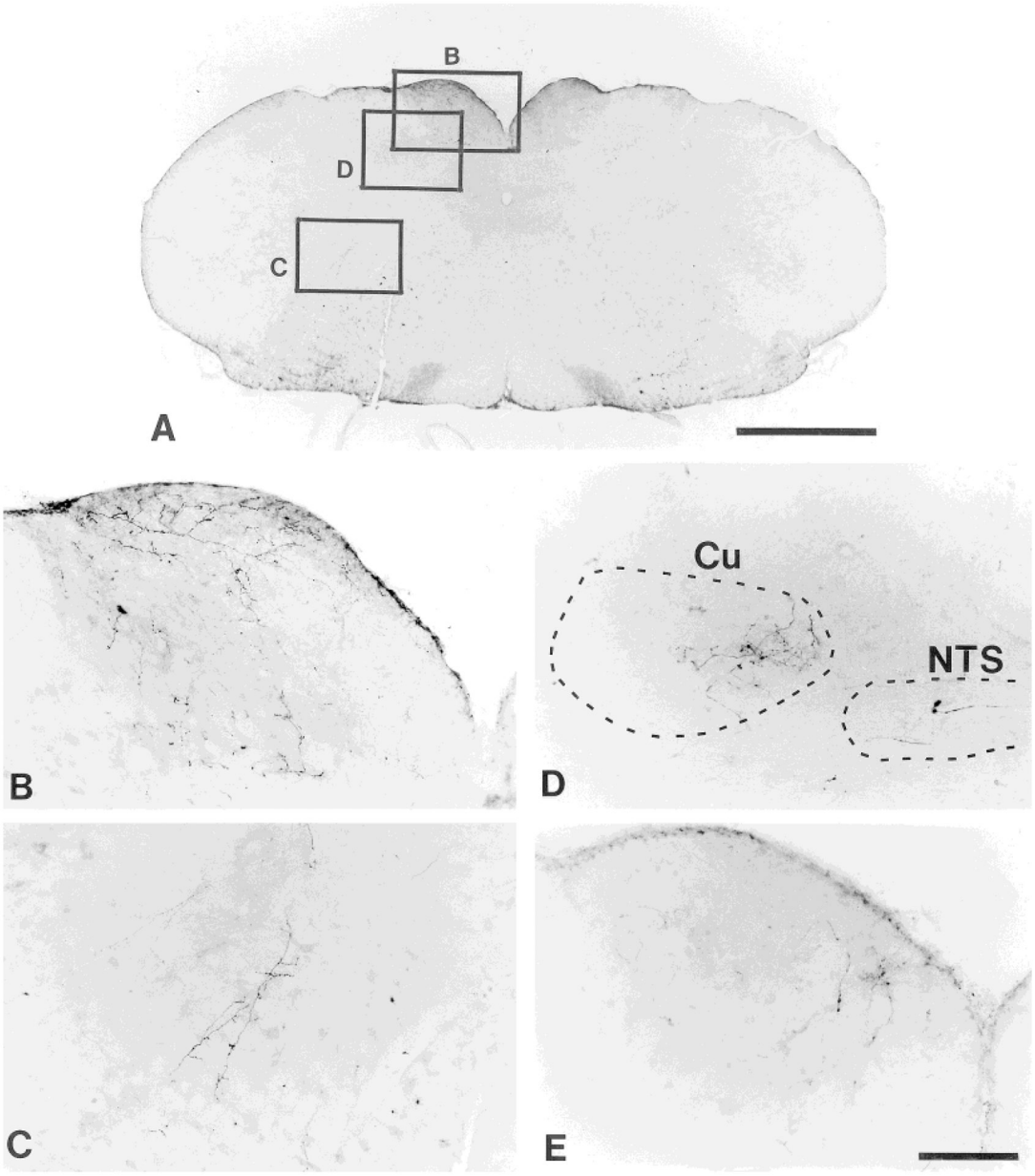


Fig. 4.

Photomicrographs illustrating PHA-L-labeled fibers and terminals in a transverse section at the caudal medulla level as shown in **A** from a midthoracic injection animal (experiment 110; from the section in level 4, Fig. 3). The labeling shown in the gracile nucleus (**B**) and the medullary reticular formation (**C**) is from the sites marked in A. **D:** The labeling in the medial aspect of the cuneate nucleus and the nucleus of solitary tract is from another midthoracic injection animal (experiment 45). The labeled fibers and terminals in the dorsomedial portion of the gracile nucleus shown in **E** are from experiment 27 in lumbosacral injection group. Dorsal is up, ipsilateral is left. Scale bars = 1 mm for A; 100 μm for B–E.

