THE JOURNAL OF COMPARATIVE NEUROLOGY 208:67-84 (1982)

Raphespinal Projections in the North American Opossum: Evidence for Connectional Heterogeneity

G.F. MARTIN, T. CABANA, F.J. DITIRRO, R.H. HO, AND A.O. HUMBERTSON, JR.
Department of Anatomy, College of Medicine, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

Retrograde transport studies revealed that the nuclei pallidus, obscurus, and magnus raphae as well as the adjacent reticular formation innervate the spinal cord in the opossum. HRP-lesion experiments showed that a relatively large number of neurons within the nucleus obscurus raphae and closely adjacent areas of the nucleus reticularis gigantocellularis project through the ventrolateral white matter and that many cells within the nucleus magnus raphae, the nucleus reticularis gigantocellularis pars ventralis, and the nucleus reticularis pontis pars ventralis contribute axons to the dorsal half of the lateral funiculi. Neurons within the rostral pole of the nucleus magnus raphae and the adjacent nucleus reticularis pontis pars ventralis may project exclusively through the latter route.

Each of the above-mentioned raphe and reticular nuclei contain non-indolaminergic as well as indolaminergic neurons (Crutcher and Humbertson, '78). When True-Blue was injected into the spinal cord and the brain processed for monoamine histofluorescence evidence for True-Blue was found in neurons of both types.

Injections of ^{3}H-leucine centered within the nuclei pallidus and obscurus raphae and/or the closely adjacent nucleus reticularis gigantocellularis labeled axons within autonomic nuclei and laminae IV–X. Labeled axons were particularly numerous within the intermediolateral cell column and within laminae IX and X. Injections of the caudoventral part of the nucleus magnus raphae or the adjacent nucleus reticularis gigantocellularis pars ventralis labeled axons in the same areas as well as within laminae I–III. When the injection was placed within the rostral part of the nucleus magnus raphae or the adjacent nucleus reticularis pontis pars ventralis axons were labeled within laminae I–III and external zones of laminae IV–VII, but not within lamina IX. The immunohistofluorescence method revealed evidence for indolaminergic axons in each of the spinal areas labeled by injections of ^{3}H-leucine into the raphe and adjacent reticular formation. They were particularly abundant within the intermediolateral cell column and within laminae IX and X. These data indicate that raphe spinal systems are chemically and connectionally heterogeneous.

The present paper is the third in a series dealing with spinal projections from the brainstem core of the adult opossum. Such studies are of comparative interest and also provide a basis for interpreting the developmental history and significance of bulbospinal connections in the same species (e.g., Martin et al., '78; Humbertson and Martin, '79; Cabana and Martin, '81). The reader is referred to Martin et al. ('78) for a discussion of the advantages offered by the opossum for such studies. In the preceding communications (Martin et al., '79b, '81b) we reported the

Accepted April 15, 1982.

0021-9967/82/2081-0067$05.00

results of experiments using axonal transport methods which indicated that reticulospinal connections are more extensive and complex than suggested by degeneration techniques (Beran and Martin, '71; Martin and Dom, '71; Martin et al., '75). Raphe neurons also innervate the spinal cord in the opossum (Crutcher et al., '78), but the course and terminal distribution of raphespinal axons has not been studied. In fact, the organization of raphespinal projections as to the distribution of axons from different nuclei has not been determined for any species. Since the raphe nuclei and closely adjacent reticular formation have a similar cytoarchitecture (see discussion by Taber et al., '60, on the cat) and indolamine neurons are found in both areas (the opossum, Crutcher and Humbertson, '78; the rat, Dahlström and Fuxe, '64; Steinbusch, '81; the cat, Poitras and Parent, '78; Wiklund et al., '81; the rhesus monkey, Schofield and Everett, '81), they will be considered together in this account.

Our strategy was first to identify the raphe and adjacent reticular nuclei which project to different levels of the spinal cord using the retrograde transport of horseradish peroxidase (HRP) and fluorescent markers. The contribution of axons from each nucleus to different funiculi was studied using HRP-lesion experiments and the direct application of HRP to injured axons. Since raphe and adjacent nuclei contain non-indolaminergic as well as indolaminergic neurons (Crutcher and Humbertson, '78) we attempted to determine if both types project to the spinal cord. For those studies fluorescent tracers were used in conjunction with the Falck-Hillarp method (Björklund and Skagerberg, '79). In parallel experiments injections of ^{3}H-leucine were made into those regions of the raphe and adjacent reticular formation which innervate the spinal cord so that the spinal distribution of their axons could be studied autoradiographically. The location of labeled axons within the spinal cord was then compared with that of axons containing 5-HT immunoreactivity.

METHODS

Horseradish peroxidase injections were made either by pressure or iontophoresis (Graybiel and Devor, '74) into different levels of the spinal cord in 20 deeply anesthetized opossums. All animals were stabilized in a spinal stereotaxic frame so as to minimize movement of the cord. In three animals lumbar injections were made after sectioning different portions of the white matter at midthoracic levels and in two animals HRP was applied directly to injured axons in the dorsal part of the lateral funiculi. Horseradish peroxidase was also injected iontophoretically into the superior cervical sympathetic ganglion of two opossums in order to label neurons within the intermediolateral cell column. After a 2–3 day survival all animals were reanesthetized and sacrificed by perfusion with a 1% paraformaldehyde-1.5% glutaraldehyde solution. Frozen sections of the injection sites and brainstems were processed histochemically for HRP with either 0-dianisidine (de Olmos, '77) or tetramethylbenzidine (Mesulam, '76) as chromagens. The processed sections were stained lightly with neutral red and examined with the aid of light and darkfield condensers. The location of brainstem neurons labeled by the spinal injections was plotted on drawings of selected sections. The terminology used for nuclei of the opossum's brainstem was taken from Oswaldo-Cruz and Rocha-Miranda ('68). Brains of animals with no injections or with lumbar injections placed after complete transection of the thoracic cord served as controls.

Evans-Blue, 4′,-6′-diamidino-2 phenyl-indol 2HCl (DAPI), bisbenzimide, True-Blue, and Nuclear-Yellow were also used to label neurons projecting to different spinal levels (Van der Kooy et al., '78; Bentivoglio et al., '79a,b; Kuypers et al., '79). Volumes of 3–4 μl (1–10% solutions) were injected into the spinal cords of 15 anesthetized animals by using a glass pipette attached to a Hamilton syringe. Many animals were used for double-labeling experiments (Martin et al., '81a), so one marker was injected into the cervical enlargement and another into either the thoracic or lumbar cord. Care was taken to avoid damaging those areas of the white matter known to contain bulbar axons (Martin et al., '79b, '81b). One to 7 days after surgery the animals were deeply anesthetized and their brains and spinal cords removed without perfusion. Blocks of the brainstem as well as the injected spinal segments were frozen with dry ice and cut into glass slides with a cryostat. Unstained sections were coverslipped with a nonfluorescent mounting medium and examined with a Leitz fluorescence microscope at excitation wavelengths of 360 and 550 nm. The position of single- and double-labeled neurons was recorded on drawings of selected sections using an X-Y plotter interfaced with the microscope stage by potentiometers.

Five animals with injections of True-Blue were treated with L-tryptophan and pargyline (Sigma) prior to sacrifice. After being cut into suitable blocks the brains were frozen in isopentane cooled with liquid nitrogen, placed into a lyophilizer for 10–14 days, and exposed to paraformaldehyde vapors. Paraffin-embedded sections were cut on a rotary microtome, mounted, coverslipped with Entellan (Merck), and examined with a Leitz fluorescence microscope at excitation wavelengths of 360 and 490 nm. Every third section was stained for Nissl substance. The position of neurons showing the fluorescence characteristic of True-Blue and/or serotonin was plotted on drawings of the sections using an X-Y recorder.

For the autoradiographic experiments pressure injections of ^{3}H-leucine (0.1–0.3 μl, concentrated to 100–400 μCi/μl) were centered within the raphe or closely adjacent reticular formation of 27 opossums. After 8–14 days each animal was reanesthetized and perfused through the heart with saline followed by 10% formalin. Frozen sections of the brainstem and spinal cord were mounted, coated with a diluted NTB-2 liquid emulsion and refrigerated for 4–12 weeks. The slides were developed with a D-19 high-contrast developer and stained through the emulsion with cresyl violet before being coverslipped and examined with darkfield optics. We have chosen to include the halo around the focus of intense activity as part of the injection site and have defined terminal label as a dispersion of silver grains over areas of gray matter to which labeled axons can be traced. The injection sites and the distribution of spinal label were drawn from the darkfield image.

For the most part we employed the indirect immunohistofluorescence method of Coons ('58) to localize serotonin (5-HT) immunoreactivity in the spinal cord. Four adult opossums were anesthetized and sacrificed by intracardiac perfusion with phosphate-buffered saline (PBS), followed by ice-cold, freshly prepared 4% paraformaldehyde in phosphate buffer (Pease, '62). The spinal cords were promptly removed and placed in the same fixative for 6–8 hours (4°C), followed by immersion in 5% sucrose-Sorenson's

phosphate buffer at 4°C for 2–3 days. Cryostat sections were obtained from representative levels of the cord and mounted on chrome-alum-gelatin-coated slides. The tissues were rehydrated with PBS and incubated consecutively with antiserotonin serum (194D supplied by Dr. Robert Elde, University of Minnesota, diluted 1:100, 1:200, and incubated overnight at 4°C) and with fluorescin-isothiocyanate-conjugated goat antirabbit Immunoglobulin-G (GAR) antiserum (diluted 1:30 and incubated 1 hour at room temperature). The diluent for all antisera was PBS containing 0.3% triton X-100 (Octyl Phenoxy Polyethoxyethanol). The sections were rinsed and immersion washed in PBS after each incubation. Following the incubation with the secondary antiserum (GAR), the sections were washed in PBS, coverslipped with a PBS-glycerine solution (1:3), and studied with a Zeiss fluorescence microscope at excitation wavelengths of 460–490 nm. Adjacent sections of some cases were processed using the same antiserum (194D) at a higher dilution (1:1,000) by the indirect antibody peroxidase-antiperoxidase (PAP) technique of Sternberger et al. ('70). In order to establish immunostaining specificity, adjacent sections were incubated with control sera and processed in parallel with those receiving the 5-HT antibody. The control serum consisted of the diluted anti-5HT serum that was pretreated overnight (4°C) with an excess of synthetic antigen (10 μg/ml of diluted antiserum).

