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RESEARCH ARTICLE

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# Association of spinal lamina I projections with brainstem catecholamine neurons in the monkey

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**Abstract** In addition to giving primary projections to the parabrachial and periaqueductal gray regions, ascending lamina I projections course through and terminate in brainstem regions known to contain catecholaminergic cells. For this reason, double-labeling experiments were designed for analysis with light and electron microscopy. The lamina I projections in the *Cynomolgus* monkey were anterogradely labeled with *Phaseolus vulgaris* leucoagglutinin (PHA-L) and catecholamine-containing neurons were labeled immunocytochemically for tyrosine hydroxylase (TH). Light level double-labeling experiments revealed that the terminations of the lamina I ascending projections through the medulla and pons strongly overlap with the localization of catecholamine cells in: the entire rostrocaudal extent of the ventrolateral medulla (A1 caudally, C1 rostrally); the solitary nucleus and the dorsomedial medullary reticular formation (A2 caudally, C2 rostrally); the ventrolateral pons (A5); the locus coeruleus (A6); and the subcoerulear region, the Kölliker-Fuse nucleus, and the medial and lateral parabrachial nuclei (A7). At the light microscopic level, close appositions between PHA-L-labeled lamina I terminal varicosities and TH-positive dendrites and somata were observed, particularly in the A1, A5 and the A7 cell groups on the contralateral side. At the electron microscopic level, examples of lamina I terminals were found synapsing on cells of the ventrolateral catecholamine cell groups in preliminary studies. The afferent input relayed by these lamina I projections could provide information about pain, temperature, and metabolic state as described previously. Lamina I input could impact interactions of the catecholamine system with higher brain centers modulating complex autonomic, endocrine, sensory, motor, limbic and cortical functions such as memory and learning. Nociceptive lamina I input to catecholamine cell regions with projections back to the spinal cord could form a feedback loop for control of spinal sensory, autonomic and motor activity.

**Key words** Nociception · Autonomic · Locus coeruleus · Norepinephrine · Catecholamine

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## Introduction

A spinal pathway arising from cells in lamina I and deeper laminae has primary terminations in the brainstem, parabrachial region and periaqueductal gray (Cechetto et al. 1985; Hylden et al. 1986; Standaert et al. 1986; Menétrey and Basbaum 1987; Wiberg et al. 1987; Lima et al. 1991; Craig 1992, 1995; Kitamura et al. 1993; Slugg and Light 1994; Bernard et al. 1995; Feil and Herbert 1995). In a previous study it was noted that the path taken by the ascending lamina I projection courses through and terminates in regions known to contain catecholamine neurons (Craig 1995). The present study seeks confirmation of a possible association of lamina I and the catecholamine system using combined anterograde tracing of the lamina I projection and immunocytochemical localization of catecholamine neurons.

The specific regions of the catecholamine system involved include noradrenergic cells in the medulla and pons as well as a collection of adrenergic cells located in the rostral medulla. These cell groups have been designated the A1–A7 noradrenergic cell groups and the C1–C2 adrenergic cell groups based on formaldehyde-induced histofluorescence and immunofluorescence (Dahlström and Fuxe 1965; Hökfelt et al. 1984). As one component of the reticular systems located in the general visceral column, the catecholamine system has complex, interactive projections to a wide variety of sites in the neuraxis affecting somatic, visceral, motor, endocrine and behavioral responses.

Neurons in lamina I and deeper laminae of the spinal (and trigeminal) dorsal horn have been retrogradely labeled from several brainstem regions known to contain catecholaminergic cells (e.g., Cechetto et al. 1985; Hylden et al. 1986; Standaert et al. 1986; Menétrey and Basbaum 1987; Wiberg et al. 1987; Lima et al. 1991; Kitamura et al. 1993). Anterograde studies have also demonstrated projections from the spinal cord (and trigeminal) dorsal horn and specifically from lamina I to regions known to contain catecholamine cells (Craig 1992; Slugg and Light 1994; Bernard et al. 1995; Feil and Herbert 1995). A previous article (Craig 1995) describes in detail the distribution of lamina I projections in the brainstem. Lamina I projections relay information about noxious, thermal and metabolic perturbations (Craig and Kniffki 1985). It is suggested that this is a major route through which the brainstem autonomic centers are informed of external and internal environmental challenges. One of the major noradrenergic cell groups, the locus coeruleus, is responsive to a variety of noxious sensory inputs (Foote et al. 1983). This responsiveness is paradoxical in the light of findings by Aston-Jones and colleagues (1986), who have made the case that the afferent inputs to the locus coeruleus are restricted to two nuclei in the rostral medulla. These include the nucleus paragigantocellularis in the rostral ventral medulla (C1 region) and the medial nucleus prepositus hypoglossi in the rostral dorsomedial medulla. These regions have also been linked to cardiovascular, respiratory and nociceptive control. The addition of specific dorsal horn projections that function to relay noxious, thermal and metaboreceptive signals to the previously rather limited list of locus coeruleus inputs provides a logical explanation for the ability of the locus coeruleus to play a major role in the attentional processes related to orienting behaviors, memory and defense reactions, in addition to its roles in sensory, autonomic and motor control.