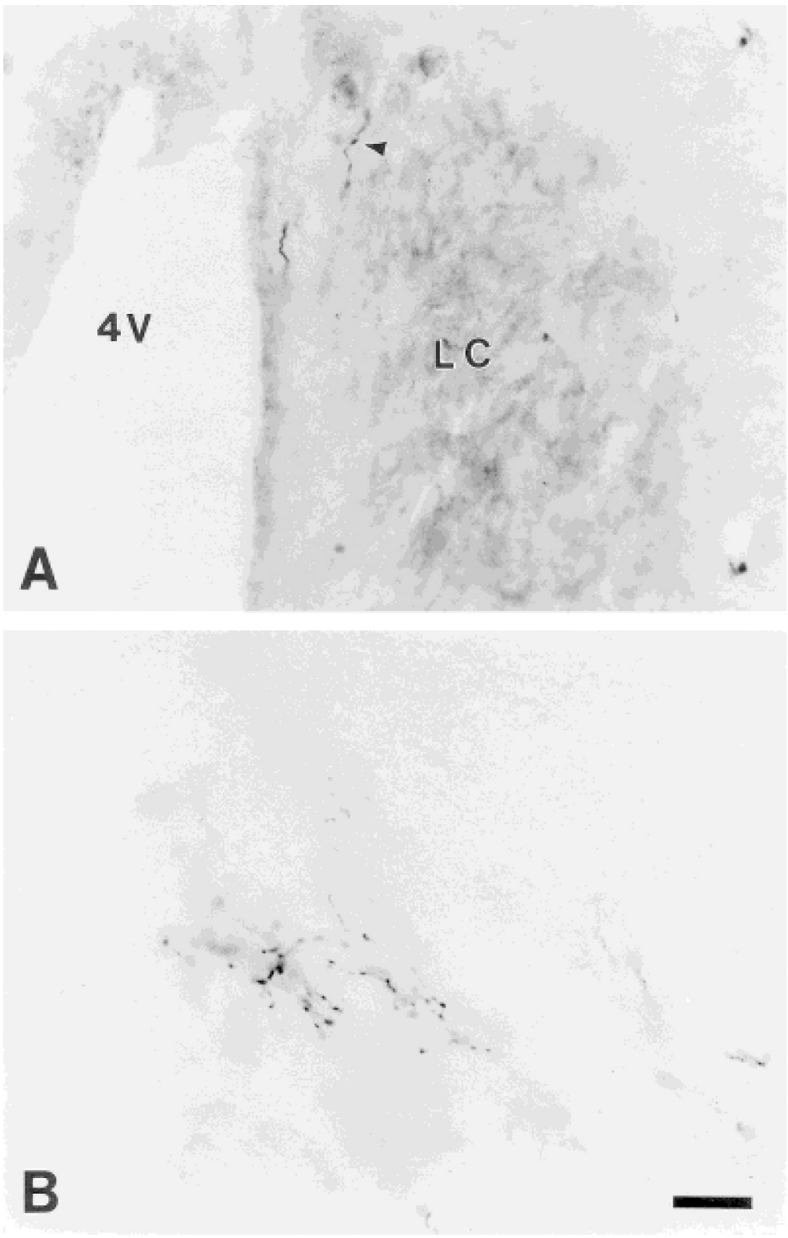


Fig. 5.

Photomicrographs of labeled fibers and terminations from neurons in the area adjacent to the spinal cord central canal in the contralateral locus coeruleus (experiment 36; **A**) and ventrolateral pons (A5 region; experiment 110; **B**; from the section in level 13 of Fig. 3). In A, the arrowhead indicates a PHA-L-labeled fiber located just medial to the locus coeruleus. Scale bar = 25 μm.

