Postsynaptic Dorsal Column Pathway of the Rat. III. Distribution of Ascending Afferent Fibers

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The distribution in the dorsal column nuclei (DCn) of postsynaptic dorsal column (PSDC) fibers was examined in rats following injections of *Phaseolus vulgaris* leucoagglutinin (PHA-L) in the spinal cord. Lemniscal neurons in the DCn were retrogradely labeled in the same animals by injecting the thalamus with Fluoro-Gold. In some experiments, primary afferent fibers were also labeled by injecting dorsal root ganglia with cholera toxin-conjugated HRP. Injections of PHA-L into the cervical enlargement labeled many fibers and varicosities throughout most of the ipsilateral cuneate nucleus. Labeled fibers were also present in the external cuneate and internal basilar nuclei. Injections of PHA-L into the thoracic cord labeled fibers and varicosities in the medial cuneate and lateral gracile nuclei, as well as the external cuneate nucleus. Injections into the lumbar enlargement labeled fibers and varicosities throughout most of the gracile nucleus. Injections in sacral cord labeled fibers in the most medial part of the gracile nucleus. Dense labeling of PSDC fibers was found in areas with high densities of retrogradely labeled lemniscal neurons and areas with high densities of primary afferent fibers. In all regions of the DCn and in the external cuneate nucleus, fibers and varicosities labeled for PHA-L were seen in apposition to retrogradely labeled lemniscal cells. The distribution of postsynaptic afferent fibers in the DCn of the rat and its relationship to lemniscal neurons and primary afferent fibers contrast sharply with these features in cats.

The dorsal column nuclei (DCn) were traditionally considered to receive somatosensory input solely from primary afferent fibers that ascend in the dorsal columns. More recently, evidence has accumulated that the DCn also receive important input through the dorsal columns from a large number of neurons located throughout the length of the ipsilateral dorsal horn of the spinal cord (Uddenberg, 1966, 1968a, b; Petit, 1972; Rustioni, 1973, 1974, 1976; Angaut-Petit, 1975a, b; Rustioni and Kaufman, 1977; Rustioni et al., 1979; Bennett et al., 1983, 1984;

Lu et al., 1983; Giesler et al., 1984; Kamogawa and Bennett, 1986). Because these dorsal horn neurons are postsynaptic to primary afferent fibers, the term postsynaptic dorsal column neurons has been frequently used to describe them. The cells of origin of the postsynaptic dorsal column (PSDC) pathway in cats have been shown to transmit nociceptive information (Uddenberg, 1966, 1968b; Petit, 1972; Angaut-Petit, 1975b; Lu et al., 1983; Bennett et al., 1984; Kamogawa and Bennett, 1986). In addition, nociceptive responses have been recorded in the portion of the DCn of cats that receives PSDC input (Dart and Gordon, 1973; Angaut-Petit, 1975b). Postsynaptic afferent fibers appear to terminate exclusively in the rostral and ventral regions of the DCn in cats, avoiding the "cell cluster" regions of both nuclei (Rustioni, 1973, 1974). The cell cluster regions in cats contain the highest concentrations of both primary afferent projections (Hand, 1966; Rustioni and Macchi, 1968; Keller and Hand, 1970) and neurons that project to the thalamus (Berkley, 1975; Cheek et al., 1975; Blomqvist and Westman, 1975; Blomqvist, 1980; Bull and Berkley, 1984).

Some potentially important differences in the PSDC pathway between cats and rats have been noted. The neurons of origin of the PSDC pathway in rats are located more superficially in nucleus proprius of the spinal cord than are those of cats (Bennett et al., 1983; Giesler et al., 1984). Consistent with their position in the dorsal horn and in contrast to PSDC cells in cats, very few in rats receive a discernable input from nociceptors (Giesler and Cliffer, 1985). In light of these previously observed differences, we have examined the distribution of postsynaptic afferent fibers in the DCn of rats.

Materials and Methods

Male Sprague-Dawley rats, between 280 and 470 gm, (Biolab Corp., St. Paul, MN) were deeply anesthetized with sodium pentobarbital and placed in a stereotaxic apparatus. Supplementary injections of sodium pentobarbital or Brevital (sodium methohexital) were used to maintain anesthesia. Following laminectomy, a 2.5% solution of *Phaseolus vulgaris* leucoagglutinin (PHA-L; Gerfen and Sawchenko, 1984) in sodium PBS (pH, 7.2) was iontophoretically applied with multiple injections through a glass micropipette with a tip of 10–15 μ m (5 μ A for a total of 40 μ A-min each injection, pulsed 8 sec on, 2 sec off). In most cases, 3–6 injections were confined to a single transverse plane. In some cases, 2–4 planes were injected, with up to 10 injections/plane. The illustrated cases injected in cervical or lumbar cord each received a total of 4–8 injections in 1 or 2 planes.

Four days prior to perfusion, a 2% solution of Fluoro-Gold (Schmued and Fallon, 1986) was injected into the contralateral thalamus. The animal was again anesthetized and placed in a stereotaxic apparatus. The skull was opened and a series of 10 injections of about 0.1 μ l each were made in 2 planes through a 5 μ l Hamilton syringe into the ventrobasal complex (VBC).