The spinal cord of one anesthetized opossum was transected at T-8 in order to determine if spinal 5-HT could be depleted caudal to the lesion. The animal was allowed to survive for 10 days after which it was sacrificed and its spinal cord processed for 5-HT immunohistofluorescence.

RESULTS

The location of raphe and adjacent reticular neurons projecting to different levels of the spinal cord and the funicular trajectory of their axons

Spinal injections of HRP retrogradely labeled neurons within the nuclei pallidus and obscurus raphae (Fig. 1E–G), the nucleus reticularis gigantocellularis (Fig. 1E), the nucleus magnus raphae (Fig. 1A–D), the nucleus reticularis gigantocellularis pars ventralis (Fig. 1C,D), and the nucleus reticularis pontis pars ventralis (Fig. 1A,B). Examples of HRP-positive neurons are shown in Figures 3 and 4. A few neurons were also labeled within the nucleus dorsalis raphae after injections of rostral cervical segments. All of the above nuclei contain indolaminergic neurons (Fig. 6). Cervical injections of HRP labeled neurons throughout the length of the above nuclei (Fig. 1), whereas lumbar and sacral injections labeled few raphe neurons caudal to midolivary levels (Fig. 2, F and G) and only an occasional one rostral to the facial nucleus (Fig. 2, A,B). These data suggest the existence of a loose topographical organization. Comparable results were obtained using the retrograde transport of fluorescent tracers. The double labeling experiments using fluorescent markers revealed that some neurons within the nuclei obscurus and magnus raphae, as well as the nuclei reticularis gigantocellularis and gigantocellularis pars ventralis, innervate both cervical and lumbar levels by way of axonal collaterals. These data are reported elsewhere (Martin et al., '81a).

When HRP was injected into the lumbar cord after bilateral transection of the lateral funiculi at thoracic levels, HRP-positive neurons were numerous within the nucleus obscurus raphae and the adjacent nucleus reticularis gigantocellularis. In contrast, they were *relatively* sparse within the nucleus magnus raphae, the nucleus reticularis gigantocellularis pars ventralis, and absent within the nucleus reticularis pontis pars ventralis. When HRP was applied to injured axons in the dorsal half of the lateral funiculus at cervical levels, labeled neurons were abundant within the latter nuclei but *relatively* sparse within the nucleus obscurus raphae and the nucleus reticularis gigantocellularis. The results obtained from an HRP injection of the dorsal extreme of the lateral funiculus are plotted in Figure 5. It should be noted that labeled neurons were present within the rostral part of the nucleus magnus raphae and the adjacent reticular formation (Fig. 5 A,B), but not within the nuclei obscurus raphae and reticularis gigantocellularis (Fig. 5 D–F).

The origin of spinal axons which transport serotonin and evidence that non-serotoninergic neurons of the raphe innervate the spinal cord

When True-Blue was injected into the spinal cord and the brain processed by the Falck-Hillarp method, evidence for the fluorescent marker was found within indolaminergic neurons of the nuclei pallidus and obscurus raphae, the nucleus reticularis gigantocellularis, the nucleus mag-

Abbreviations

CcD	nucleus cochlearis dorsalis	RaM	nucleus magnus raphae
cr	corpus restiformis	RaO	nucleus obscurus raphae
CuL	nucleus cuneatus lateralis	RGc	nucleus reticularis gigantocellularis
DF	dorsal funiculus	RGcv	nucleus reticularis gigantocellularis: pars ventralis
Fac	nucleus n. facialis	RL	nucleus reticularis lateralis
Hg	nucleus n. hypoglossi	RP	nucleus reticularis pontis
LF	lateral funiculus	RPv	nucleus reticularis pontis: pars ventralis
LR	nucleus lateralis reticularis	RV	nucleus reticularis medullae oblongatae ventralis
Lumb	lumbar cord	Sac	sacral cord
Ol	nucleus olivaris inferior	TrMo	nucleus motorius n. trigemini
OSL	nucleus olivaris superior lateralis	tz	nucleus corporis trapezoidei
OSM	nucleus olivaris superior medialis	VF	ventral funiculus
pyr	tractus pyramidalis	VstI	nucleus vestibularis inferior

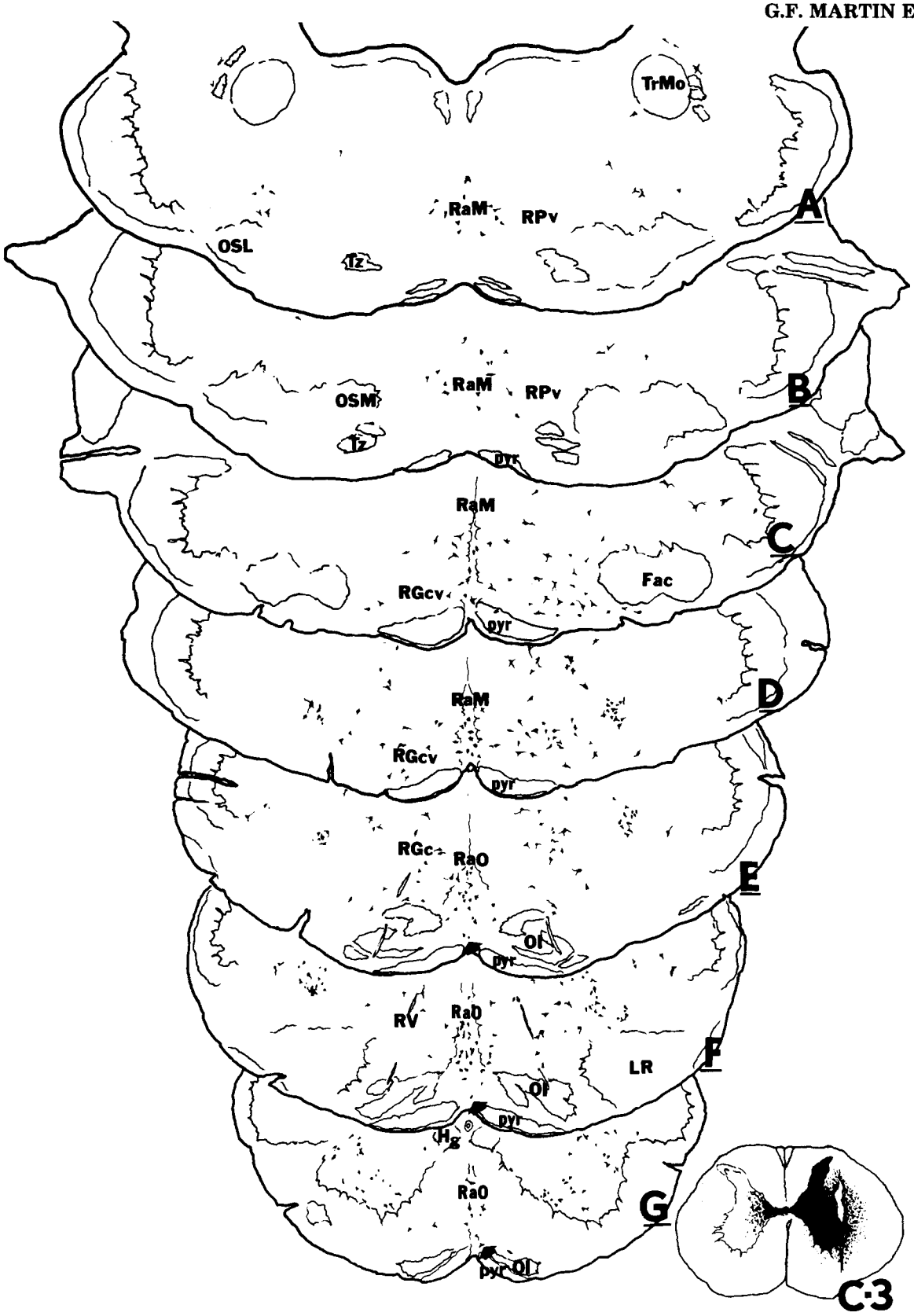


Fig. 1 Plotting of the neuronal labeling produced by an injection of horseradish peroxidase into the third cervical segment (C-3) of the spinal cord (lower right). The sections are stacked from rostral (A) to caudal (G). The labeled neurons were plotted from five adjacent sections cut at 20 μm. The small arrows indicate labeled neurons in the nucleus pallidus raphae.