The location of lamina I axonal projections traversing the ventrolateral medulla, and the dorsal sweep of this projection to travel in the dorsolateral pons through the Kölliker-Fuse nucleus, suggest that there is an association with the catecholamine cell column. Additionally, the terminals of the lamina I pathway strongly overlap the distribution of most of the catecholamine cell groups of the medulla and pons. Based on the distribution of the ascending lamina I projections through the brainstem shown previously (Craig 1995), double-labeling experiments were designed for analysis at both the light and electron microscopic levels. Anterogradely transported *Phaseolus vulgaris* leucoagglutinin was used to trace the spinal lamina I projection through the brainstem. Catecholaminergic cells were identified immunocytochemically with an antibody to tyrosine hydroxylase. Ultrastructural demonstration of synaptic contacts between lamina I terminals and catecholamine cells of the pontine A7 cell group provided evidence for a direct association.

The innervation of catecholamine cell groups established in this study suggests that the lamina I projection can relay incoming nociceptive, thermoreceptive and metaboreceptive information to the catecholamine system. It is plausible that nociceptive input to the dorsolateral pons could activate the descending catecholamine-spinal pathways known to modulate sensory, autonomic and motor function. As one example of ascending influences, lamina I influences on catecholaminergic activation of hippocampal circuits could impact memory of the painful event. In addition, nociceptive input would affect the many interactions that the catecholamine system has with brainstem regions responsible for central cardiovascular, respiratory and homeostatic control (Loewy and Spyer 1990). A preliminary report of these data has been made (Westlund and Craig 1993).

## Materials and methods

### Light microscopy

For these studies the lamina I projection was identified in five *Cynomolgus* monkeys by injecting the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L; 2.5% in 0.05 M phosphate buffer, pH 8.0; Vector) into the cervical or lumbar enlargement while the animal was anesthetized. A more detailed description of the procedures used has been given in a previous report (Craig 1995). After a 3–8 week survival period, the animals were killed with an overdose of sodium pentobarbital. Animals were perfused transcardially with 1 l of warm, heparinized phosphate-buffered saline (PBS, pH 7.5), followed by 1 l of 4% paraformaldehyde and 0.2% picric acid in acetate buffer (pH 6.3), and finally by 1.5 l of cold 4% paraformaldehyde and 0.05% glutaraldehyde with 10% sucrose in phosphate buffer (PB) (pH 8.0). The tissue blocks were stored in cold fixative with 30% sucrose for 4 h and then overnight in cold 30% sucrose. Frozen or vibratomed sections (40 or 50 μm) were cut through the entire brainstem in either the transverse or the sagittal plane.

Double-labeled material was prepared for fluorescence or brightfield microscopy using immunohistochemical procedures. A one-in-four series of sections was processed immunohistochemically and another was stained with thionin. In two cases, both a fluorescence and a brightfield series were obtained. Since the antibodies used to localize PHA-L and the catecholamine cell system were produced in different species, the antibodies were applied simultaneously. PHA-L was identified with goat anti-PHA-L primary antiserum (Vector, 1:1350) and catecholamine cells with an antibody against tyrosine hydroxylase (rabbit anti-TH, Pel-Freez, 1:1000). Tissue sections were incubated for 40 h at room temperature. Immunoreactive PHA-L was visualized first with either the avidin/Texas Red procedure (in TRIS buffer containing 0.1% Triton-X) or with a similar avidin–biotin peroxidase protocol (ABC, Vector Elite) using a stable intensified chromagen [3,3′-diaminobenzidine (DAB, Sigma) in PBS with Triton-X]. Biotinylated donkey anti-goat serum (Accurate, 1:200, 4–8 h) was used as the secondary antibody, followed by avidin/Texas Red (Vector, 1:500, 4 h) and then avidin and biotin blocking steps. The catecholamine cell population was then identified with either fluorescein isothiocyanate (FITC)-labeled donkey anti-rabbit serum (Accurate) or biotinylated donkey or horse anti-rabbit serum (Accurate or Vector; 1:200), followed by avidin/FITC (Vector, 1:500, DCS grade). This resulted in red-fluorescent PHA-L-labeled lamina I terminals and green-fluorescent TH-positive catecholamine cells. Both standard (B-2A, G-2A) and more selective (B-2E for FITC) filter cubes were used with a Nikon microscope. Dual-color DAB material was obtained by using a nickel-enhanced DAB reaction to produce black PHA-L-labeled terminals and a standard ABC/DAB reaction to produce brown TH-positive cells (Hancock 1986). The sections were mounted, dried, cleared in xylene, and coverslipped with DPX mountant (Gallard-Schlesinger). Labeling and cytoarchitectural detail in every fifth section were plotted with an MD-2

computerized system (Minnesota Datametrics). Color photomicrographs were taken with Fujichrome 400 (push-processed one stop) and Kodak EPY-160.