In some animals in which both PHA-L and Fluoro-Gold had been injected, and in others that had received only Fluoro-Gold, 0.2% chol-

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eragenoid-conjugated HRP (B-HRP; List Biological Laboratories, Inc., Campbell, CA) was injected into a dorsal root ganglion (DRG) 2 or 3 d prior to perfusion (for cervical or lumbar injections, respectively). The DRG of the spinal segment in which PHA-L had been injected was exposed and injections totaling about 1 μ l were made through a glass micropipette (tip diameter, 10–50 μ m) attached to a 5 μ l Hamilton syringe. During these injections B-HRP that leaked out of the injection site was absorbed by a cotton wick. Adjacent DRGs were examined after processing to verify that neurons in them were not labeled. Results from cases in which there was diffuse labeling of the edge of the spinal cord or brain beyond the immediate vicinity of the injection were not used. Six cases were satisfactorily labeled with both PHA-L and B-HRP. Three cases labeled only with B-HRP were used for comparison with those labeled with PHA-L.

After survival periods of up to 41 d after injection of PHA-L, animals were deeply anesthetized with sodium pentobarbital and perfused with cold oxygenated calcium-free Tyrodes solution, followed by 4% paraformaldehyde (pH, 6.5), then 4% paraformaldehyde with 0.05% glutaraldehyde (pH, 9.4; Berod et al., 1981). Satisfactory survival times were determined to be approximately 14 d for injections in the cervical enlargement, and 35 d for injections in the lumbar enlargement. Tissue to be stained immunohistochemically for PHA-L only was postfixed 12–24 hr in the high-pH fixative solution. Tissue to be reacted for HRP was not postfixed for more than 4 hr. After postfixation, tissue was stored in 10% sucrose in Sorenson's phosphate buffer (pH, 7.2).

The medulla and spinal cord were cut at 20 μ m on a freezing microtome in either the horizontal or transverse plane. Sections taken at intervals up to 100 μ m were placed in PBS for immunohistochemical staining for PHA-L. In cases in which B-HRP was injected, adjacent sections were placed in either distilled water or 0.2 M phosphate buffer for TMB staining (Mesulam, 1978; Gibson et al., 1984).

PHA-L was immunohistochemically labeled using the avidin-biotin fluorescence technique. Goat anti-PHA-L (Vector Laboratories, Inc., Burlingame, CA) was applied at a dilution of 1/1000 with 2% normal rabbit serum (NRS) overnight at 4°C. Subsequent steps were performed at room temperature. Biotinylated rabbit anti-goat IgG (Vector) was applied for 1 hr at 1/200 dilution with 2% NRS. Fluorescein-conjugated-avidin (cell sorter grade; Vector) was applied for 1 hr at a dilution of 1/100. In some cases indirect fluorescent labeling was done using a dilution of 1/200 for the primary antibody and fluorescein-conjugated swine anti-goat IgG (Tago, Inc., Burlingame, CA), diluted at 1/10. After mounting, immunohistochemically stained sections were counterstained with ethidium bromide (Schmued et al., 1982).

Fluorescent material was examined microscopically using reflected illumination. Double-exposed photomicrographs of fluorescein-labeled fibers and Fluoro-Gold-labeled cells were made using a 560 nm short-pass barrier filter and a 590 nm long-pass barrier filter, respectively, to enhance color contrast. TMB-stained HRP was viewed with dark-field illumination; contrast was enhanced using magenta filters. Dark-field illumination was used to determine architectonic boundaries. Drawings were made using a camera lucida drawing attachment on the microscope, with a 20x objective. Borders and retrogradely labeled cells were drawn using colored paint pens and the drawings were photographed with filters to produce gray tones.

Results

Fluoro-Gold

A representative injection of Fluoro-Gold into the thalamus is illustrated in Figure 1; all such injections were similar. They extensively involved the ventrobasal, posterior thalamic and lateral geniculate nuclei (B). Rostrally, injections extended into the laterodorsal thalamic nucleus (A), and caudally into the medial geniculate nucleus (at a level not illustrated) and the rostral ventrolateral midbrain (C). The injections involved the pretectal area (B) and, in the midbrain, substantia nigra (C). The most rostral part of the ventrobasal complex was not sub-

Figure 1. Photomicrographs of an injection of Fluoro-Gold into the thalamus. A–C, Rostral to caudal. Asterisks, Third ventricle (B); cerebral aqueduct (C). Abbreviations: CG, central gray; CP, cerebral peduncle; F, fornix; FR, fasciculus retroflexus; Hn, habenular nuclei; Hy, hypo-

thalamus; IC, internal capsule; LDn, laterodorsal nucleus of the thalamus; LGn, lateral geniculate nucleus; MGn, medial geniculate nucleus; ML, medial lemniscus; MT, mammillothalamic tract; P, posterior nucleus of thalamus; PC, posterior commissure; PT, pretectal nuclei; RTn, reticular nucleus of the thalamus; SN, substantia nigra; VB, ventrobasal nuclei of the thalamus; ZI, zona incerta. Scale bar, 1 mm.

Figure 2. Photomicrographs of examples of injection sites of PHA-L. *Top*, Injection into segment C8, consisting of 8 individual injections in 4 tracks in 2 rostrocaudal planes. *Dashed lines* indicate edge of gray matter. *Asterisk*, Central canal. *Bottom*, Injection into segment L4, consisting of 3 injections in a single plane. Scale bar, 0.5 mm (*top*); 0.25 mm (*bottom*).

stantially involved (*A*). Ventrally in the diencephalon, zona incerta was partially involved (*B*), but the hypothalamus was not. Little, if any, Fluoro-Gold spread into the medial thalamus (*B*).