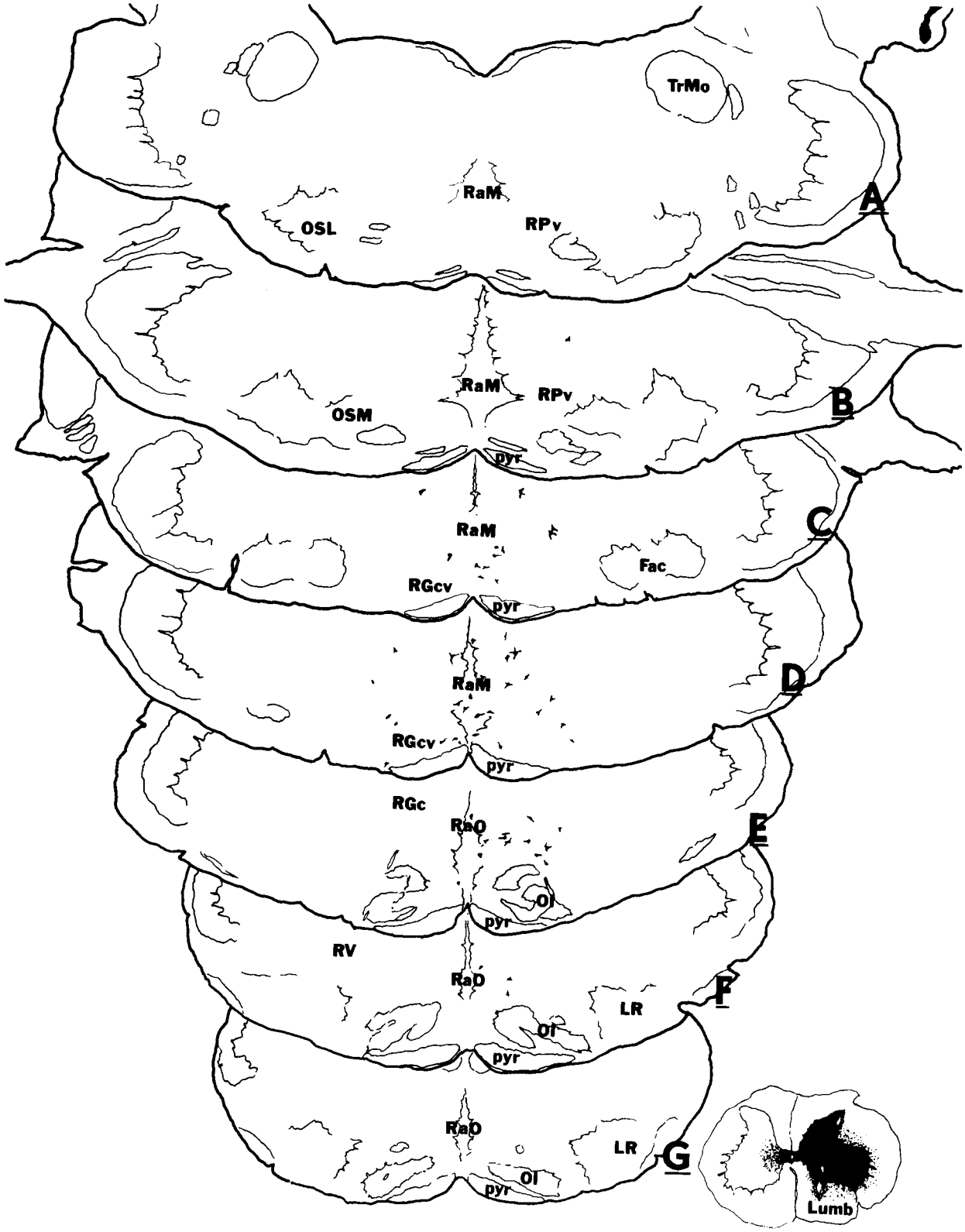


Fig. 2. Plotting of the neuronal labeling produced by an injection of horseradish peroxidase into the lumbar (Lumb) cord. The sections are comparable to those in Figure 1 and labeled neurons are charted from five adjacent sections cut at 20 μm.

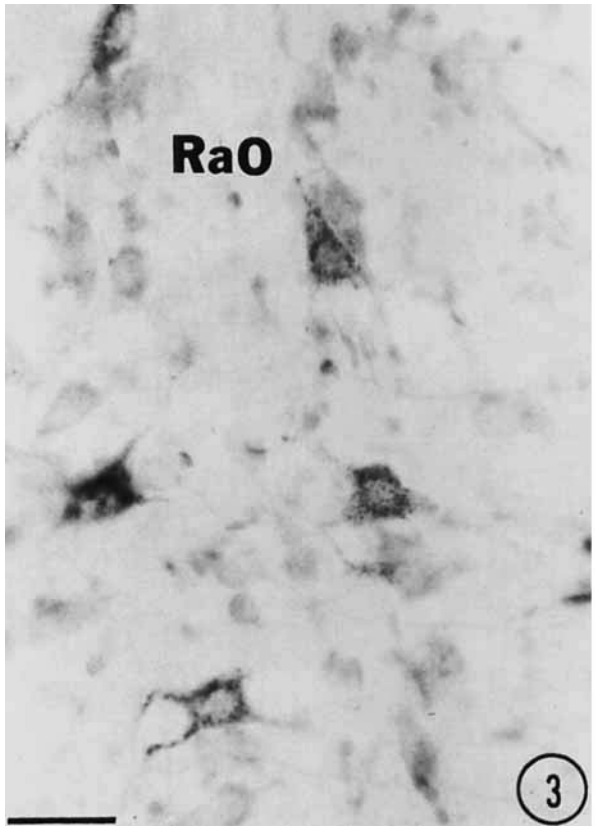


Fig. 3. Photomicrograph of neurons within the nucleus obscurus raphae (RaO) labeled by the injection of horseradish peroxidase shown in Figure 1. The section is lightly counterstained with neutral red. The bar indicates 27 μm and can also be used in Figure 4.

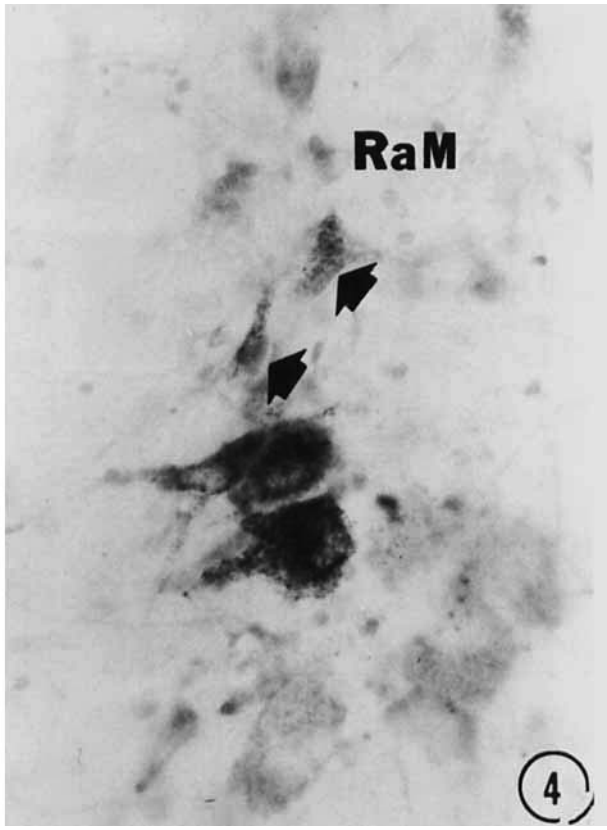


Fig. 4. Photomicrograph of neurons within the caudal part of the nucleus magnus raphae (RaM) labeled by the injection of horseradish peroxidase shown in Figure 1. The arrows point to some of the smaller neurons which are HRP-positive.

nus raphae, the nucleus reticularis gigantocellularis pars ventralis, and the nucleus reticularis pontis pars ventralis (Fig. 6). Our data were not conclusive concerning the nucleus dorsalis raphae. True-Blue was viewed at 360 nm and was not visible at the 490-nm wavelength used to identify indolamines. Unfortunately, the intense fluorescence of indolamines can be seen at 360 nm sometimes masking that of True-Blue. For that reason the number of neurons containing both True-Blue and indolamines is probably greater than plotted on Figure 6. It was clear, however, that many non-indolaminergic neurons were also labeled by the fluorescent marker (Figs. 6–8). Such neurons were most numerous in the reticular formation adjacent to the raphe, but they were also abundant within the raphe proper (Figs. 7, 8).

The spinal projections of raphe and closely adjacent reticular cells

Injections of ^{3}H-leucine centered within the nuclei obscurus and pallidus raphae or related portions of the nucleus reticularis gigantocellularis produced the spinal labeling illustrated for OP-509 in Figure 9. Comparable results were seen when the injection included the caudalmost part of the nucleus magnus raphae (OP-535, Fig. 9). At cervical and thoracic levels labeled axons were most numerous within the lateral funiculi but they were also present within the ventral and sulcomarginal funiculi. Axonal labeling was even present within the dorsal funiculi, but it has not been plotted because of its sparsity. At lumbar and sacral levels labeled axons were most numerous within dorsolateral areas. Terminal label was present within laminae IV–VIII, but it was most abundant within the area judged to be the intermediolateral cell column (Fig. 12), within lamina IX (Figs. 13, 14), and within lamina X (Fig. 12). The labeling in lamina IX often outlined the somata and proximal dendrites of presumed motoneurons (Figs. 13, 14). Labeled axons could also be traced to the sacral parasympathetic nucleus (Sac sections in Fig. 9) as defined by DeGroat et al. ('78) in the cat. Cases with injections limited to caudal levels of the nucleus obscurus raphae and the adjacent reticular formation provided evidence for projections to laminae V–X at cervical levels and to the intermediolateral cell column. As predicted from the HRP studies there was little evidence for projections to lumbosacral segments.