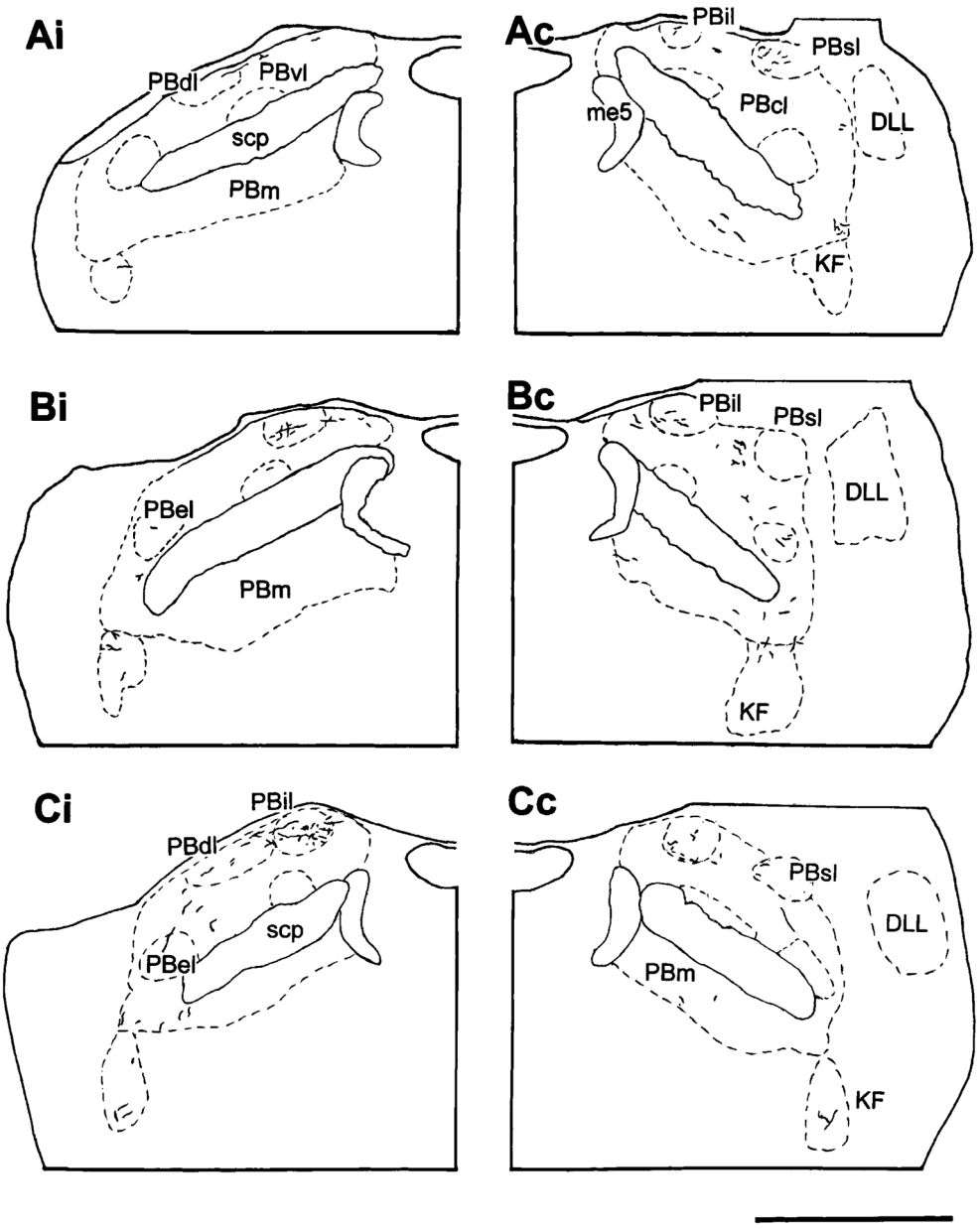


Fig. 6.

Camera lucida drawings illustrating the labeling in the ipsilateral (i) and contralateral (c) parabrachial nuclei and the Kölliker-Fuse nucleus near the pontomesencephalic junction following an injection in the area surrounding the spinal cord central canal from experiment 110 (Ai-Bi; midthoracic) and 118 (Ci; lumbosacral). The distance between Ai and Bi is 200 μm. For abbreviations, see list. Scale bar = 1 mm.