The distribution of cells retrogradely labeled with Fluoro-Gold in the DCn and cervical spinal cord resembled those reported in previous studies after injections of HRP into the thalamus of rats (Tan and Lieberman, 1978; Giesler et al., 1979a; Feldman and Kruger, 1980). Labeled cells were distributed contralateral to the injection throughout the DCn. In addition, cells were labeled in the external cuneate nucleus, primarily in the caudal portion. The density of labeled cells was especially high

at the level of the obex¹ and for about a millimeter rostrally and caudally (see reconstructions in Figs. 3, 4). The population of labeled cells extended into the cervical spinal cord, with labeled cells in the dorsal columns and the most medial part of the dorsal horn. Labeled cells in these locations were common in C1, especially rostrally, and relatively sparse through C2. Caudal to C2, few labeled cells were seen in the dorsal columns or in the most medial part of the dorsal horn. In other studies, substantial numbers of cells in the medial part of the dorsal horn in C2–4 have been found to project to the thalamus (Giesler et al., 1979a; Granum, 1986; Kemplay and Webster, 1986); the small number of labeled neurons in this study may be the result of our injections not reaching the most rostral VBC. The most rostral part of the VBC is the part that receives the densest projection from spinothalamic tract neurons caudal to C2 (Lund and Webster, 1967b). Retrogradely labeled cells were also seen in the contralateral lateral cervical nucleus, in the contralateral spinal trigeminal nucleus, and in the ipsilateral nucleus of the solitary tract.

PHA-L injections

Examples of multiple injections of PHA-L into the spinal cord are illustrated in Figure 2. The injection illustrated at the top consisted of a total of 8 individual injections in 4 tracks in 2 transverse planes in segment C8 (the case shown in Fig. 7). As a result, nearly the entire dorsal horn was heavily labeled, including nucleus proprius, the region in which almost all PSDC neurons are found (Giesler et al., 1984). The injection illustrated at the bottom consisted of 3 individual injections within a single plane in segment L4. Labeled neuronal cell bodies could be seen within injection sites. After long survival periods, the diffuse labeling at injection sites in the gray matter of lumbar cord was often relatively light compared with injection sites in the gray matter of cervical cord after shorter survival periods. In contrast, labeling at injection sites remained relatively intense in white matter, even over long survival periods.

Cases with injections of PHA-L into the spinal cord that resulted in substantial numbers of labeled fibers in the DCn included the following: 14 cases with 2–30 injections in the cervical enlargement (C6–T1), survival times 13–40 d; 4 cases with 3–35 injections in mid-thoracic cord (T8–10), survival times 26–36 d; 17 cases with 1–27 injections in the lumbar enlargement (L3–5), survival times 23–39 d; 3 cases with 2–30 injections in sacral cord, survival times 39–41 d.

Absence of retrograde labeling with PHA-L

In 13 animals that received injections into the gray matter of the dorsal horn (8 cervical and 5 lumbar injections), we processed and examined DRGs with attached portions of the dorsal roots from the segments of the injections and from adjacent segments. In a few cases, a few labeled fibers were observed in the proximal dorsal roots after injections that impinged on the dorsal root entry zone. We did not observe labeled fibers close to or in the ganglia. Labeled neurons were not seen in any DRG. Within the brain and spinal cord, neurons labeled with PHA-L were almost always confined to the immediate vicinity of the injection site. Labeled neurons were not encountered further than the dorsal horn contralateral to the injection at the same level (one instance).

¹ Following Papez (1929; pp. 121, 196), we use the term “obex” to mean the most rostral level where the 2 gracile nuclei abut one another.

Figure 3. Drawings of labeled fibers in transverse sections through the dorsal column nuclei after 4 injections of PHA-L in a single plane of segment L4. *Inset* drawings indicate locations of illustrated areas. Cells retrogradely labeled with Fluoro-Gold from injections in the thalamus are depicted in gray. Levels indicated by approximate distance in millimeters from obex (calculated by numbers and thicknesses of sections); positive is rostral. Borders drawn from architectonic structure seen with dark-field illumination. *AP*, Area postrema; *Cf*, cuneate fasciculus; *Cn*, cuneate nucleus; *ECn*, external cuneate nucleus; *Gf*, gracile fasciculus; *Gn*, gracile nucleus. Scale bars, 0.2 mm. (See following 2 pages for continuation of this figure.)

Figure 3. Continued.

Figure 3. Continued.

Figure 4. Drawings of labeled fibers in horizontal sections through the dorsal column nuclei after 6 injections of PHA-L in a single plane of segment L4. *Inset* drawings indicate locations of illustrated areas progressing from dorsal to ventral (A–F) in sections separated by approximately 100 μ m. Cells retrogradely labeled with Fluoro-Gold from injections in the thalamus are depicted in gray. Borders drawn from architectonic structure seen with dark-field illumination. Abbreviations as in Figure 3. Scale bars, 0.2 mm. This page: *Figure 4A*.

Figure 4B.

Figure 4C.

Figure 4D.

Figure 4. E (left) and F (right).

Figure 5. Photomicrographs of transverse sections showing immunohistochemical staining for PHA-L in the DCn. Levels of injections are indicated at the top. Approximate distance from the obex is indicated at the left in millimeters (calculated by numbers and thicknesses of sections); positive is rostral. Dot, Dorsal intermediate sulcus, between gracile and cuneate complexes; arrowhead, midline; asterisk, central canal. Scale bar, 400 μ m.