Since the intermediolateral cell column is not distinct in Nissl-stained sections of the opossum's cord, it was defined by injections of HRP into the superior cervical sym-

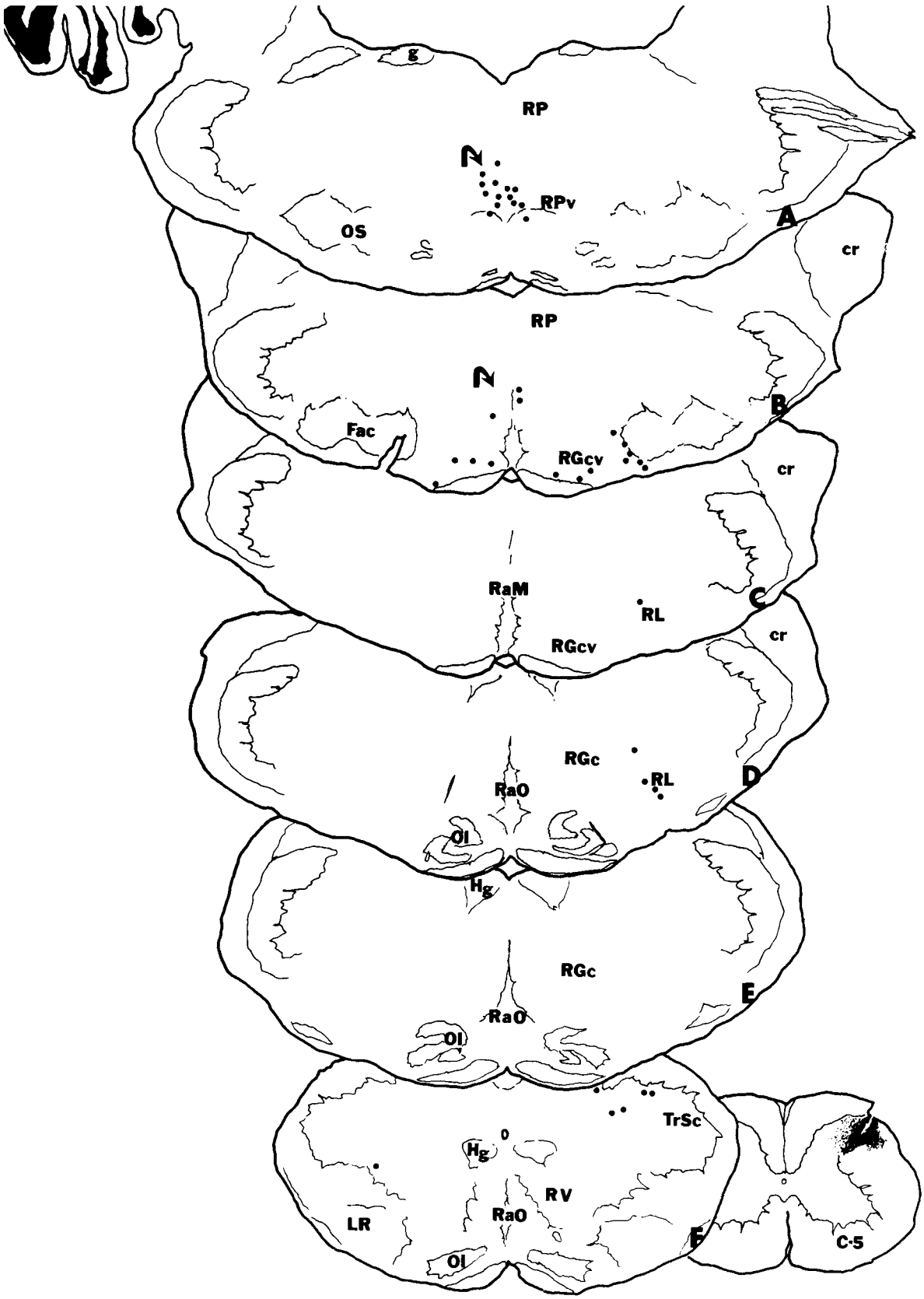


Fig. 5. Plotting of the neuronal labeling produced by an injection of horseradish peroxidase into the dorsal part of the lateral funiculus at the fifth cervical segment (lower right). The sections are stacked from rostral (A) to caudal (F) and labeled neurons (arrows) are plotted from five adjacent sections cut at 20 μm.

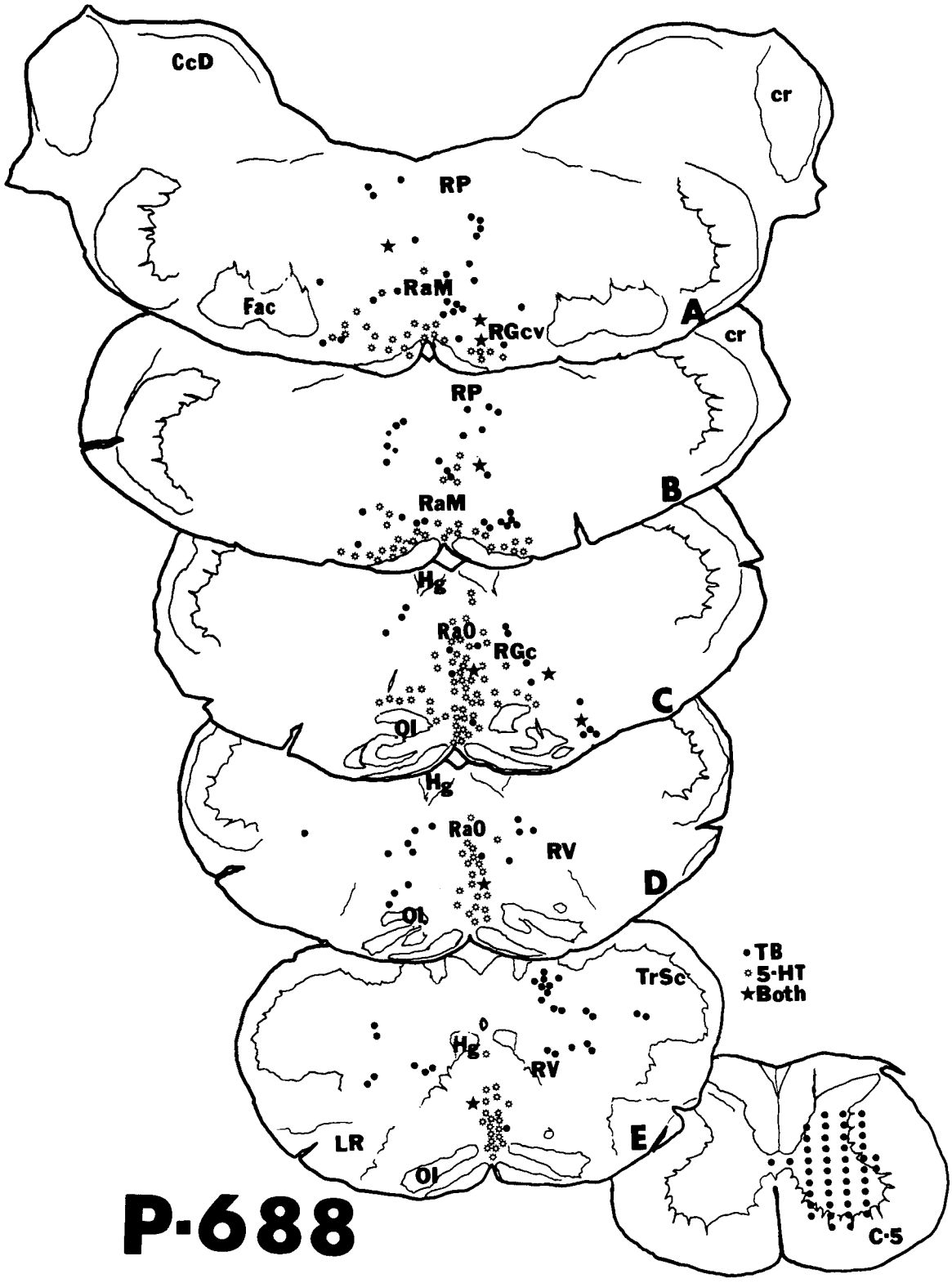


Fig. 6. Plotting of indolaminergic and/or True-Blue-filled neurons in selected sections from rostral (A) to caudal (E). Neurons containing only True-Blue (·), True-Blue plus indolamines (*), as well as only indolamines (○) are indicated.

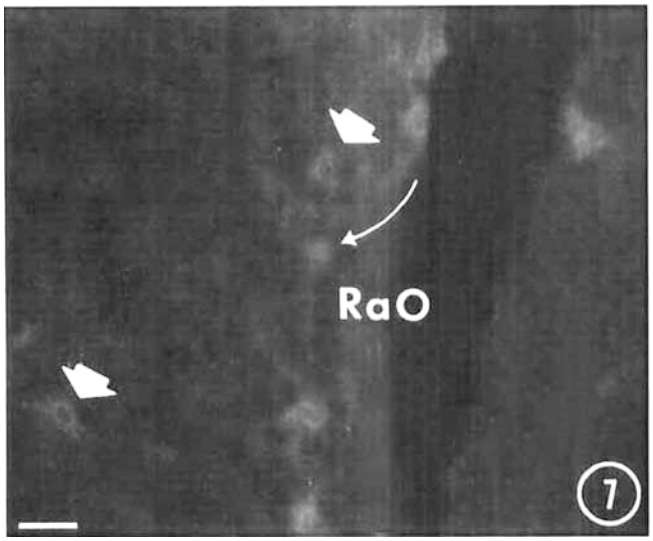


Fig. 7 Photomicrograph of neurons within the nucleus obscurus raphae (RaO), some of which display at 360 nm the fluorescence characteristic of True-Blue as it appears in Falck-Hillarp-processed material. Comparison with Figure 8, photographed at 490 nm to show 5-HT fluorescence, should make it clear that the neurons indicated by the block arrows exhibit the fluorescence of True-Blue but not of indolamines. Notice, however, that the intense fluorescence of indolamines shows through at 360 nm. One indolamine neuron is indicated by the curved arrow to emphasize its proximity to those containing only True-Blue. The bar equals 40 μm and can be used for Figure 8.

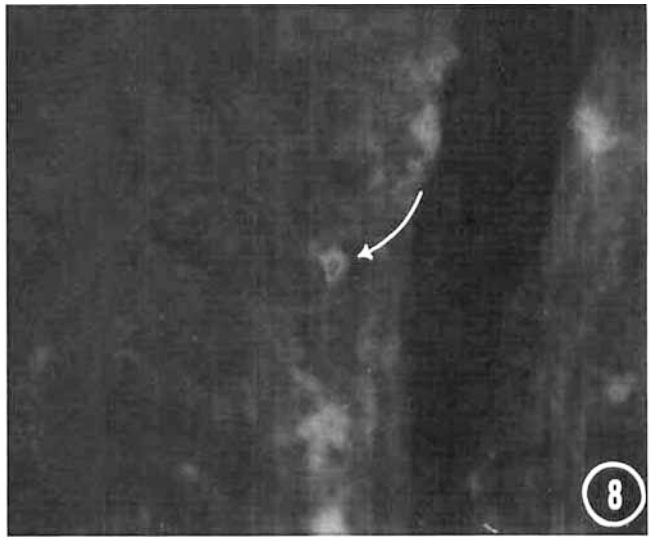


Fig. 8. Photomicrograph of the field shown in Figure 7 but viewed at an excitation wavelength of 490 nm. Many of the neurons in the field display the fluorescence characteristic of indolamines. The neuron indicated by the curved arrow is intensely fluorescent for indolamines and is similarly indicated in Figure 7.

pathetic ganglion. Labeled neurons (Fig. 18) were found within the dorsolateral white matter, within the principal part of the intermediolateral cell column, between the latter area and lamina X, and within lamina X. Such areas have been referred to in the rhesus monkey as pars funicularis, pars principalis, pars intercalatus, and pars paraependymalis of the nucleus intermediolateralis thoracolumbalis (Petras and Cummings, '72). As expected, these were the regions labeled most extensively in the autoradiographic material (Fig. 12).