### Electron microscopy

Three additional monkeys with PHA-L injections in the cervical lamina I were perfused with 3% paraformaldehyde, 0.5% glutaraldehyde and 0.2% picric acid. The best preservation was obtained when tissue blocks were postfixed for 24 h at 4°C. Fifty-micrometer parasagittal sections were cut through the contralateral brainstem with a vibratome. One-in-four series were processed for dual immunohistochemical staining for both light and electron microscopy. Sections for light microscopy were processed as described above. The sections used for electron microscopy were soaked for 30 min in 1% sodium borohydride to reduce aldehyde cross-linking (Schachner et al. 1977) and were passed through graded alcohols (10%, 25%, 40%, 25%, 10% ethanol, 5 min each) to increase permeability. Triton-X was excluded from the diluent buffer solution of 1% or 3% normal horse serum in PBS (pH 7.6) while 0.05% thimerosal was added. After thorough rinsing (6×) in PB, tissue sections were blocked in avidin (Vector, 1:10, 15 min) and biotin (Vector, 1:10, 15 min) in 3% diluent buffer. Sections were then incubated in primary antibodies anti-PHA-L (Vector, 1:2000) and anti-TH (Pel-Freez, 1:1000) simultaneously in 1% buffer for 2–4 days. Subsequently, the sections were rinsed in PBS (10 min) and 1% buffer (30 min). The sections were incubated in the secondary antibodies, horse anti-goat/biotin (1:200) and donkey anti-rabbit (1:200), in 1% diluent buffer for 2 h. After another rinse series, the sections were incubated in extrAvidin-HRP (Sigma, 1:1100) in 1% buffer for 2 h. The PHA-L was visualized after reaction in 0.025% DAB in PB with 0.02% hydrogen peroxide for 6 min. The TH-positive catecholamine cells were subsequently labeled by incubation of the sections in rabbit peroxidase-antiperoxidase (PAP, 1:500, 2 h) and visualized as a dense black reaction product with the procedure of Weinberg and Van Eyck (1991). This included rinsing in PB (2×, pH 6.0) and a 30 min incubation in TMB-tungstate solution (1 ml PB at pH 6.0, 50 µl 1% ammonium paratungstate in dd$H_2O$, and 25 µl 0.2% tetramethyl benzidine in 100% ethanol). The addition of $H_2O_2$ (10 µl, 0.03%, 6 min) initiated the reaction. After rinsing in PB, the reaction product identifying the TH was stabilized in 20 µl of 1% cobalt chloride, 20 µl of 2.5% DAB, and 20 µl of 0.03% $H_2O_2$ in 1 ml PB and rinsed again. The tissue sections prepared for light microscopy were mounted, dried, cleared in xylene and coverslipped with DPX. The sections prepared for electron microscopy were osmicated (1% osmium tetroxide in 1% sodium cacodylate, 45 min), dehydrated and wafer-embedded. Examples of labeled cells in the A5 (one cell) or A7 (five cells) catecholaminergic cell group that appeared to have lamina I contacts were identified in the osmicated sections with light microscopy and photographed. The labeled cells were dissected from the double-labeled plastic sections using a dissection microscope. The cells were re-embedded on a blank chuck, and thin-sectioned serially. Between 150 and 200 serial, silver-gold (100 nm) thin sections were examined sequentially for each of the six cells with the electron microscope, requiring enormous technical effort. The sections were stained with uranyl acetate (1%) during dehydration and with lead citrate (0.4% aqueous, 2 min) following embedding, after thin sectioning.

## Results

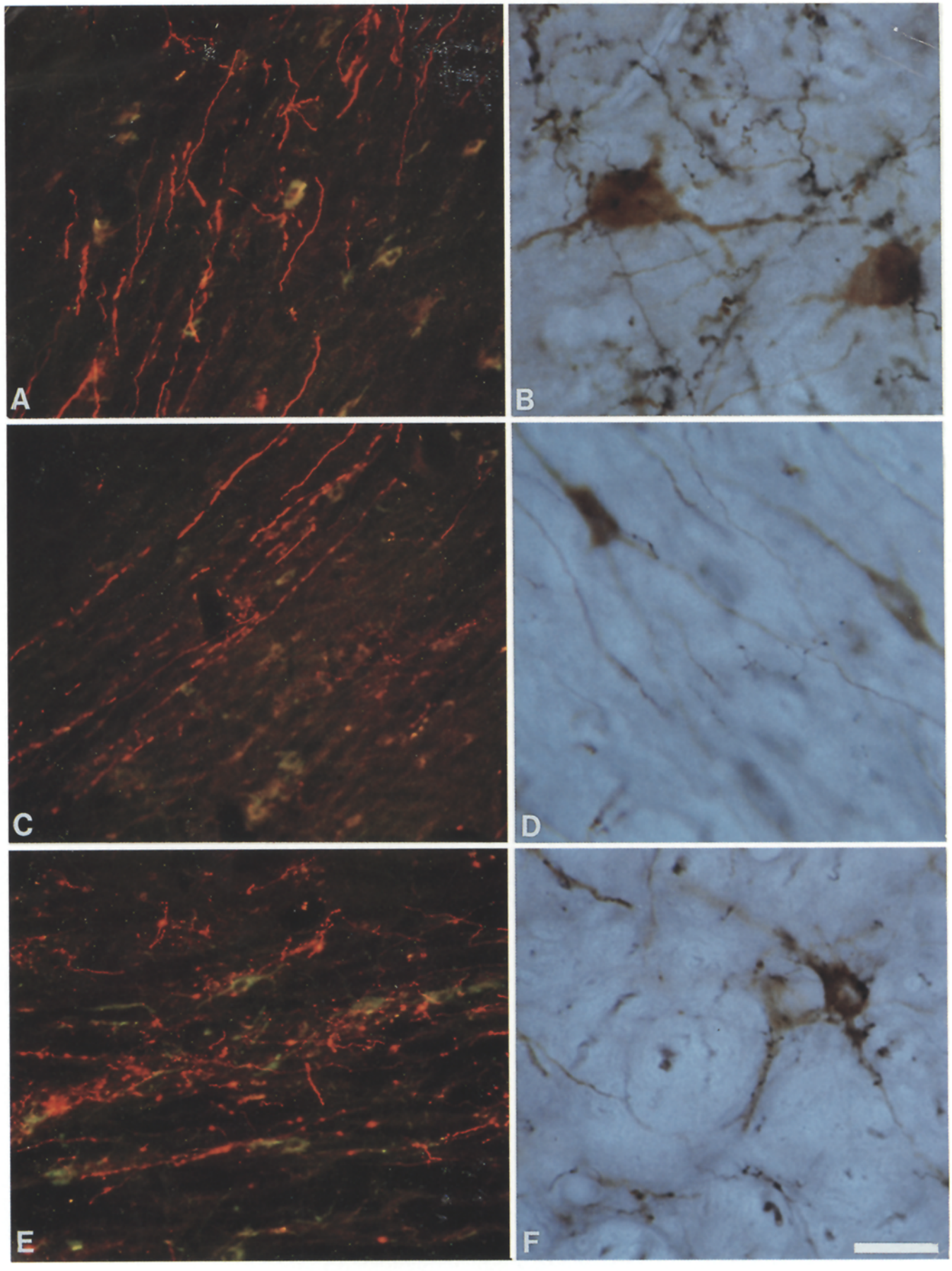
### Comparison of the lamina I distribution pattern with catecholaminergic cell labeling