Distribution of labeled fibers

Lumbar injections. Nearly all of the ipsilateral gracile nucleus contained labeled fibers after unilateral injections made in single transverse planes in the lumbar enlargement. Figures 3 and 4 are reconstructions of the locations of all labeled fibers in selected transverse and horizontal sections of the dorsal column fasciculi and nuclei after injections of PHA-L in segment L4 of the spinal cord (4 and 6 injections for the cases illustrated in Figs. 3 and 4, respectively). Architectonic boundaries and neu-

rons retrogradely labeled with Fluoro-Gold are shown in gray. The wide distribution of labeled fibers in the ipsilateral gracile nucleus after injections in the lumbar enlargement can be seen also in Figure 5. Labeled varicosities were observed throughout all nuclear regions of the gracile complex. Injections into the lumbar cord also resulted in occasional labeled fibers contralateral to the injection and in the ipsilateral cuneate fasciculus and nucleus (Figs. 3–5).

Thoracic injections. After injections of PHA-L into thoracic spinal cord, labeled fibers were observed primarily in the lateral part of the gracile nucleus and the medial part of the cuneate

Figure 6. Photomicrographs of horizontal sections through the dorsal column nuclei, showing labeling from an injection of PHA-L in segment T10. Note that labeled fibers are at or near the border between the gracile and cuneate nuclei. Note also fibers extending rostrally from the area of densest labeled fibers. *A* is more dorsal; *C*, cuneate nucleus; *Cf*, cuneate fasciculus; *G*, gracile nucleus. Scale bar, 500 μ m.

Figure 7. Photomicrographs of labeling in horizontal sections following 5 injections of PHA-L in a single plane in segment C6 (*A–E*; same case as in Fig. 8, *C, D*) or following injection of B-HRP into the DRG of segment C7 (*F, G*; same case as in Fig. 8*F*). *A–D*, Double-exposed photomicrographs of labeling in locations indicated by dots in *E*, showing anterogradely labeled fibers and varicosities (green/yellow) apposed to retrogradely labeled cells (red). *E*, Photomicrograph of single horizontal section showing extent of anterograde labeling from external cuneate nucleus through C2. Location shown in drawing to left of photomicrograph. *F*, Photomicrograph of similar view to *E* of anterograde labeling by B-HRP. This is a composite photomicrograph taken from several sections since the angle of sectioning was not proper to get the entire extent in one section. *G*, Similarly composite photomicrograph, caudal to area shown in *F*. Locations of *F* and *G* are indicated in drawing to left of *F*. In *E–G*, divisions between segments are indicated by arrowheads, corresponding to those in the drawings. Abbreviations in drawings: *C*, cuneate nucleus; *D*, dorsal columns; *E*, external cuneate nucleus; *G*, gracile nucleus; *L*, lateral funiculus; *M*, medulla; *SpV*, spinal trigeminal nucleus. Scale bars, *A, B*, 100 μ m; *C, D*, 50 μ m (lower left); *E–G*, 1 mm (lower right).

Figure 8. *A*, Photomicrograph of labeling in a transverse section through the cuneate nucleus approximately 0.5 mm rostral to obex, following 6 injections of PHA-L in one plane in segment C6. Location of *B* is indicated by dots at corners. *B*, Higher power photomicrograph of heavily labeled area indicated in *A*. *C*, Photomicrograph of labeling in a horizontal section through the external cuneate nucleus following 5 injections of PHA-L in a single plane in segment C6 (same case as for Fig. 7, *A–E*). Location of *D* is indicated by dots in corners. *D*, Higher power, double-exposed photomicrograph of area indicated in *C*, showing retrogradely labeled cells (red) in an area of dense anterogradely labeled fibers. *E*, Photomicrograph of labeling in a horizontal section through the ventral part of the cuneate nucleus following 8 injections of PHA-L in 2 transverse planes about 400 μ m apart in segment C6. Location of *G* is indicated by dots in corners. *F*, Photomicrograph of similar section to that in *E* following injection of B-HRP into DRG of segment C7 (same case as in Fig. 7, *F*, *G*). Locations of *E* and *F* indicated in drawing. *G*, Higher power, double-exposed photomicrograph of area indicated in *E* showing retrogradely labeled cells (red) in an area of dense anterogradely labeled fibers. *H–J*, Double-exposed photomicrographs of labeling in horizontal sections through the dorsal column nuclei in same case depicted in Figure 4. Locations of photomicrographs indicated in drawing. *K*, Double-exposed photomicrograph of labeling in transverse section of ventral, caudal part of gracile nucleus (level about 0.4 mm caudal to obex) showing labeled varicosities apposed to retrogradely labeled cell. Location indicated in drawing. *L*, Double-exposed photomicrograph of labeling in horizontal section through the LCn (same case as in *E*) showing anterogradely labeled fibers and

nucleus, on the ipsilateral side (transverse sections, Fig. 5; horizontal sections, Fig. 6).

Cervical injections. Injections of PHA-L into the cervical enlargement labeled a large number of fibers throughout most of the ipsilateral cuneate nucleus. Labeled fibers were also seen in the external cuneate nucleus and the internal basilar nucleus (IBn; Ramón y Cajal, 1909; Torvik, 1956). The extent of labeled fibers after injections into the cervical enlargement is illustrated in photomicrographs of transverse sections (Fig. 5) and of a single horizontal section (Fig. 7E); in both cases the injections were in C6. In these cases labeling was observed from caudal C2 through the rostral poles of the cuneate and external cuneate nuclei. Horizontal sections from 2 additional cases in which PHA-L was injected into the cervical enlargement (C8) were taken caudally through C6; we observed labeled fibers extending within the IBn as far as the caudal border of C6. Large varicosities (approximately 3–5 μ m diameter) were labeled in the external cuneate nucleus; few large varicosities were seen in the remainder of the DCn (Fig. 7, compare *A*, *B*). A few labeled fibers were found in the ipsilateral gracile nucleus and in the contralateral cuneate nucleus.