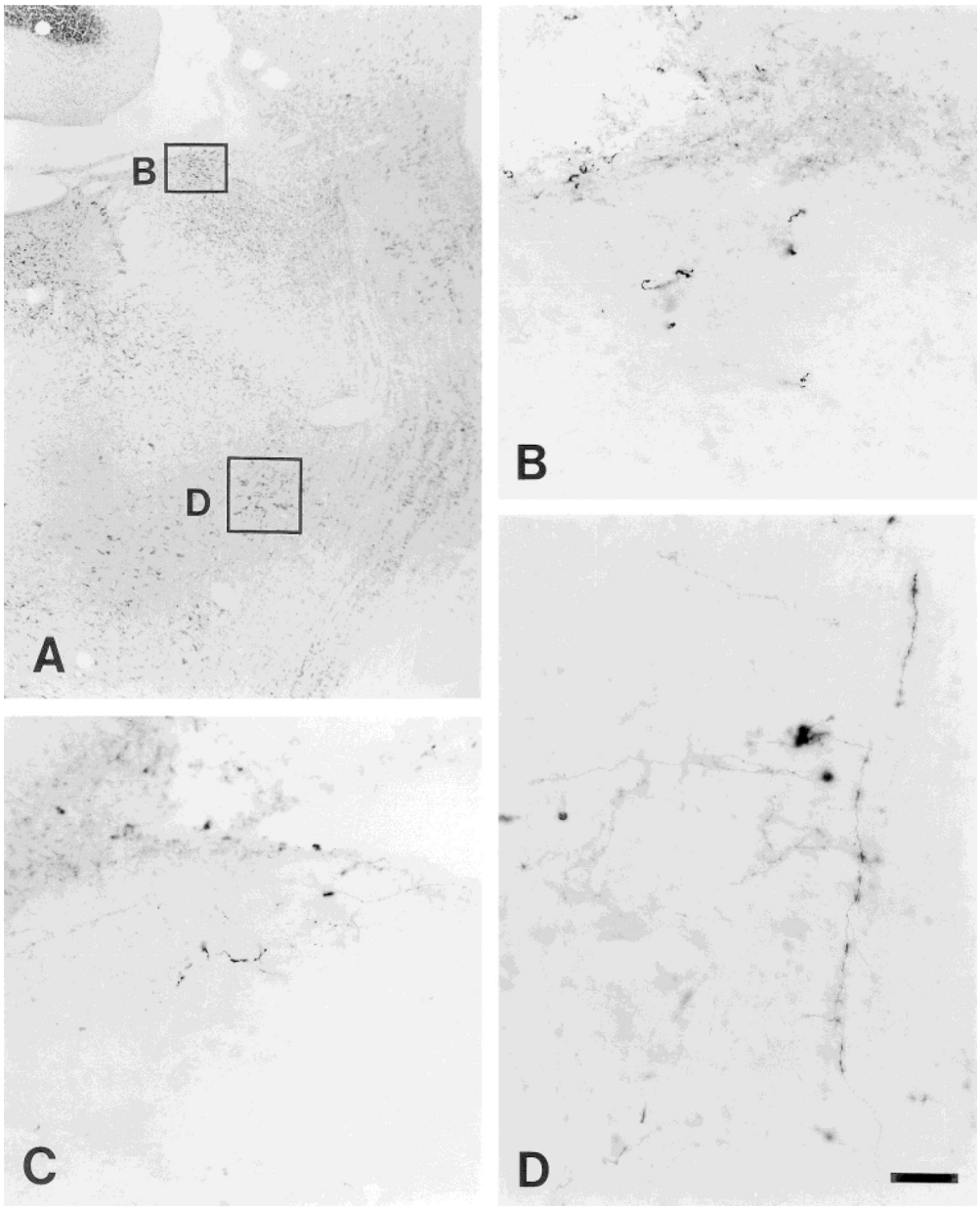


Fig. 7.

Photomicrographs showing the PHA-L labeling in the internal lateral parabrachial subnuclei ipsilateral (**B**) and contralateral (**C**) to the injection site in experiment 110 and in the Kölliker-Fuse nucleus (**D**) in experiment 27. The labeling shown in B and D is from the sites marked in the adjacent transverse Nissl-stain section shown in **A**. Scale bar = 250 μm for A; 50 μm for B–D.