Simultaneous light microscopic visualization of PHA-L-labeled lamina I terminations and TH-positive catecholaminergic cells in the brainstem was performed in five monkeys: four with cervical injections and one with a lumbar injection (table 1 in Craig 1995). Monkeys were chosen for the present experiments because, in the previous study, the PHA-L labeling was consistently more intense in monkeys than in cats. Double fluorescent labeling (with Texas Red and FITC) was obtained in all five cases (Fig. 1A,C,E), and double-labeling with black and brown DAB reaction products was obtained in two cases (Fig. 1B,D,F).

The pattern of fluorescent labeling in representative single sections is shown in the transverse plane in Fig. 2 and in the sagittal plane in Fig. 3. Each dot represents three to five PHA-L-labeled varicosities. Similarly, each open circle represents three to five TH-positive cells. Axons have not been plotted but are described here and illustrated with low-power photomicrographs. The charts have the major catecholamine (A1, A5, A6, A7 and C1) cell groups indicated.

As illustrated in Figs. 1–3, the lamina I projection coursed through and terminated in the TH-containing catecholaminergic cell column of the medulla and pons. Both *en passant* and terminal arborizations were observed. The pattern of lamina I terminations in the cases reported here was identical to the pattern described in the previous study. No double-labeled elements were observed. The TH staining identified the catecholamine cell column (composed of labeled somata, dendrites and terminals) that extends rostrocaudally through the medulla and pons, represented by plots of the cell bodies in the sagittal plane in Fig. 3. In the transverse plane, these structures formed a dorsomedially to ventrolaterally oriented band (Fig. 2). The pattern of TH labeling observed was consistent with published maps of the catecholamine system for monkey and cat brainstem (Maeda et al. 1973; Schofield and Everitt 1981; Felten and Sladek 1983; Westlund et al. 1984; Ciriello et al. 1986).

Within the medulla, lamina I terminations were intermingled with the TH-positive cells and dendrites throughout the ventrolateral medulla, the dorsomedial reticular formation and the solitary complex. That is, lamina I terminations were present in the A1 (Fig. 2, levels 1–3; Fig. 3, levels 3–6) and C1 (Fig. 2, levels 3–5; Fig. 3, levels 2–4) cell groups in the ventrolateral medulla. In the dorsomedial medullary reticular formation, lamina I projections also terminated in the A2 (Fig. 2, levels 1–3; Fig. 3, levels 7–8) and C2 (Fig. 2, levels 3–4; Fig. 3, level 6) groups. Within the pons, lamina I terminations also occurred in the regions that contain catecholaminergic cells and their dendrites. These regions included the ventrolateral pons caudally (A5 cell group: Fig. 2, levels 6–7; Fig. 3, levels 2–3), the locus coeruleus (LC) dorsally (A6 cell group: Fig. 2, levels 7–9; Fig. 3, levels 5–7), and the subjacent subcoeruleus, the parabrachial nuclei (PB) (Fig. 2, levels 8–9; Fig. 3, levels 1–4) and Kölliker-Fuse (KF) (Fig. 2, level 8 at arrowhead; Fig. 3, level 1) nuclei in the rostral dorsolateral pons. The ventral subcoeruleus, parabrachial and Kölliker-Fuse nuclei together compose the A7 cell group. Sequential ranking in order of lamina terminal density associated with catecholamine


A
B
C
D
E
F

cell groups is as follows: A1>Kölliker-Fuse >A5 >C1 >parabrachial>subcoeruleus>locus coeruleus>A2>C2.

Axons of the ascending lamina I projection coursed rostrally through the ventrolateral medulla and arched dorsally into the pons to assume a dorsolateral position. Labeled lamina I ascending axons coursed directly through the A1, C1, A5 and A7 catecholaminergic cell groups bilaterally with a contralateral predominance. Ascending axons also coursed ventrally past the TH-positive A1/C1 cells along the surface of the ventrolateral medulla and laterally past the A5 and A7 cells along with the lateral lemniscus (Mehler et al. 1960; Craig 1995). A ventrolateral to dorsomedially (diagonal) oriented region extends through the medullary reticular formation with scattered catecholaminergic cells and dendrites situated between the A1 and A2 cell groups (Fig. 2, levels 1–3). It is through this region that lamina I projections migrate dorsally through this diagonally oriented band to terminate in the dorsal catecholaminergic cell groups (A2, C2, A6).