Although labeling was present throughout the cuneate nucleus, the density of labeled fibers often varied from one location to another. Figure 8*A* illustrates a section showing variability in density of the PHA-L-labeled fibers, with a view at higher magnification (*B*) of an area containing an especially high density of labeled fibers.

After cervical injections, labeled fibers were distributed widely in the cuneate fasciculus (Fig. 9). A few were observed within the area occupied by the pyramidal tract in the ventral part of the dorsal columns (Fig. 9). Fibers in this position were not as common more rostrally (Fig. 5, level –1.7), suggesting either that they were propriospinal fibers or that they shifted to positions outside of this tract. A tail of labeled fibers could be observed at rostral cervical levels, extending from the main body of labeled axons ventromedially toward the medial ventral border of the dorsal columns (Fig. 5, level –1.7; Fig. 9, C2). As these fibers were followed further rostrally, they appeared to lead to the ventral part of the gracile nucleus (see below).

Sacral injections. Injections of PHA-L in the sacral cord labeled fibers within the most medial part of the ipsilateral gracile nucleus (Fig. 10). In these cases, labeling was not seen in the gracile nucleus rostral to the area postrema.

Relationship of PSDC fibers and varicosities to retrogradely labeled lemniscal cells

Injections of PHA-L into the spinal cord labeled many fibers and varicosities in the areas of the DCn in which retrogradely labeled lemniscal cell bodies were located. In cases in which PHA-L was injected into lumbar or cervical cord, the varicosities of labeled fibers were closely apposed to retrogradely labeled neurons in all parts of the gracile nucleus (Figs. 3; 4; 8, *H–K*) or cuneate nucleus (Fig. 7, *A–D*), respectively. Notably, many retrogradely labeled neurons were present in areas with especially high densities of fibers labeled with PHA-L. Figure 8, *D* and *G*, shows retrogradely labeled lemniscal neurons in

such areas, both in the cuneate (*G*) and external cuneate nuclei (*D*). The many labeled fibers in Figure 8*B* also surrounded labeled neurons (not shown).

Relationship of PSDC fibers to primary afferent input

Injections of B-HRP into DRGs of the cervical enlargement labeled many fibers in a dense and widespread distribution throughout the cuneate nucleus. This labeling extended in the IBn at least as far caudally as the C5/6 border (Fig. 7, *F*, *G*). The distribution of labeled primary afferent fibers corresponded closely to the distribution of PSDC fibers labeled with PHA-L (Fig. 7, *E*, *F*). Both B-HRP and PHA-L also labeled many fibers throughout the most ventral part of the cuneate nucleus (Fig. 8, *E*, *F*). These results show that the distributions of postsynaptic afferent fibers and of primary afferent fibers to the cuneate nucleus occur in equivalent areas, with some areas having dense concentrations of both types of fibers.

To examine directly the distributions of primary and postsynaptic afferent fibers in the same animal, adjacent sections were processed for PHA-L or B-HRP. The results from one such case are illustrated in Figure 11. Although not all areas had equivalent densities of afferent fibers from the 2 sources, the overlap was extensive throughout the nucleus, and in many areas both types of fibers were particularly dense.

Primary afferent fibers and postsynaptic fibers overlapped not only within the DCn, but also as they ascended in the dorsal columns. Widespread distributions of labeled fibers observed after cervical injections of PHA-L are illustrated in Figures 5 (level –1.7, left) and 9. Overlap of the distributions is directly indicated in adjacent sections in Figure 11, especially at level –1.4.

Dense labeling of primary afferent fibers in the IBn extended continuously from the segment of the injection to the cuneate nucleus (Fig. 7, *F*, *G*). Figure 12 illustrates examples of such labeling in transverse sections at C6 and C4 in a case that received injections into the DRGs of segments C7 and C8. In C6 dense labeling of primary afferent fibers was distributed widely within much of the dorsal horn (Fig. 12). However, the substantia gelatinosa and the lateral reticulated area contained almost no labeled fibers. Robertson and Grant (1985) previously reported that injections of B-HRP do not label a large number of fibers in the superficial dorsal horn and lateral reticulated area, a finding they attributed to the selectivity of B-HRP for larger diameter fibers. In C4, the densest label was restricted to the ventromedial dorsal horn, including the IBn. Comparison with Figures 9 (C2) and 11 (C2/3) reveals the similarity of the location of the concentrated labeling in transverse sections to that produced by PHA-L injections. Like Robertson and Grant (1985), we never saw cell bodies labeled by B-HRP in the dorsal horn after injections of DRGs, even among dense accumulations of labeled fibers.

Deeper labeling

The most ventral part of the gracile nucleus (ventral to the gracile fasciculus; Fig. 3), from the caudal limit of the pyramidal decussation rostrally to the obex, consistently contained a few

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varicosities in apposition to retrogradely labeled cells. Note varicosity apposed to dendrite of cell at upper right, as well as many apposed to cell at center. Rostral is up. Abbreviations in drawings: *Cf*, cuneate fasciculus; *NTS*, nucleus of solitary tract; other abbreviations as in Figure 7. Scale bar, 240 μ m (*A*); 120 μ m (*B*); 340 μ m (*C*, *F*); 320 μ m (*E*); 60 μ m (*D*, *G*, *L*); 70 μ m (*H*); 35 μ m (*I–K*).