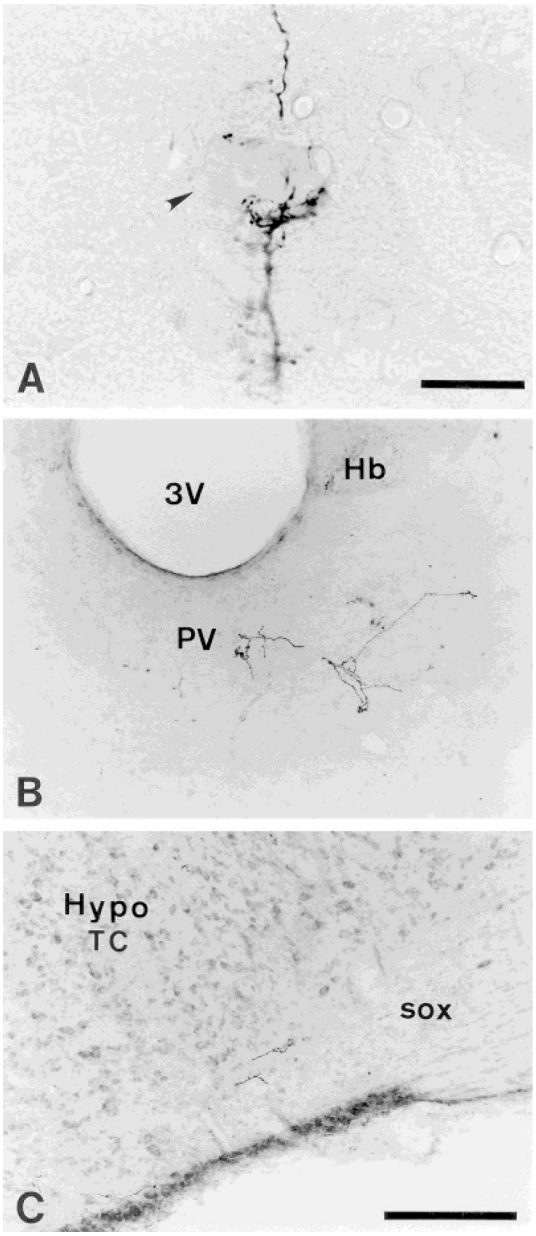


Fig. 8.

Labeled fibers and terminals in transverse sections from the neurons in the area around the central canal in experiment 107 (lumbosacral) are seen in the mesencephalic trigeminal nucleus at the periaqueductal gray level (**A**), the paraventricular thalamic nucleus (**B**), and the supraoptic decussation (**C**). B: Labeled terminal fibers below the habenular complex are localized in the mediodorsal thalamic nucleus. Arrowhead in A is the mesencephalic trigeminal neuron. For abbreviations, see list. Scale bars = 25 μm for A; 200 μm for B,C.