Catecholamine-containing cells were found in few medullary or pontine areas without nearby lamina I terminations (note Fig. 2, level 5, ventromedial medulla). Conversely, few lamina I terminations occurred in medullary and pontine areas without catecholaminergic cells or their dendrites (note the dorsomedial labeling in the extreme rostral pole of the solitary nucleus in Fig. 2, level 4 and in Fig. 3, level 1). The region caudal to the locus coeruleus and parabrachial nuclei in which lamina I terminations did not appear to be associated with catecholaminergic cells [Fig. 3, level 2 (arrow) and level 4] is in fact associated with catecholaminergic dendrites extending caudally from the locus coeruleus neurons (illustrated in Fig. 3, levels 5–6). Only in the lateral and rostral parabrachial regions and within the mesencephalon, periaqueductal gray (PAG) and intercollicular nucleus were lamina I axons and terminations clearly not associated with catecholaminergic cells or their dendrites. The dopaminergic cell group in the ventrolateral mesencephalic tegmentum (A10 cell group) received very few lamina I terminations.

◀ **Fig. 1A–F** Color photomicrographs of material double-labeled for *Phaseolus vulgaris* leucoagglutinin (PHA-L; red on the *left*, black on the *right*) and tyrosine hydroxylase (TH; green on the *left*, brown on the *right*) from monkey STM-19 (*left column*, sagittal sections) and from STM-21 (*right column*, transverse sections). The lower-power fluorescence images on the *left* show the close association of ascending lamina I fibers and labeled terminations with the catecholaminergic cells in the A7 (**A**), A5 (**C**) and A1 (**E**) cell groups. The higher-power images on the *right* show close appositions between lamina I terminations and TH-positive cells in the A7 (**B**), C1 (**D**) and A1 (**F**) cell groups. The locations of each of these photomicrographs are indicated by the arrowheads in the charts in Figs. 2 and 3. Dorsal up, rostral right (*left column*) and dorsal up, lateral right (*right column*). Scale bars represent 30 μm (**B, F**), 38 μm (**D**), 95 μm (**A, E**), or 150 μm (**C**)

## Association of PHA-L-labeled boutons with catecholaminergic cells

The close association of the ascending PHA-L-labeled lamina I fibers with these medullary and pontine catecholamine-containing cells in many instances was best observed in sagittal sections (Fig. 3, STM-19). The color photomicrographs from one monkey (STM-19), shown in Fig. 1, illustrate particularly well the passage of ascending axons near and through the region of TH-positive cell groups in A1 (Fig. 1E), A5 (Fig. 1C) and A7 (Fig. 1A). The sagittal plots in Fig. 3 show the clear association of the distribution of catecholaminergic cells with the ascending termination pattern of the lamina I projection through the medulla and pons. With light microscopy, it was also evident that PHA-L-labeled lamina I varicosities in the catecholaminergic cell groups of the medulla and pons were not always directly associated with TH-positive elements. In many instances, however, lamina I boutons in the ventrolateral medulla and in the dorsolateral pontine tegmentum were found closely associated with TH-positive dendrites or somata on the contralateral side. On the ipsilateral side, they were always separated by a small gap, visible at high power, suggesting that an unlabeled structure was present, or that a process from an adjacent small autofluorescent cell was interposed. Such near terminations were also observed in the contralateral ventrolateral medulla. However, close appositions between PHA-L-labeled boutons and TH-positive elements were consistently observed in the contralateral ventrolateral medulla, particularly in the regions of the A1 (Fig. 1F) and C1 (Fig. 1D) cell groups, as well as in the pons in the A5 cell group. Close appositions were most frequently found in the A7 (Fig. 1B) region in the pons. These putative synaptic contacts were found not only on dendrites but also on somata that were TH-positive.

To confirm that catecholamine cells were contacted by lamina I projections, serial sections of six TH-positive cells in the A5 and A7 groups from three additional monkeys that had received optimal cervical lamina I injections were examined with electron microscopy. These cells were selected based on the appearance at the light level of close contacts by PHA-L-labeled lamina I terminals. Between 150 and 200 serial, silver-gold (100 nm) thin sections were examined sequentially for each of the six cells with the electron microscope. The PHA-L-labeled lamina I preterminal axons associated with the six cells were followed through the tissue serially in the electron microscope until they either: (1) contacted a TH-positive element; (2) approached a TH-positive cell but did not make contact because a thin glial process interposed; or (3) contacted adjacent structures but not TH-positive elements. Many examples of lamina I contacts with TH-positive dendrites and somata were noted in these preliminary studies. An example of a synaptic contact with a distinct cleft between a densely labeled PHA-L-labeled lamina I bouton and a TH-positive dendrite is shown in Fig. 4. The example illustrated is from the A7