Figure 9. Photomicrographs of transverse sections through levels C7 and C2 of the spinal cord following 8 injections of PHA-L in 2 transverse planes about 0.6 mm apart in segment C8 (left) and following a single injection of PHA-L in a single plane in segment C6 (right; same case as in Fig. 5, left). Note locations of ascending labeled fibers. Some burst artifacts are evident in dorsal columns of C2; these are not uncommon. Asterisks, Central canal. Scale bar, 200 μ m.

labeled fibers in cases that received injections at cervical, thoracic, or lumbar levels (Fig. 3, levels -0.4 , -0.8 , -1.2 , -1.7 ; Fig. 5, levels -0.7 , -1.7). These fibers were primarily ipsilateral to the injections, but a few were contralateral. Occasionally they could be seen to cross the midline in a transverse section. Fibers and varicosities could be found in this region apposed to retrogradely labeled neurons (Fig. 8K). Retrogradely labeled neurons in this region were contralateral to the injections of Fluoro-Gold, in contrast to those in the ventrally adjacent nucleus of the solitary tract, which were ipsilateral to the injections. Feldman and Kruger (1980) have previously observed cells retrogradely labeled from the ipsilateral thalamus in the nucleus of the solitary tract.

After cervical injections, labeled fibers were commonly seen in the reticular formation immediately ventral and ventrolateral to the cuneate nucleus, often spreading widely in the reticular formation. Sparse labeling in this area was also seen after lumbar injections. After cervical injections, some labeled fibers were seen in the rostral part of the spinal trigeminal nucleus, immediately medial to the spinal trigeminal tract.

Rostral DCn

The medial part of the rostral DCn was also labeled after injections at several levels of the spinal cord. This region, too,

contained some labeled fibers after injections into all but sacral levels of the cord. Both in transverse (Fig. 3, levels $+1.0$, $+1.3$; Fig. 5, level $+1.1$) and horizontal (Figs. 4, *A–D*; 6) sections, fibers were consistently labeled after injections into mid-thoracic or lumbar cord coursing rostrally from the main part of the gracile nucleus along the medial edge of the cuneate nucleus. Lumbar afferent fibers have previously been described in cats in the medial part of the rostral cuneate nucleus (primary afferent fibers: Hand, 1966; postsynaptic afferent fibers: Rustioni, 1973). After cervical and thoracic injections, but not after lumbar injections, labeled fibers extended into the external cuneate nucleus (Figs. 3, 6, 7).

Lateral cervical nucleus

Labeled fibers and varicosities were seen in the ipsilateral lateral cervical nucleus (LCn) after injections in cervical, thoracic, and lumbar cord. Especially in horizontal sections, these elements could be seen in apposition to neurons in the LCn that were retrogradely labeled with Fluoro-Gold (Fig. 8L). Spinocervical tract axons have not been previously observed directly apposed to retrogradely labeled lemniscal neurons in the LCn.

We observed no clear difference in the mediolateral distribution of afferents to the LCn from various segmental levels of

Figure 10. Photomicrographs of labeled fibers in a transverse section of the gracile nucleus at the level of the obex following injection of PHA-L in sacral spinal cord. *B* is a higher power photomicrograph of the area designated in *A*. Note medial position of labeled fibers. Some bright edge artifact has been retouched. *Dot*, Dorsal intermediate sulcus at lateral edge of gracile nucleus. *Arrowhead*, Midline; *Gn*, gracile nucleus. Scale bar, 225 μ m (*A*); 113 μ m (*B*).

the spinal cord. This finding confirms the results from a study with degeneration techniques of the termination of the spino-cervical tract in the rat (Giesler et al., 1988) and is consistent with the finding that many neurons throughout the LCN of rats have receptive fields that cover most or all of the body surface (Giesler et al., 1979b).

Discussion

Technical considerations

A large number of fibers and terminals were labeled in the dorsal column nuclei following our injections of PHA-L into the dorsal horn of the spinal cord. In addition to PSDC fibers, one or more of the following types of fibers may have been inadvertently labeled. (1) Primary afferent fibers passing through the injection site could have incorporated and transported PHA-L. Such labeling was probably not an important problem in this study. Other investigators have failed to find substantial labeling of axons of passage with PHA-L (Gerfen and Sawchenko, 1984; Grove et al., 1986). Also, we directly examined the ability of axons in the dorsal columns to transport PHA-L (Cliffer and Giesler, 1988). In approximately half of the cases, we failed to find labeling in the DCn after injections of PHA-L into the dorsal columns. In the other cases, only a few fibers were weakly labeled. The quality of the labeling clearly differed from that reported here. (2) Primary afferent axons could have been labeled via terminating collateral branches in the dorsal horn. This possibility also appears to be unlikely since we did not see labeled axons or cell bodies in the DRGs. (3) Cell bodies in other regions of the brain and spinal cord that project to the DCn might have been retrogradely labeled and then anterogradely transported PHA-L to the DCn. This possibility is also unlikely since we searched widely for labeled cell bodies and did not find any outside the vicinity of the injections. (4) PHA-L could have

been transported transsynaptically from axons of spinal origin to neurons in the brain that projected to the DCn. This is also unlikely since we did not find any labeled postsynaptic cell bodies, even in areas of intense terminal labeling. Therefore, we conclude that the axonal labeling we observed in the DCn was due overwhelmingly to labeling of postsynaptic afferent fibers originating in or near the injections in the spinal cord.