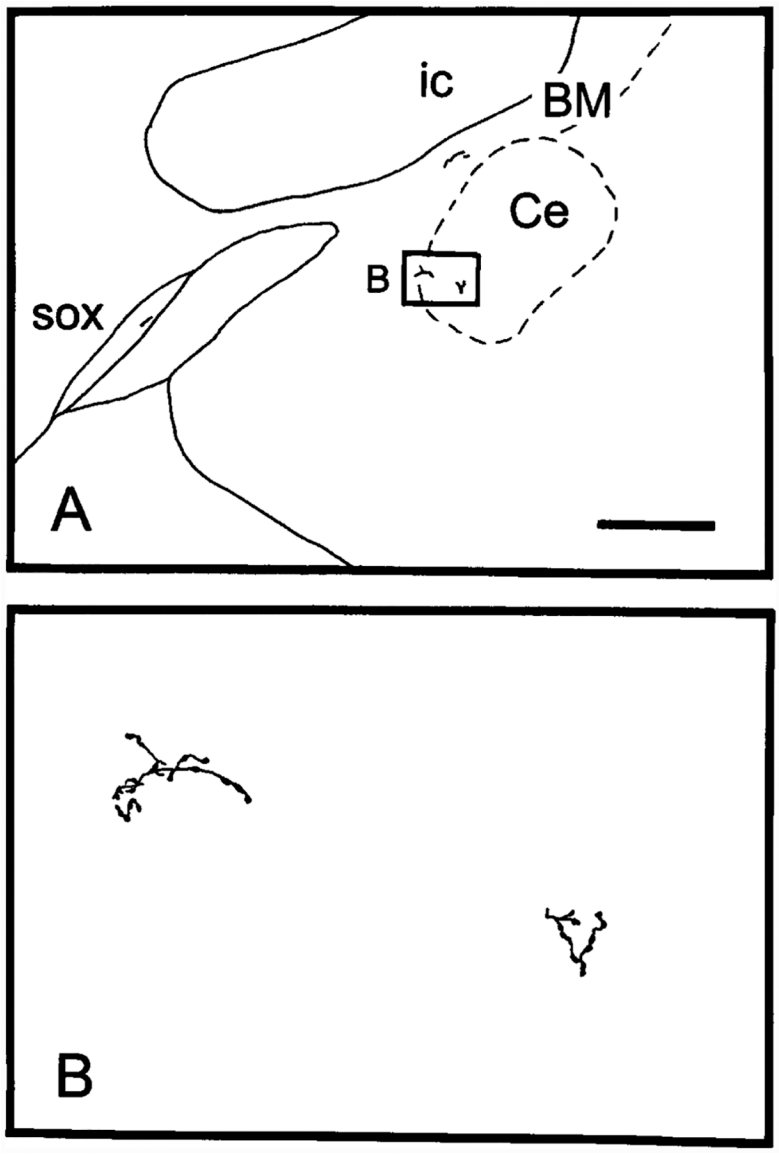


Fig. 9.

A: Camera lucida drawing illustrating PHA-L labeling in the central nucleus of the amygdala, the basal nucleus of Meynert, and the supraoptic decussation (experiment 107), contralateral to the injection site. **B:** Higher magnification of a camera lucida drawing illustrating PHA-L-labeled axons and varicosities in the central nucleus of amygdala. For abbreviations, see list. Scale bar = 500 μm for A; 50 μm for B.

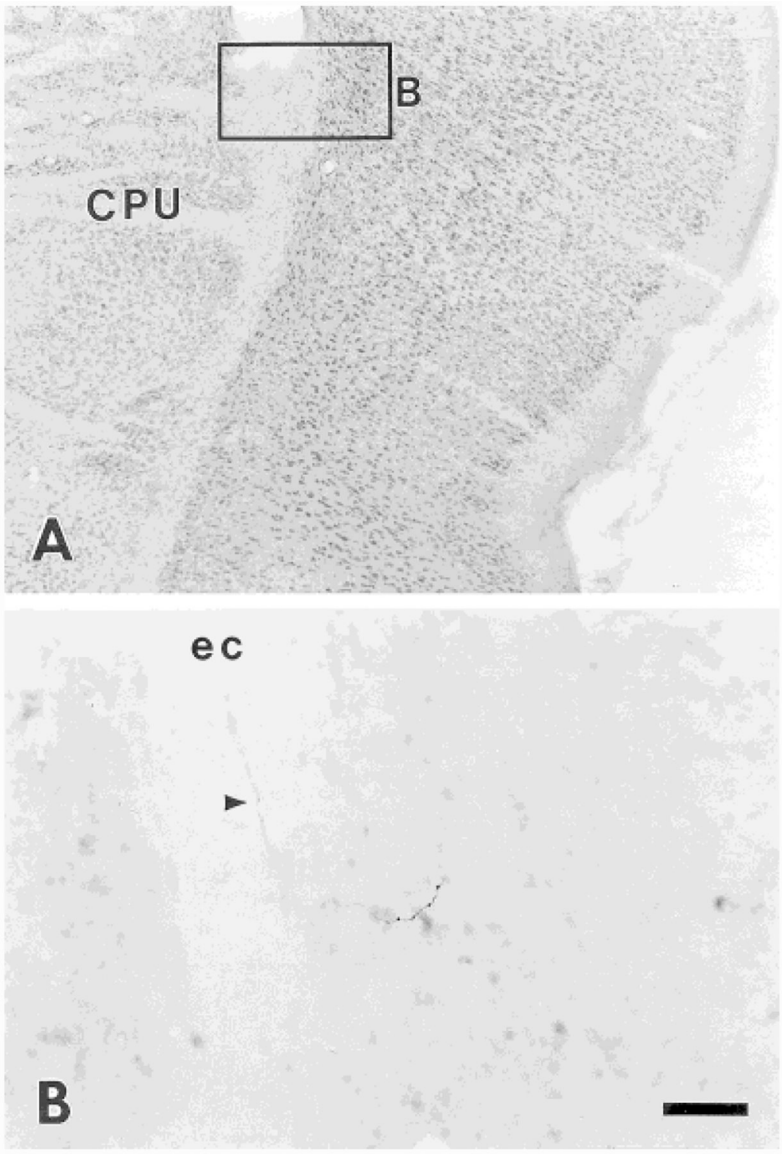


Fig. 10.