Figure 10 shows plots of the spinal labeling produced by injections of ^{3}H-leucine into the nucleus magnus raphae (OP-537) and the nucleus reticularis gigantocellularis pars ventralis (OP-526). There was no spread of either injection to the nuclei obscurus raphae and reticularis gigantocellularis or to the rostral part of the nucleus magnus raphae and adjacent reticular formation included in the cases illustrated in Figure 11. In both of the cases shown in Figure 10 labeling was present within autonomic nuclei and laminae III–X although different amounts were found at caudal levels of the cord. In addition, labeled axons could be traced from dorsal areas of the lateral funiculi to lamina I and the outer portions of lamina II (Fig. 15).

Figure 11 illustrates the axonal labeling observed after injections of the rostral part of the nucleus magnus raphae (OP-547) and the nucleus reticularis pontis pars ventralis (OP-517). Neither injection spread to the areas covered by those illustrated in Figures 9 and 10. In both cases shown in Figure 11 labeled axons could be traced from the extreme dorsal parts of the lateral funiculi to laminae I and II (sparse) and to portions of laminae III–VI. Some labeling was also present within laminae VII and VIII and within autonomic nuclei, but not within lamina IX. As predicted from the retrograde studies labeled axons were sparse at lumbosacral levels. Injections limited to the dorsomedial part of the nucleus reticularis pontis with no involvement of the nucleus magnus raphae or the nucleus reticularis pontis pars ventralis produced spinal labeling which was restricted to the sulcomarginal and ventral funiculi and laminae VII and VIII.

Location of serotoninergic (5-HT) immunoreactivity at spinal levels

The results obtained by the indirect immunohistofluorescence and PAP immunohistochemical techniques were essentially comparable and are plotted in Figure 16. At all levels 5-HT immunoreactive elements were numerous within the lateral funiculus, but only scattered more ventrally. In some sections a few were present in the dorsal funiculi. The most compact bundle of 5-HT immunoreactive fibers (arrow in Fig. 16) was located in the dorsolateral portion of the lateral funiculus. The comparable region was heavily labeled in some of the autoradiographic cases (e.g., see arrows in Fig. 9). Indolaminergic varicosities were present within laminae IV–VIII, but they were most abundant within the intermediolateral cell column, lamina IX, and lamina X (Fig. 16). The distribution of 5-HT immunoreactivity within the intermediolateral cell column at T-3 can be compared with that of the neurons labeled at the same level by an injection of HRP into the superior cervical sympathetic ganglion (Figs. 17, 18). The 5-HT immunoreactive elements within lamina IX tended to cluster around the somata and proximal dendrites of presumed motoneurons (Fig. 19). Indolaminergic immuno-

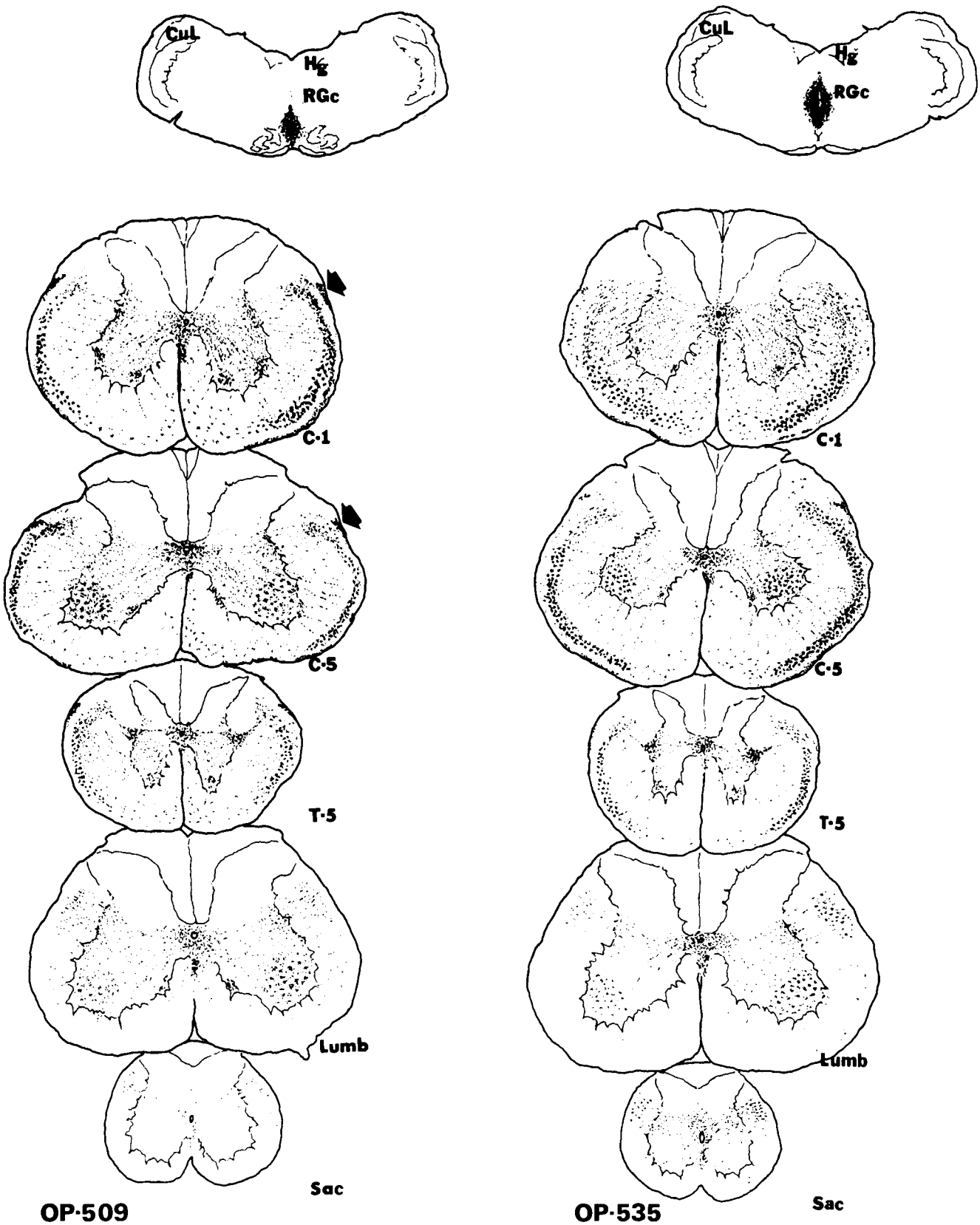


Fig. 9. Two drawings of cervical (C-1, C-5), thoracic (T-5), lumbar (Lumb), and sacral (Sac) sections of the spinal cord showing the labeling produced by the brainstem injections of ^{3}H-leucine illustrated at the top for each case. In OP-509 the injection was centered within the nuclei obscurus and pallidus raphae, whereas in OP-535 it included the caudal part of the nucleus magnus raphae and the nucleus obscurus raphae. The illustrations shown here and in Figures 11 and 12 were drawn from material exposed for 12 weeks and taken from animals which survived for 10 days after the injection.