All fibers labeled by injection of B-HRP into the DRGs were probably primary afferent fibers, not postsynaptic fibers labeled as a result of transsynaptic transport. We, like Robertson and Grant (1985), failed to observe labeled cells in the dorsal horn following injection of B-HRP into DRGs, even among dense accumulations of labeled fibers. In contrast, wheat germ agglutinin-conjugated HRP (WGA-HRP), for which evidence of transsynaptic transport exists, can be observed within cell bodies postsynaptic to stained fibers (Robertson and Grant, 1985; Pechanski and Ralston, 1985). Also, if transsynaptic transport of B-HRP had occurred in the dorsal horn, one would expect to find labeling in regions such as the brain-stem reticular formation which receives a large projection from dorsal horn neurons. No such labeling was evident in our tissue.

The cells retrogradely labeled with FG may belong to more than one subpopulation of lemniscal cells. The injections were mainly within the ventrobasal complex of the thalamus (VBC), but spread to the pretectum and the ventro-lateral midbrain, as well as to the zona incerta. In the cat, populations of cells in the DCn that project to the VBC appear to be different from those that project to the tectum, to the pretectum, or to the zona incerta (Blomqvist et al., 1978; Berkley et al., 1980, 1986; Bull and Berkley, 1984; Wiberg and Blomqvist, 1984). No such disjunction of populations of lemniscal cells has been shown in rats, and several studies have indicated that cells projecting to the thalamus in rats are distributed more uniformly throughout the DCn (Lund and Webster, 1967a; Tan and Lieberman, 1978;

Figure 11. Pairs of photomicrographs of adjacent sections labeled for PHA-L (left) and HRP (right) after 8 injections of PHA-L in 2 transverse planes about 0.6 mm apart and injection of B-HRP into the DRG in segment C8. Drawings at right show locations of the photomicrographs, with approximate distance from the obex indicated in millimeters (calculated from numbers and thicknesses of sections; positive is rostral). Abbreviations as in Figure 8. Scale bar, 0.5 mm.

Figure 12. Photomicrographs of labeling in transverse sections through levels C6 and C4 after injection of B-HRP into DRGs of segments C7 and C8. Scale bar, 0.5 mm.

Feldman and Kruger, 1980; Massopust et al., 1985). The pretectum and zona incerta receive projections only from rostral portions of the DCn (Lund and Webster, 1967a). However, it is not known whether single cells in the DCn of rats project exclusively to the pretectum or zona incerta. If so, some such cells may have been retrogradely labeled in our experiments.

Distribution of PSDC fibers

Although we did not examine the issue directly in the present study, it is likely that almost all of the labeled fibers within the dorsal column nuclei ascended to the medulla within the dorsal columns. We previously found that retrograde labeling of spinal cord neurons following injections of HRP into the gracile nucleus was completely blocked by restricted lesions of the dorsal columns (Giesler et al., 1984). We also found that dorsal column lesions prevented the labeling of more than 90% of the neurons that project to the cuneate nucleus. A small number of axons was found to project to the cuneate nucleus in the dorsal lateral funiculus (Giesler et al., 1984).

The postsynaptic projection to the dorsal column nuclei was found to be somatotopically organized. Injections of PHA-L into the cervical enlargement labeled fibers throughout most of the cuneate nucleus. Injections into thoracic cord labeled fibers in the medial cuneate and lateral gracile nuclei. Injections into the lumbar enlargement labeled fibers throughout most of the gracile nucleus. Injections in the sacral cord labeled fibers in the

most medial part of the gracile nucleus. These results confirm our previous description of the somatotopic organization of the PSDC projection in rats that was based on retrograde tracing (Giesler et al., 1984). In addition, the present findings indicate that the organization of the PSDC system in rats is very similar to that of primary afferent projections in rats (Ranson et al., 1932; Basbaum and Hand, 1973; Beck, 1981; Imamura et al., 1986; Pfaller and Arvidsson, 1988), and of primary (Carpenter et al., 1968; Shriver et al., 1968; Wild, 1985; additional references in Norton and Kruger, 1973) and postsynaptic (Rustioni, 1973, 1974; Rustioni et al., 1979) afferent projections in other species. Physiological mapping has shown that the neurons of the DCn are similarly organized (Norton and Kruger, 1973; Dykes et al., 1982).

The collective wide distribution of postsynaptic fibers throughout the DCn in rats contrasts sharply with the distribution that has been described for the cat. Taken together, cases in which we injected only very restricted regions of the spinal cord at sacral, lumbar, thoracic, or cervical levels contained labeled fibers throughout the DCn ipsilateral to the injections (Figs. 5, 7). In contrast, in cats cells in the lumbar and cervical spinal cord have been reported to project sparsely (Pierce et al., 1983; Pierce and Rustioni, 1985) or not at all (Rustioni, 1973, 1974) to large portions of the DCn. The areas in the DCn of cats that do not receive a projection from PSDC fibers are the cluster regions, the areas that contain the highest concentrations

of neurons that project to the ventrobasal complex (Berkley, 1975; Cheek et al., 1975; Blomqvist and Westman, 1975; Blomqvist, 1980; Bull and Berkley, 1984). Therefore, in cats relatively little information transmitted by the PSDC pathway appears to be carried directly to neurons that project to the thalamus. In contrast, our results show postsynaptic afferent input to the DCn in the rat in all areas with high densities of medial lemniscal neurons. In addition, although we were able to see only the most proximal dendrites of labeled neurons, labeled fibers and varicosities were observed closely apposed to lemniscal neurons in all areas of the DCn. In fact, our results probably underrepresent the richness of the direct projection of PSDC afferent fibers to medial lemniscal neurons. In rats, ascending afferent fibers make synaptic contact almost exclusively on the dendrites of lemniscal neurons (Valverde, 1966; Walberg, 1966).