A: Nissl-stained section illustrating the location of labeled fibers from a coronal section shown in B from experiment 118. **B:** Labeled fiber (arrowhead) travels in the external capsule, and terminations are seen in deep layers of the granular insular cortex. For abbreviations, see list. Scale bar = 250 μm for A; 50 μm for B.

TABLE 1.

Comparative Density of PHA-L-Labeled Fibers in Supraspinal Structures in Selected Experiments[1]

	Midthoracic injections (Exp. No.)					L6-S1 injections (Exp. No.)				
	45	**59**	**110**	**111**	**121**	**27**	**33**	**36**	**107**	**118**
Survival time (days)	25	37	26	26	28	32	38	34	45	40
Medulla										
Gr	++	++	+++	+++	+++	++	+	+	++	+++
Cu	+++	++	++	+++	++	*	0	+	+	0
LRt	+++	+	+++	+++	+++	++	+++	++	++	++
NTS	+	+	+	+	+	+	+	+	+	+
SRV	++	+	+++	++	+	+	++	+	++	+
SRD	+	*	+	+	+	+	+	*	+	+
Sp5C	*	0	0	*	*	+	0	0	0	0
LPGi	+	+	+++	++	++	++	+++	++	+++	++
Gi	++	+	++	++	++	++	++	++	++	++
ROb	*	0	+	+	+	*	++	+	+	+
RMg	+	0	+	+	+	+	+	+	+	+
Pons										
PB	+	0	+	+	++	++	++	++	++	++
KF	+	*	++	+	+	++	++	+	+	+
SubC	++	+	++	++	++	++	++	+	++	++
Pn	+	+	+	+	++	+	++	+	+	++
LC	0	0	+	+	0	+	*	+	+	*
Bar	0	0	0	0	0	+	*	+	+	+
Mesencephalon										
PAG	+++	+	+	+	+	++	+++	++	+++	++
CnF	++	++	++	+	+	+	+	+	+	++
DpMe	++	+	++	++	+	++	++	+	+	+
SC	+	+	+	0	+	+	+	+	+	*
InC	+	++	+	+	+	+	+	+	+	+
SNR	0	0	*	*	0	0	0	0	0	0
R	0	0	+	+	+	*	*	0	*	0
APT	0	+	+	*	+	+	+	+	+	+
Thalamus										
PIL + PP	0	+	+	+	+	++	+	0	+++	*
SPFPC	+	+	+	*	+	+++	+++	+	+++	++
LHb	0	0	+	0	0	+	+	+	0	+
PV	+	+	+	*	*	*	+	+	+	+
PF	0	0	++	+	++	+	+	+	+	++
PrC	0	0	0	0	0	0	+	+	+	+
Po	*	0	+	0	+	0	+	+	*	+
VPPC	0	0	0	0	0	0	+	0	0	0

	Midthoracic injections (Exp. No.)					L6-S1 injections (Exp. No.)				
	45	**59**	**110**	**111**	**121**	**27**	**33**	**36**	**107**	**118**
VL	0	0	+	0	+	+	0	+	+	+
AVVL	0	0	0	0	+	0	+	+	0	0
VM	0	0	*	0	+	0	+	+	+	0
Re	0	0	0	0	*	0	+	0	0	0
CL	0	0	+	+	+	0	+	+	0	++
LD	0	0	+	0	+	0	0	*	0	0
MD	0	0	0	0	0	0	+	0	+	0
CM	0	0	*	+	*	0	+	+	0	0
Hypothalamus										
LH	+	+	++	+	+	+	+	+	++	+
ZI	+	+	++	+	+	+++	+	+	+	*
Subl	0	0	0	0	0	0	+	0	++	0
SUM	+	0	+	+	*	+	0	*	+	+
DA	+	+	0	0	+	+	+	+	+	0
PVH	0	0	0	0	+	+	0	0	++	0
PH	*	+	++	+	++	+	++	+	+	+
MPA	0	0	0	0	0	+	0	0	*	0
LPO	+	+	+	+	+	0	0	*	*	0
AH	*	0	0	0	0	0	0	0	0	0
sox	0	+	+	+	+	0	+	+	+	+
Telencephalon										
Ce	0	0	0	0	0	0	0	+	+	0
CPU	0	0	0	0	+	0	0	0	0	0
GP	0	0	+	+	+	0	+	0	0	0
SI	0	+	+	+	+	0	0	+	+	0
BM	+	0	0	+	0	0	+	+	0	0
GI	0	0	0	0	0	0	0	0	0	+

[1] ++++, Very high density; +++, high density; ++, medium density; +, low density; *, sparse density; 0, no fibers found. For abbreviations, see list.