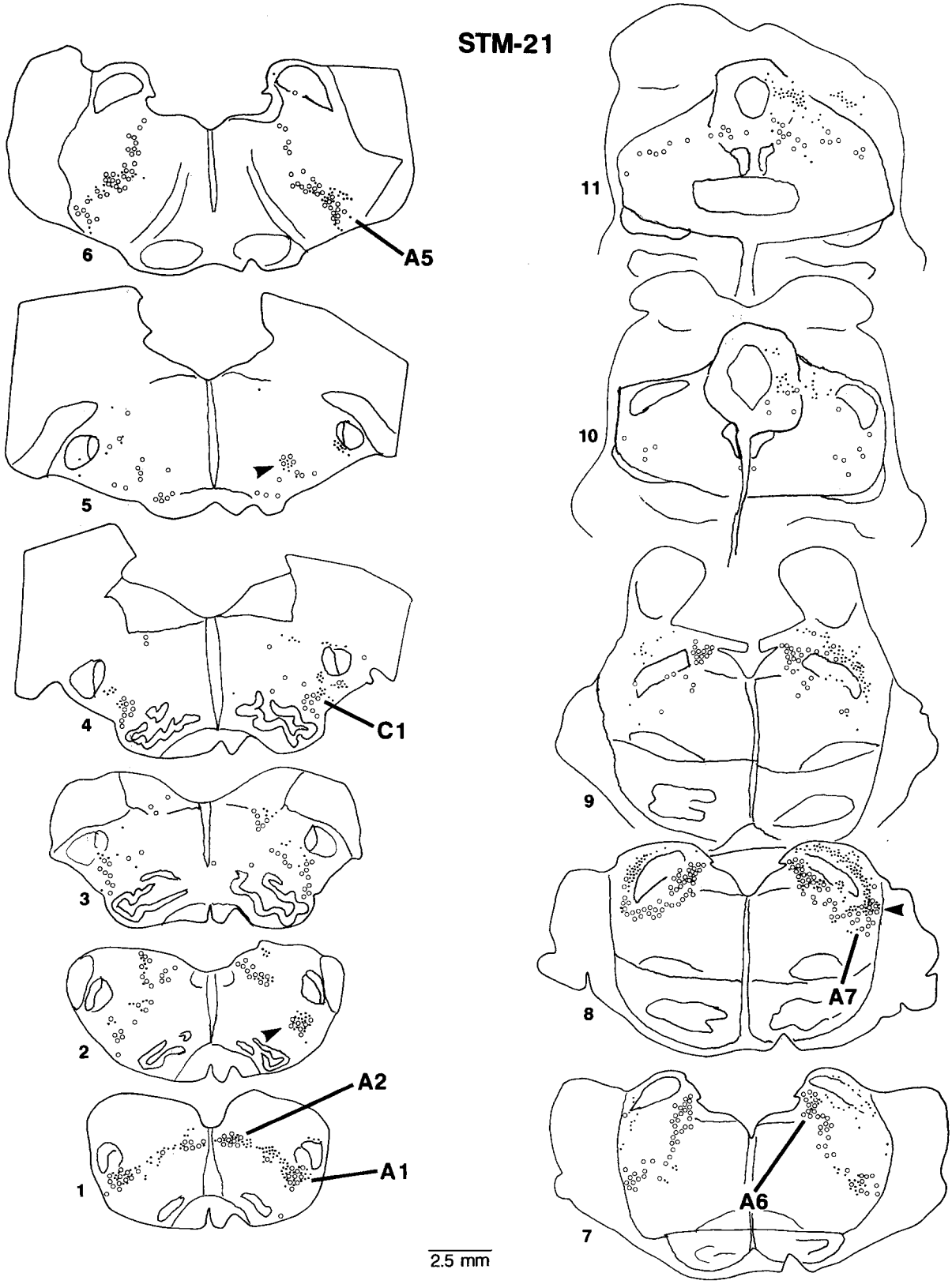



**Fig. 2** Plots of PHA-L-labeled lamina I terminations (*dots*) and TH-positive neurons (*open circles*) in a series of single transverse sections from monkey STM-21 (cervical, optimal) in which double-labeling was performed. The sequence is *numbered* from caudal to rostral; *left* is ipsilateral to the injection site. The major catecholaminergic cell groups are marked. The *arrowheads* indicate the locations of the photomicrographs shown in Fig. 1

cell group in the ventrolateral pons. Contacts with distal dendrites were most common. In some cases direct contact with TH-positive elements was not observed when the terminal appeared at light level to be in close apposition. Examples of contacts with other neuronal and glial elements were also noted.