In the cat, areas appearing devoid of postsynaptic input have been observed to receive the heaviest input from primary afferent fibers (Hand, 1966; Rustioni and Macchi, 1968; Keller and Hand, 1970). In contrast, our results in the rat indicate extensive overlap of input from both postsynaptic and primary afferent fibers. This overlap is striking even in regions where primary afferent fibers are most heavily concentrated (Figs. 7, 8, 11, and Basbaum and Hand, 1973). We have observed retrogradely labeled lemniscal neurons split between sections that appeared to be contacted by both types of afferent fibers (unpublished observations). These observations suggest that individual lemniscal neurons in rats may receive input from both primary and postsynaptic afferent fibers.

In monkeys, the distribution of PSDC fibers in the gracile nucleus appears to differ from that in the cuneate nucleus. PSDC input is substantial throughout the gracile nucleus (Rustioni et al., 1979), resembling in this respect the distribution of PSDC fibers in rats. In contrast, the cuneate nucleus in monkeys, like that in cats, contains an area that appears devoid of heavy postsynaptic afferent input (Rustioni et al., 1979). It will be interesting to determine the relationship of these patterns to the locations of nociceptive neurons in the DCn of monkeys (Farrington et al., 1988).

Variations on somatotopy

Injections of PHA-L into widely separated segmental levels each labeled fibers in restricted regions of the DCn. These included the ventral part of the gracile nucleus caudal to the obex and an area in the medial rostral DCn. These overlapping projections may be associated with the relatively large receptive fields that have been encountered in the rostral and caudal portions of the DCn in rats (McComas, 1963) and cats (Gordon and Paine, 1960; Gordon and Jukes, 1964).

Villaneuva et al. (1988) recorded nociceptive cells with whole-body receptive fields in the dorsal medullary reticular formation immediately ventral to the cuneate nucleus in rats. Their findings indicate that individual neurons in this region also receive input that originates in widespread regions of the body. After injections of PHA-L into cervical or lumbar cord, we observed labeling in the same area. Lima and Coimbra (1985, and personal communication) have injected the same area with retrograde tracers and observed labeled cells in the marginal zone of the cervical and lumbar enlargements. This labeling does not appear to be due to spread of the tracer into the DCn since no neurons were labeled in the marginal zone following injections of retrograde tracer that were restricted to the dorsally adjacent

DCn (Giesler et al., 1984). The labeling of marginal zone neurons seen following injections into the dorsal medullary reticular formation was blocked by lesions of the lateral part of the dorsal column (D. Lima, J. M. Castro-Lopes and A. Coimbra, personal communication). Thus, nociceptive cells in the marginal zone may contribute input, possibly through the dorsal columns, to nociceptive cells ventrally adjacent to the DCn.

Occasional fibers were labeled with PHA-L contralateral to our injections or in the ipsilateral non-homotopic nucleus (gracile from cervical or cuneate from lumbar). We do not think that such labeling was artifactual. As discussed above, fibers of passage were probably not labeled in substantial numbers. Furthermore, the locations of the injections were incompatible with non-homotopic labeling through fibers of passage. Injections in cervical cord were far from the gracile fasciculus, where fibers from lumbar levels pass (Giesler et al., 1984). Lumbar injections were centimeters caudal to ascending cervical fibers. The unilateral nature of the injections and scarcity of retrogradely labeled cells contralateral to the injections suggest that the contralateral labeling was due to contralaterally projecting cells. These findings confirm an earlier study in which retrograde tracers were used (Giesler et al., 1984). In that study, unilateral injections of HRP in the DCn labeled small numbers of neurons in the contralateral dorsal horn and in the non-homotopic part of the spinal cord. Others have noted similar occasional apparent departures from the somatotopic arrangement for primary afferent fibers in the DCn of rats (Ueyama et al., 1985), and for primary (Hand, 1966; Rustioni and Macchi, 1968; Keller and Hand, 1970; Kuo and de Groat, 1985; Wild, 1985) and postsynaptic (Rustioni, 1973, 1974; Rustioni et al., 1979) afferent fibers in other species.

Internal basilar nucleus

The IBn was described by Ramón y Cajal (1909) and Torvik (1956) as located in the ventromedial dorsal horn of upper cervical spinal cord. The IBn receives input from several sources. In the present study, injections of B-HRP into DRGs of the cervical enlargement labeled many primary afferent fibers within the IBn throughout the length of the cervical cord. Others have also presented evidence for primary afferent input to the region of the IBn extending throughout the cervical spinal cord in rats (Webster and Kemplay, 1987) and monkeys (Shriver et al., 1968). The IBn also appears to receive projections from somatosensory cortex in cats (Armand et al., 1985) and rats (Zimmerman et al., 1964; Brown, 1971; Antal, 1984). The present studies have shown that postsynaptic afferent fibers from the cervical enlargement also project to the IBn.

The output of the IBn is not well understood. A large number of neurons in the IBn in segments C1-2 project to the ventrobasal thalamus (Giesler et al., 1979a; Granum, 1986; Kemplay and Webster, 1986). The projections of cells in the IBn in more caudal cervical segments are not known. It is also not known whether neurons in the rostral IBn have projections to areas other than the VBC.

The functional characteristics of neurons in the IBn have not been specifically investigated. Therefore, although the internal basilar nucleus receives a massive direct input from both primary and postsynaptic afferent fibers, stretching from the cervical enlargement to the medulla, the response characteristics of its neurons and the full range of their connections are not known.

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