TABLE 2.

Comparative Density of PHA-L-Labeled Fibers in Supraspinal Structures in Control Experiments[1]

	DC (Exp. No.)			Laminae I-V (Exp. No.)			Lamina VII (Exp. No.)	
	125	**124**	**128**	**25**	**51**	**129**	**32**	**31**
Survival time (days)	35	29	32	26	26	40	27	27
Medulla								
Gr	+	+++	+++	+	++++	++	0	0
Cu	0	0	++	0	*	0	0	0
LRt	0	0	+	*	++	+	++	++
NTS	0	*	+	0	0	0	0	0
SRV	0	*	*	+	*	+	++	+
SRD	0	0	+	0	*	0	+	*
Sp5C	0	0	0	0	*	0	0	0
LPGi	0	0	*	0	+	*	++	+
Gi	0	0	*	*	*	+	++	+
ROb	0	0	0	0	0	0	*	0
RMg	0	0	0	*	*	0	+	*
Pons								
PB	0	*	+	0	+	+	++	*
KF	0	0	0	0	+	+	+	0
SubC	0	0	0	*	+	+	++	*
Pn	0	0	*	*	0	*	+	+
LC	0	0	0	0	0	*	0	0
Bar	0	0	0	0	0	0	*	0
Mesencephalon								
PAG	0	*	*	0	+	+	++	*
CnF	0	*	*	0	0	*	+	0
DpMe	0	0	0	0	*	+	+	+
SC	0	0	0	0	*	+	*	0
lnC	0	0	0	0	0	*	*	0
SNR	0	0	0	0	0	0	0	0
R	0	0	0	0	*	0	0	0
APT	0	0	0	0	0	*	*	0
Thalamus								
PIL + PP	0	0	0	0	0	*	0	0
SPFPC	0	0	0	0	0	+	0	0
LHb	0	0	0	0	0	0	+	0
PV	0	0	0	0	+	+	+	0
PF	0	0	0	0	+	*	+	0
PrC	0	0	0	0	0	0	0	0
Po	0	0	0	0	0	0	+	*
VPPC	0	0	0	0	0	0	0	0

	DC (Exp. No.)			Laminae I-V (Exp. No.)			Lamina VII (Exp. No.)	
	125	**124**	**128**	**25**	**51**	**129**	**32**	**31**
VL	0	0	0	0	0	0	0	0
AVVL	0	0	0	0	0	0	0	0
VM	0	0	0	0	0	0	0	0
Re	0	0	0	0	0	*	0	0
CL	0	0	0	0	0	0	0	0
LD	0	0	0	0	0	0	+	0
MD	0	0	0	0	0	0	0	0
CM	0	0	0	*	0	+	0	0
Hypothalamus								
LH	0	0	0	0	0	0	+	0
ZI	0	0	0	0	0	+	*	0
Subl	0	0	0	0	0	0	0	0
SUM	0	0	0	0	0	0	0	0
DA	0	0	0	0	0	+	+	0
PVH	0	0	0	0	0	0	0	0
PH	0	0	0	0	+	0	0	0
MPA	0	0	0	0	0	0	0	0
LPO	0	0	0	0	0	0	0	0
AH	0	0	0	0	0	0	0	0
sox	0	0	0	0	0	0	+	0
Telencephalon								
Ce	0	0	0	0	0	0	0	0
CPU	0	0	0	0	0	0	0	0
GP	0	0	0	0	0	0	0	0
SI	0	0	0	0	0	0	*	0
BM	0	0	0	0	0	0	0	0
GI	0	0	0	0	0	0	0	0

[1] ++++, Very high density; +++, high density; ++, medium density; +, low density; *, sparse density; 0, no fibers found. For abbreviations, see list.