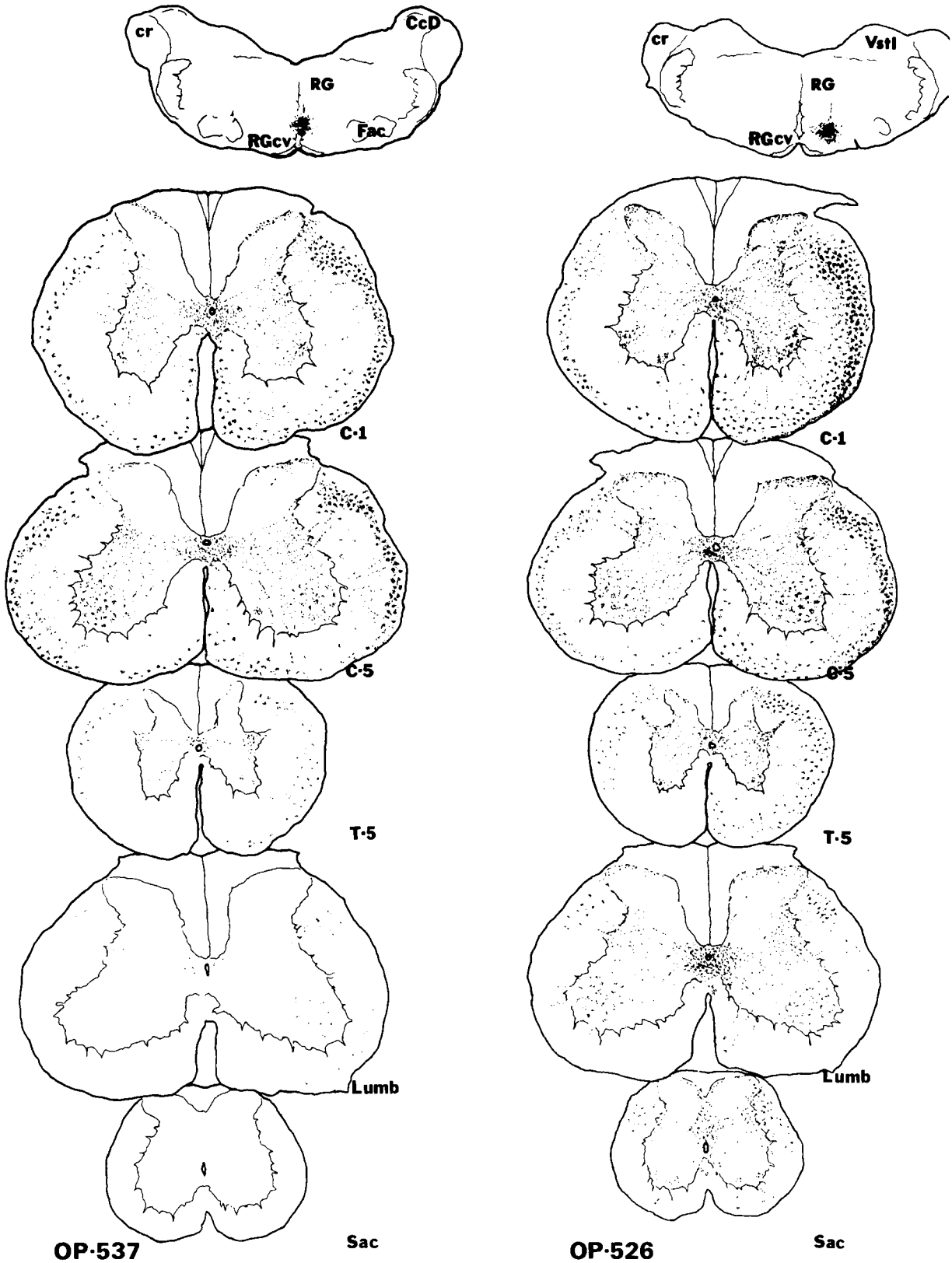


Fig. 10. Two plots of cervical (C-1, C-5), thoracic (T-5), lumbar (Lumb), and sacral (Sac) sections of the spinal cord showing the labeling produced by the brainstem injections of ^{3}H-leucine shown at the top for each case. In OP-537 the injection was centered within a part of the nucleus magnus raphae located rostral to that included in OP-535 (Fig. 9), whereas in OP-526 it was restricted to the nucleus reticularis gigantocellularis pars ventralis.

reactive varicosities were also present within laminae I–III where they tended to aggregate within lamina I and the outer part of lamina II (Fig. 20). Essentially all 5-HT immunofluorescence disappeared caudal to transection of the spinal cord at T-8. Sections incubated in the control serum did not exhibit immunostaining.

DISCUSSION

The nuclei pallidus, obscurus, and magnus raphae of the opossum's brainstem (Oswaldo-Cruz and Rocha-Miranda, '68) correspond in position and cytoarchitecture to the comparably designated nuclei in the cat (Taber et al., '60) and probably to the B_1, B_2, and B_3 groups of the rat (Dahlström and Fuxe, '64). The occurrence of indolamine neurons far outside of the raphe is not unique to the opossum and has been reported for the rat (e.g., Steinbusch, '81) and cat (Wiklund et al., '81).

In the opossum, bulbospinal projections arise from the nuclei pallidus, obscurus and magnus raphae, and from adjacent reticular nuclei (the nucleus reticularis gigantocellularis, the nucleus reticularis gigantocellularis pars ventralis, and the nucleus reticularis pontis pars ventralis). Apparently comparable results have been reported for the rat (Basbaum and Fields, '79; Satoh, '79; Zemlan and Pfaff, '79), cat (Kuypers and Maisky, '75, '77; Basbaum and Fields, '79; Tohyama et al., '79), and rhesus monkey (Kneisley et al., '78). Many areas of the above nuclei project the length of the spinal cord and some of the neurons within them innervate both the cervical and lumbar enlargements via axonal collaterals (the opossum, Martin et al., '81a; the rat, Huisman et al., '81; the cat, Hayes and Rustioni, '81). A few neurons within the nucleus dorsalis raphae also project to the spinal cord, but their axons only reach rostral cervical levels. A similar observation has been made in the rat by Bowker et al. ('81) and in the cat by Tohyama et al. ('79).

Our HRP-lesion experiments revealed that many of the neurons within the nucleus obscurus raphae and the adjacent nucleus reticularis gigantocellularis project through ventral and ventrolateral areas of the white matter and that a disproportionately large number of the neurons within the nucleus magnus raphae, the nucleus reticularis gigantocellularis pars ventralis, and the nucleus reticularis pontis pars ventralis project through the dorsal part of the lateral funiculi. Neurons within the rostral pole of the nucleus magnus raphe and the nucleus reticularis pontis pars ventralis may project exclusively through dorsolateral channels. Generally comparable results have been described for the rat (Basbaum and Fields, '79) and cat (R.F. Martin et al., '78; Basbaum and Fields, '79), suggesting a basic mammalian pattern.

The distribution of serotoninergic (5-HT) varicosities within the spinal cord of the opossum is similar to that of the rat (e.g., Fuxe, '65; Steinbusch, '81). Although it is well known that spinal 5-HT is transported in bulbar axons (Dahlström and Fuxe, '65; Bowker et al., '81), the contribution made by different nuclei to specific projections has not been dissected in any species. Comparison of the autoradiographic labeling with the distribution of 5-HT in the spinal cord suggests that in the opossum: (1) the 5-HT projections to autonomic nuclei, lamina IX, and lamina X originate within the nucleus obscurus raphae, the nucleus reticularis gigantocellularis, caudal parts of the nucleus magnus raphae and the nucleus reticularis gigantocellularis pars ventralis, and (2) the 5-HT innervation of laminae I and II originates within the nucleus magnus raphae, the nucleus reticularis gigantocellularis pars ventralis, and the nucleus reticularis pontis pars ventralis.

Spinal axons also arise from many non-indolaminergic neurons in the raphe and adjacent reticular formation. Similar findings have been reported for the rat (Björklund and Skagerberg, '79; Bowker et al., '81). These data suggest that some of the functions subserved by raphespinal connections (see below) do not involve serotonin. It is of interest that enkephalin and substance-P immunoreactive neurons of the raphe and reticular formation innervate the spinal cord (enkephalin neurons, Hökfelt et al., '79; enkephalin and substance-P neurons, Bowker et al., '81), since both peptides have been found within spinal targets of raphe axons (the opossum, DiTirro et al., '81).

The effect of raphe and reticular stimulation on sympathetic (e.g., Adair et al., '77; Coote and MacLeod, '74; Henry and Calaresu, '74) and parasympathetic (e.g., Tokonugo and Kuru, '59) activity has been studied in some detail. However, the role played by indolaminergic versus non-indolaminergic neurons is not clear. The function(s) of raphe projections to motoneurons has not been studied directly, but it seems that serotonin acts as a gain setter for excitatory inputs to such neurons (McCall and Aghajanian, '79).

The role of the nucleus magnus raphae on centrally induced analgesia has been studied extensively (see review by Basbaum and Fields, '78) and the part played by that nucleus and the adjacent reticular formation may be different (Satoh et al., '80). Although a vast literature implicates serotoninergic axons in that function (Akil and Liebeskind, '75; Besson et al., '81; Satoh et al., '80; Yaksh and Wilson, '79), 5-HT antagonists do not necessarily block the effects of raphe magnus stimulation on spinal interneurons (Belcher et al., '78; Griersmith et al., '81; Yezierski et al., '81) and stimulation-induced analgesia can be produced after depletion of spinal monoamines (Johanessen et al., '80). Our finding that non-serotoninergic as well as serotoninergic neurons of the nucleus magnus raphae innervate the spinal cord may provide an anatomical basis for these functional observations.

It is apparent from our developmental studies that raphe and reticular axons are among the first ones from the brainstem to reach the spinal cord (Martin et al., '78; Cabana and Martin, '81). We have observed 5-HT immunoreactivity in raphe and reticular neurons at birth (12 days after conception) and it is assumed that such neurons give rise to the many 5-HT immunoreactive axons present in the spinal cord (marginal zone) at the same age (unpublished results). Raphe and reticular neurons can be labeled by spinal injections of HRP by at least postnatal day 5

Fig. 11. Two plots of cervical (C-1, C-5), thoracic (T-5), lumbar (Lumb), and sacral (Sac) sections of the spinal cord showing the labeling produced by the brainstem injections of ^{3}H-leucine illustrated for each case. The injection in OP-547 was centered within the rostral part of the nucleus magnus raphae, whereas that in OP-517 was localized within the nucleus reticularis pontis pars ventralis.