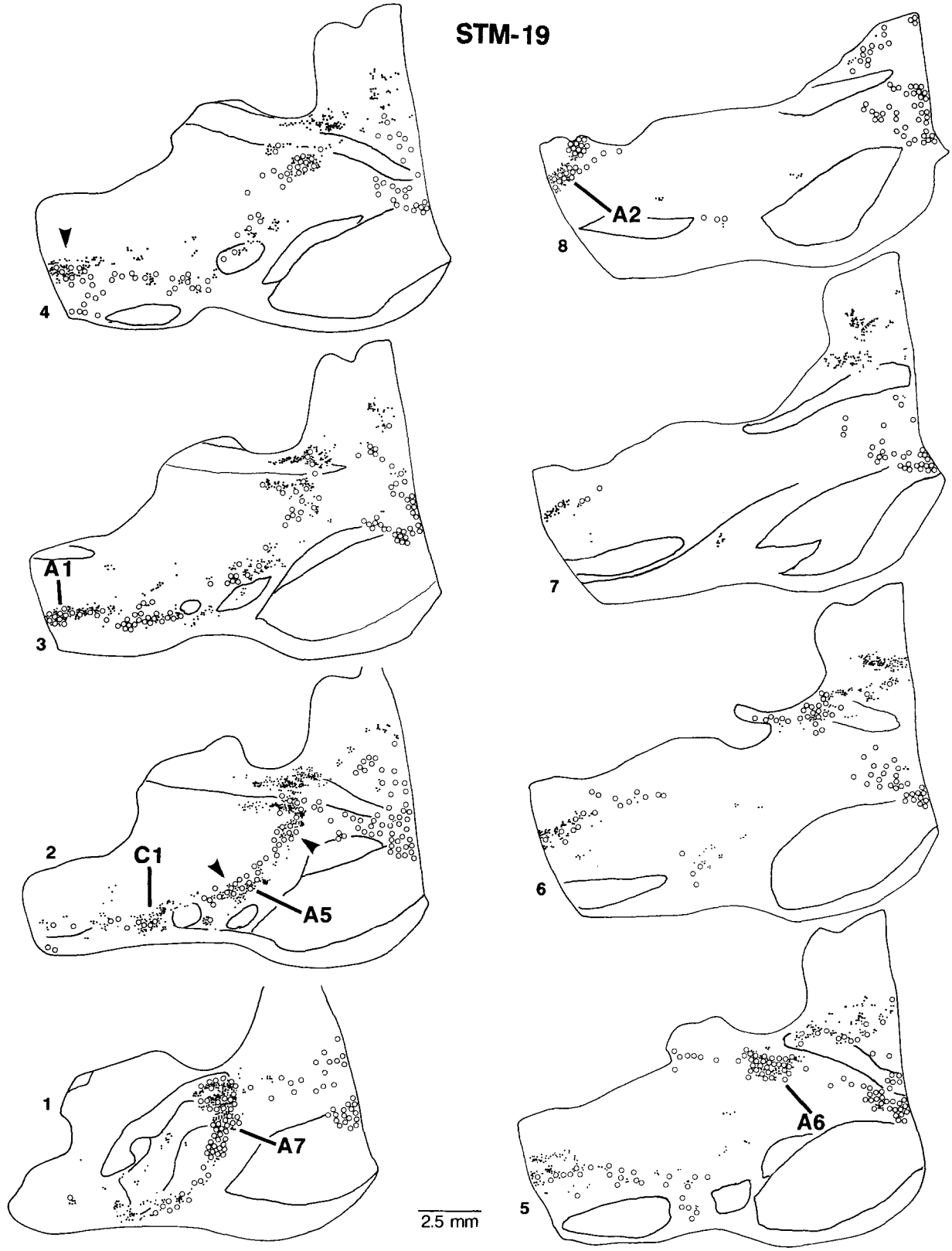



**Fig. 3** Plots of PHA-L-labeled lamina I terminations (*dots*) and TH-positive neurons (*open circles*) in a series of single sagittal sections from the right side of monkey STM-19 (cervical, nominal) on which double-labeling was performed. The sequence is *numbered* from lateral to medial; this is the side contralateral to the injection site. The major catecholaminergic cell groups are marked. The *arrowheads* indicate the locations of the photomicrographs shown in Fig. 1

## Discussion

The present observations utilizing the PHA-L anterograde transport method indicate that spinal lamina I projections en route to major non-catecholaminergic termination sites in the parabrachial and periaqueductal gray

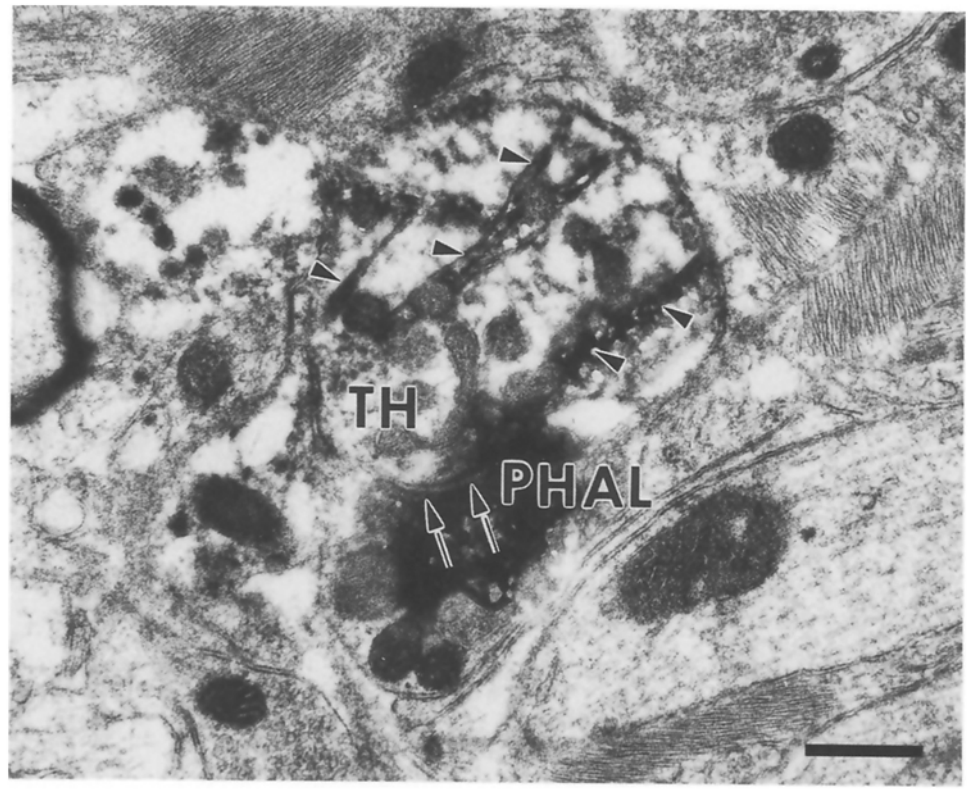



**Fig. 4** Synaptic contacts between PHA-L-labeled lamina I terminations and noradrenergic neurons of the A7 cell group observed in STM-39 (cervical injection). The PHA-L-labeled terminals found at the surface of the tissue section were covered with dense peroxidase labeling. The noradrenergic dendrite is identified by TH immunocytochemical labeling using a TMB-paratungstate reaction product, which forms needle-like crystals (*arrowheads*). Scale bar represents 0.6 μm

regions, project to and through the catecholaminergic cell column of the medulla and pons. This includes the A1 and C1 regions of the ventrolateral medulla and the A2 and C2 regions of the dorsomedial medulla. In the pons, the regions innervated include the A5 and A7 cell groups in the caudal ventrolateral and rostral dorsolateral tegmentum, respectively. Catecholaminergic cells of the A7 cell group innervated by lamina I axons were distributed in the Kölliker-Fuse, subcoeruleus and parabrachial nuclei. Terminations were also observed dorsally in the pons among the locus coeruleus neurons (A6 cell group). The lamina I projection preferentially terminated in the ventrally located catecholamine cell groups through which it traversed.