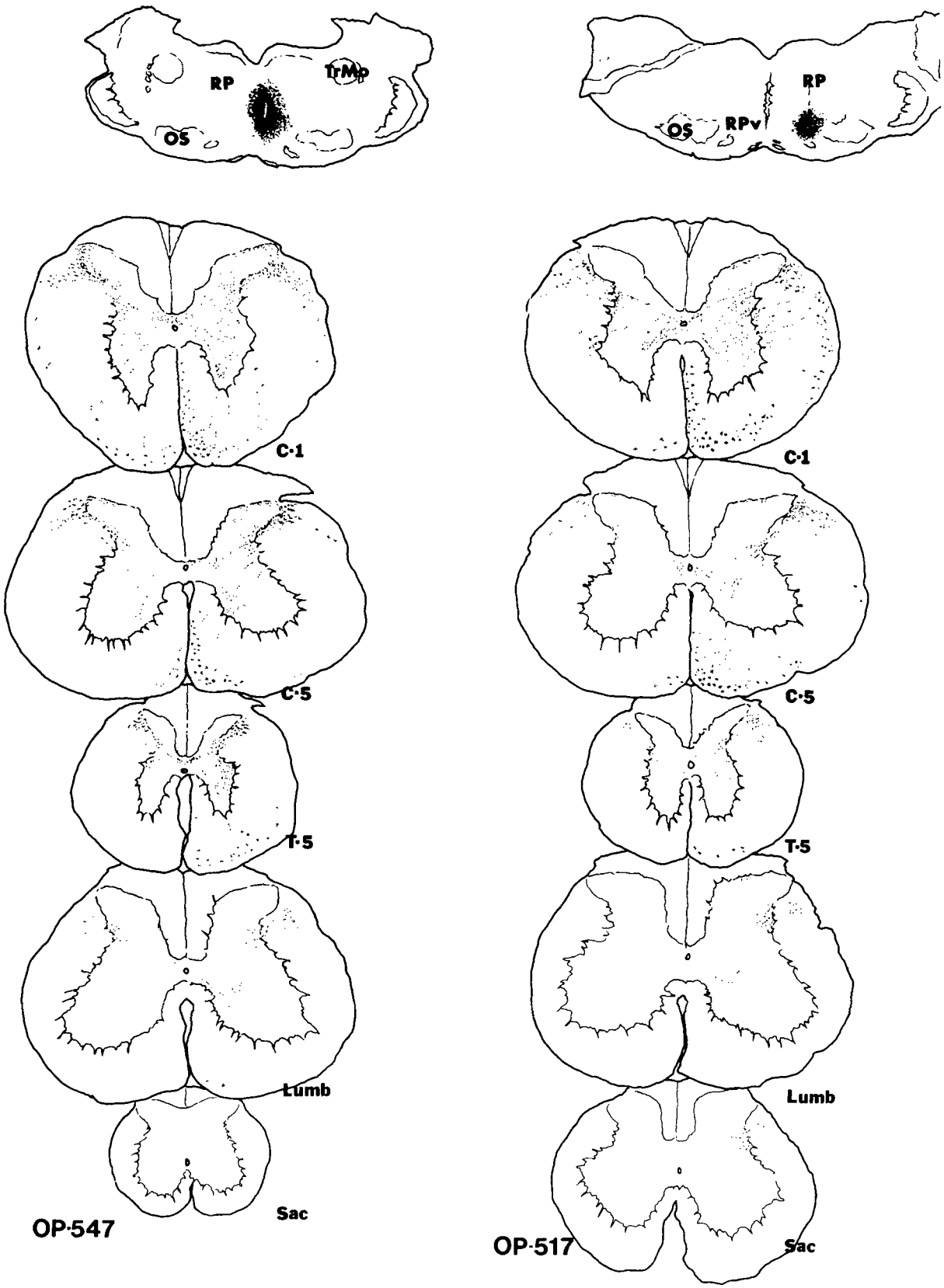
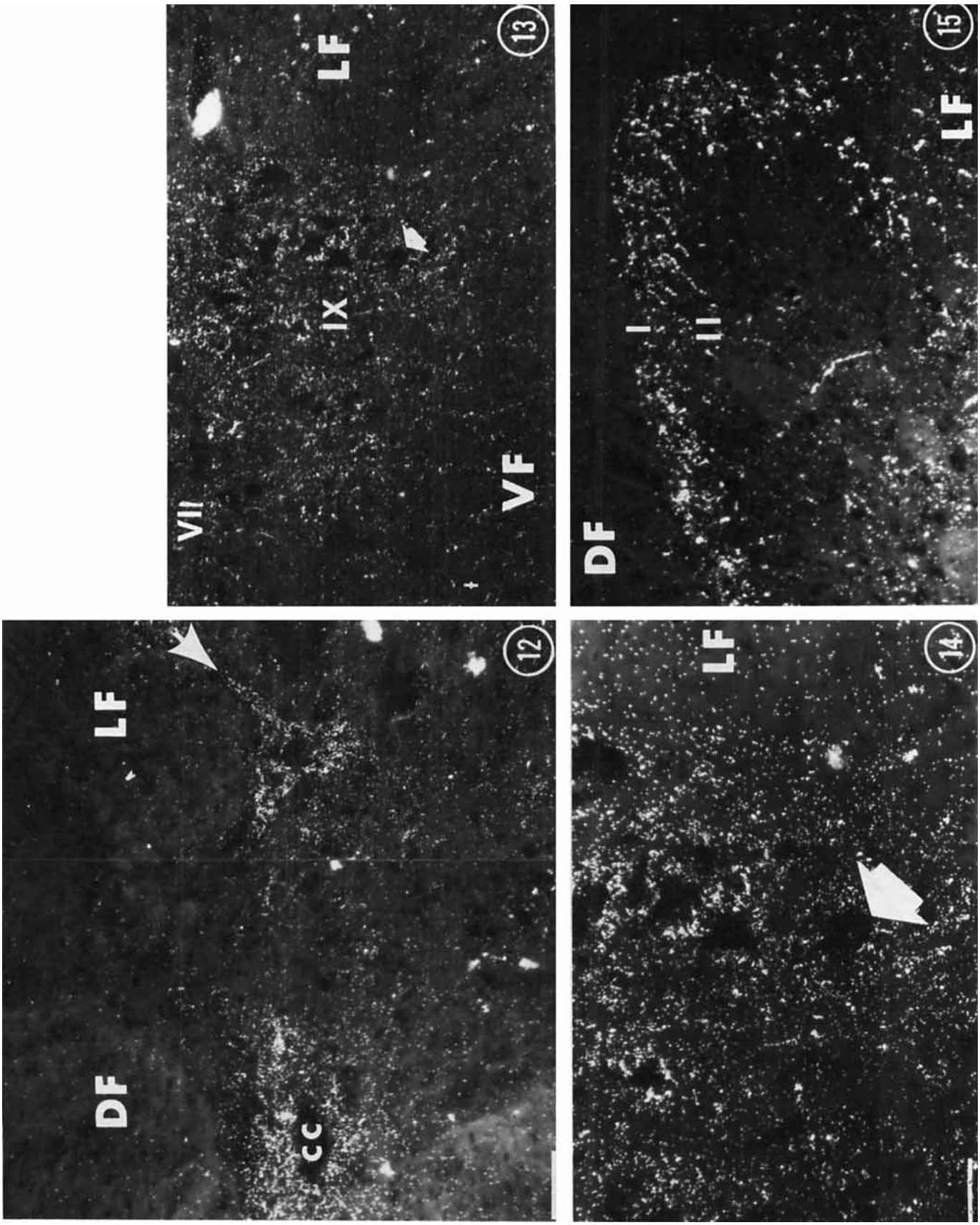

RP
TrMp
OS
RP
OS
RPv
C·1
C·5
T·5
Lumb
Sac
OP·547
C·1
C·5
T·5
Lumb
Sac
OP·517

Figure 11


12
LF
DF
CC
13
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IX
VII
VF
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LF
15
LF
I
II
DF

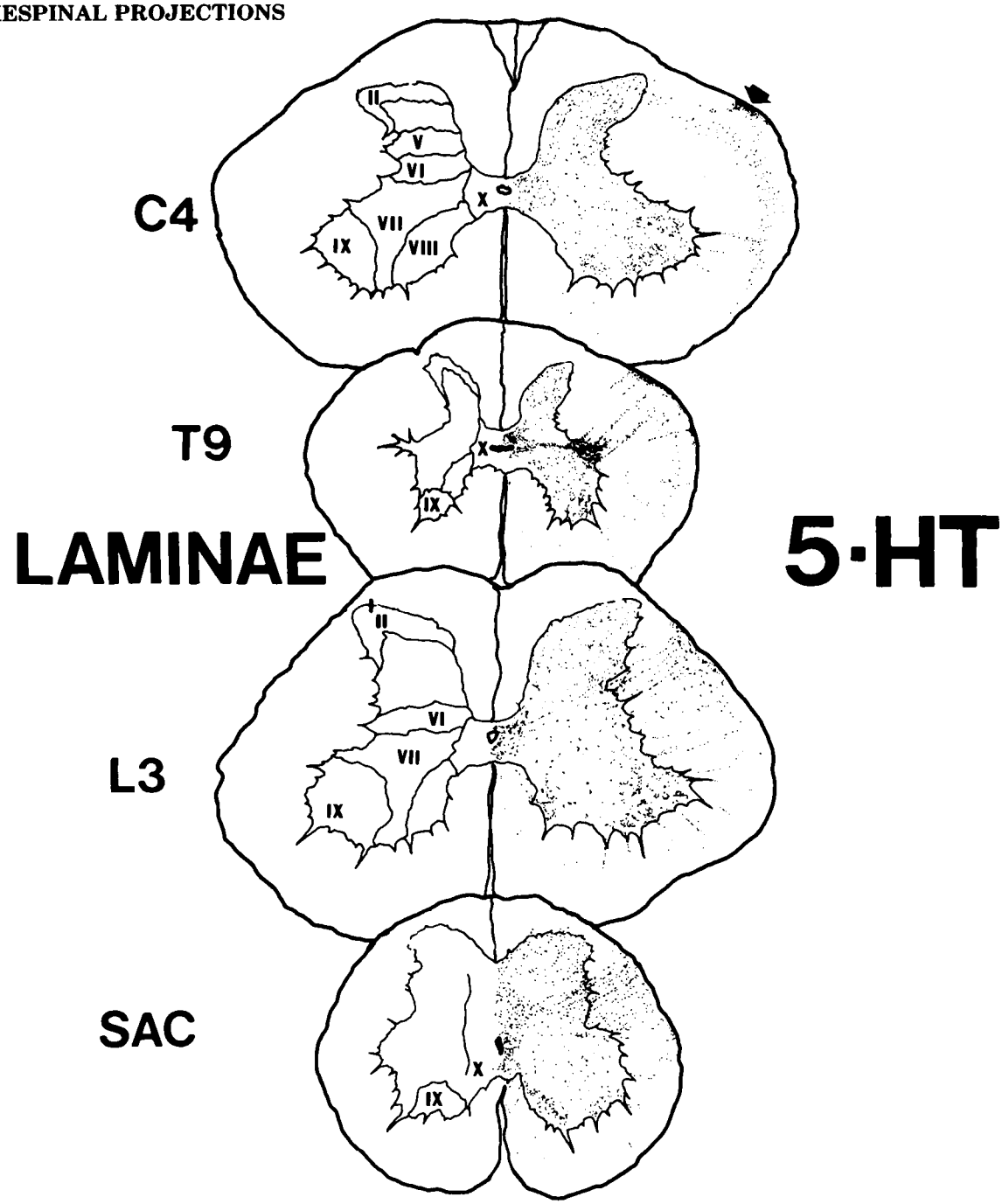


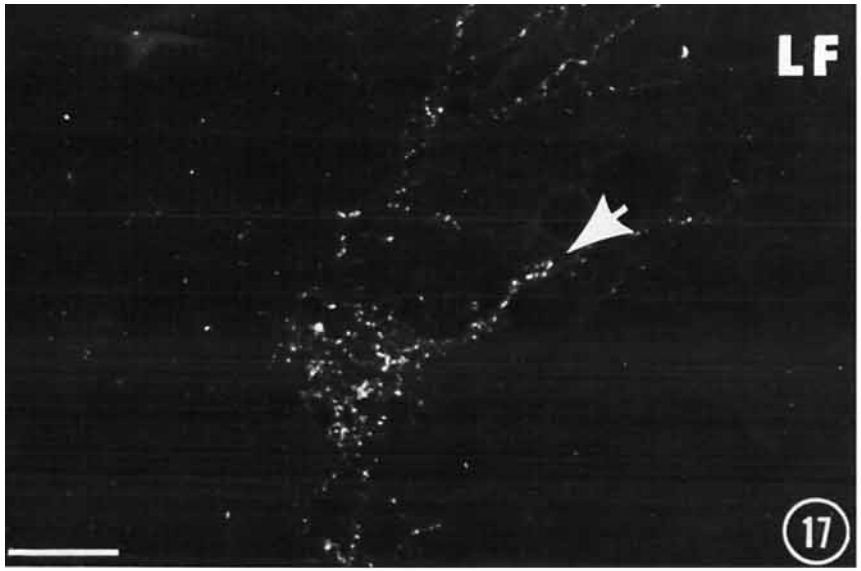
Fig. 16. Plots of the 5-HT immunofluorescence present at cervical (C-4), thoracic (T-9), lumbar (L-3), and sacral (Sac) levels. Some of Rexed's laminae are outlined on the left side. The arrow points to aggregates of 5-HT immunoreactive axons believed to be comparable to the ones similarly indicated in Figure 9.

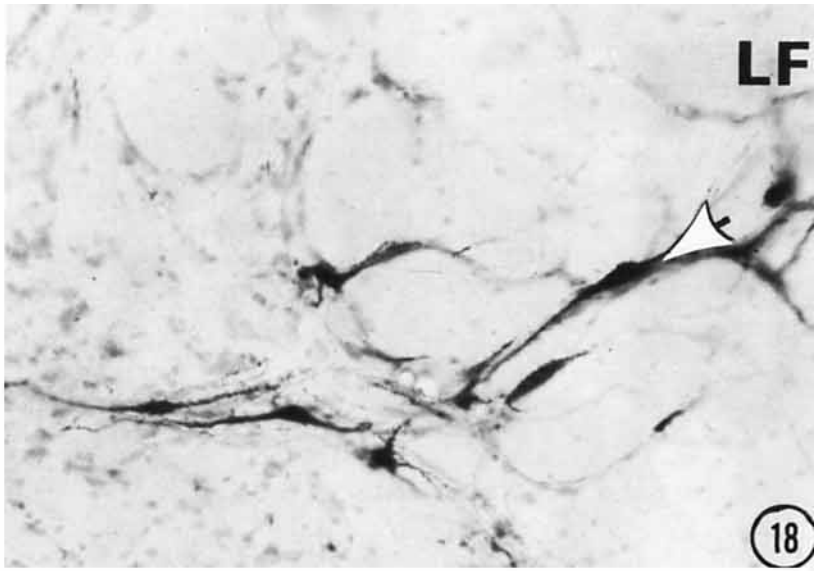
Fig. 12. Photomicrograph of the autoradiographic labeling present at T-5 after the injection of ^{3}H-leucine shown for OP-509 in Figure 9. The arrow points to labeling over pars funicularis of the nucleus intermediolateralis thoracolumbalis. The dorsal funiculus (DF), lateral funiculus (LF), and central canal (cc) are indicated. The bar equals 100 μm and can be used for Figure 13.

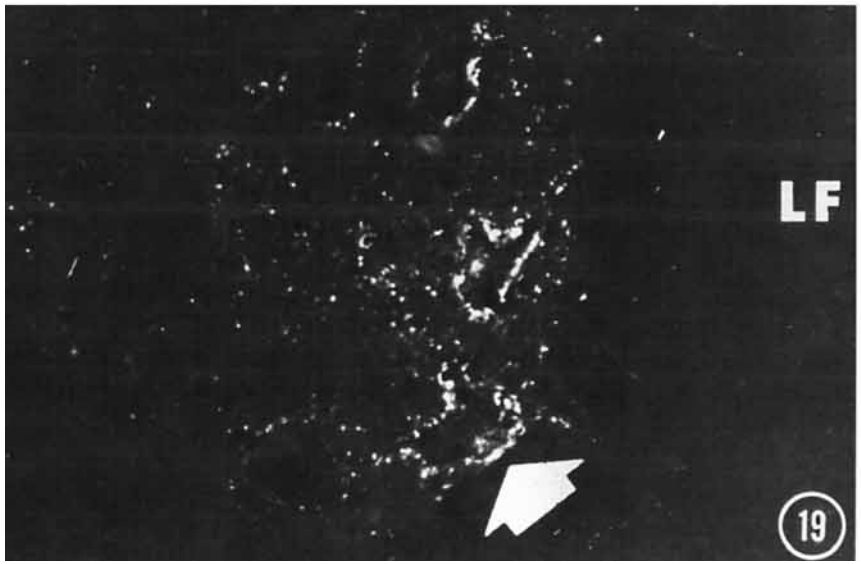
Fig. 13. Photomicrograph of the autoradiographic labeling present within the ventral horn at C-5 subsequent to the injection of ^{3}H-leucine shown for OP-509 in Figure 9. The lateral (LF) and ventral (VF) funiculi as well as laminae VIII and IX are indicated. The arrow points to the neuron similarly indicated in Figure 14.

Fig. 14. Higher-power photomicrograph of the labeling in lamina IX shown in Figure 13. The arrow points to the neuron similarly indicated in Figure 13. The bar equals 50 μm for both Figures 14 and 15.

Fig. 15. Photomicrograph of the autoradiographic labeling of the dorsal horn produced by the injection of ^{3}H-leucine shown for OP-526 in Figure 10. The dorsal funiculus (DF), the lateral funiculus (LF), as well as laminae I and II are indicated.







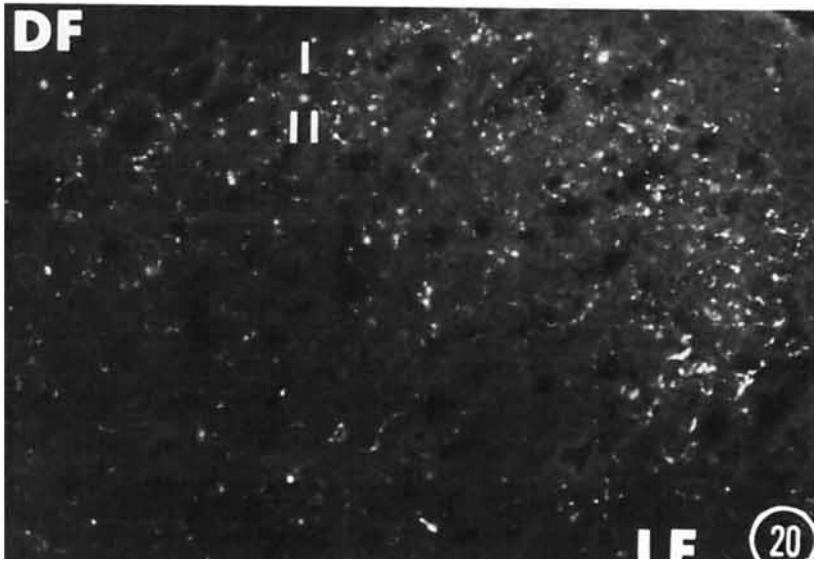


Fig. 17. Photomicrograph of 5-HT immunofluorescence within pars funicularis (arrow) and pars principalis of the nucleus intermediolateralis thoracolumbalis. The bar equals 50 μm for figures 17–20. The lateral funiculus (LF) is indicated.

Fig. 18. Photomicrograph of spinal neurons labeled by an HRP injection of the superior cervical sympathetic ganglion. The lateral funiculus (LF) is indicated.

Fig. 19. Photomicrograph of 5-HT immunofluorescence within lamina IX. The arrow points to a presumed motoneuron surrounded by fluorescent varicosities. The lateral funiculus (LF) is indicated.

Fig. 20. Photomicrograph of the 5-HT immunofluorescence present within the dorsal horn at C-4. The dorsal funiculus (DF) is indicated.

(Cabana and Martin, '81). Axons showing 5-HT immunoreactivity are soon found in somatic motor and autonomic nuclei of the spinal cord, but they are not present in laminae I and II until considerably later (Cabana et al., '81). It can be suggested from such data that serotoninergic raphe control of somatic motor and autonomic functions appears early in development, but that the sensory modulation occurring in laminae I and II is a later developing phenomenon.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Mary Ann Jarrell for excellent technical help, Ms. Malinda E. Amspuagh for typing this paper in final form and Mr. Karl B. Rubin for photographic help. We are also grateful to Dr. H. Loewe of Frankfurt, The Federal Republic of Germany, for providing the Nuclear-Yellow used in some of our experiments and to Dr. Robert Elde, The University of Minnesota for providing the 5-HT antiserum.

This investigation was supported by U.S.P.H.S. grants NS-07410 and BNS-8008675.

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