Close appositions between PHA-L-labeled lamina I terminal varicosities and TH-positive dendrites and somata were observed at the light microscopic level, particularly in the ventrolateral A1, A5 and the A7 catecholaminergic cell groups on the side contralateral to the spinal cord injection. But, in the other regions and almost always on the ipsilateral side, the PHA-L-labeled terminations were near but did not directly appose TH-positive elements. Tedious serial section analysis demonstrated examples of lamina I projections terminating directly on catecholamine cells in the A5 and A7 cell groups of the pons that were examined. In other cases where light microscopic close appositions were observed, however, serial section ultrastructural analysis revealed that many terminals synapsed on structures interposed between the PHA-L-labeled terminal and the TH-positive element.

Lamina I neurons are small neurons that are oriented rostrocaudally in the superficial marginal zone (Beal et al. 1981; Lima and Coimbra 1986; Zhang et al. 1993). The fact that spinal cord input to the catecholaminergic cell groups has been overlooked in previous retrograde studies (Aston-Jones et al. 1986) is no doubt due to the morphological orientation of many lamina I projection neurons. In transverse sections their appearance is scarcely different from microglia. Although it is not known yet what other sources may relay afferent input directly or indirectly to the catecholaminergic cells (i.e., lamina V, dorsal column nuclei, cranial nerve nuclei), the present study demonstrates that the lamina I projection could serve as an anatomical substrate providing spinal input to the catecholamine cell groups and other cells closely associated with them. The catecholaminergic cell groups in turn are the sources of input to many other brain regions. The functional significance of the lamina I innervation of these regions in autonomic control is addressed in the previous paper (Craig 1995). The relevance of lamina I input onto the catecholaminergic cell population is specifically discussed here.

## Lamina I input to brainstem catecholamine systems with ascending projections

The brainstem catecholaminergic regions innervated by the lamina I spinobulbar pathway have many ascending projections to sites such as the hypothalamus, cortex, basal forebrain and hippocampus (Ungerstedt 1971). These lamina I projections to catecholaminergic regions with connections to higher centers would impact the many functions ascribed to these centers. Cortical and

hippocampal regions receive their noradrenergic input exclusively from the locus coeruleus (Ungerstedt 1971). A role for catecholamines in focused attention and in memory formation in these regions has been reviewed by Foote, Segal and colleagues (Foote et al. 1991; Segal et al. 1991). It was believed that the locus coeruleus plays a crucial role in wakefulness and in paradoxical sleep (Jouvet 1972). In cats, lesions of the ventral noradrenergic bundle with 6-hydroxydopamine have been shown to increase deep slow wave sleep and REM sleep (Panksepp et al. 1973). In hippocampal cortex, norepinephrine enhances excitatory post-synaptic potential (EPSP)/spike coupling in dentate granular cells through $\alpha_1$ and $\beta$ noradrenergic receptors (Aston-Jones and Bloom 1981; Segal et al. 1991). Since the data indicate that noradrenergic locus coeruleus neurons are most responsive to noxious sensory stimuli, an influence of afferent input conveyed by lamina I projection cells to the locus coeruleus region and the subsequent influence of this afferent input on cortical and hippocampal function has obvious implications for selective attention and memory encoding. The complex influences of afferent stimuli on cortical activity that are mediated through the locus coeruleus (Foote et al. 1991) demonstrate the importance of the catecholaminergic system in integrative cortical functioning.

Likewise, functions related to catecholaminergic innervation of the hypothalamus and basal forebrain would be impacted by lamina I input to catecholaminergic cell groups. It has been shown that the A1 noradrenergic cell group projects to the paraventricular nucleus of the hypothalamus (Sawchenko and Swanson 1982) and the medial preoptic nucleus (Saper and Levisohn 1983). These two regions have been shown to receive particularly large numbers of noradrenergic terminals in monkeys (Ginsberg et al. 1993). The prominent termination of catecholaminergic projections in these two regions suggests that norepinephrine has a role in the hypothalamic-pituitary-adrenal stress axis and in gonadotropin release, respectively. Catecholaminergic modulation of the release of corticotropin releasing factor and its effects on the hypothalamic-pituitary-adrenal stress axis have been reviewed by Plotsky and colleagues (1989). In another study, double immunocytochemical localization experiments have revealed a close association of noradrenergic terminals with cells of the medial preoptic and ventrolateral hypothalamus that express estrogen receptor-immunoreactivity (Tetal and Blaustein 1991).

Branches of catecholaminergic projections from A1, A2 and A6 to the paraventricular hypothalamic nucleus innervate the central nucleus of the amygdala in rats (Petrov et al. 1993). Direct projections from the parabrachial region to the amygdala have also been reported (Fallon et al. 1978). Lamina I cell activation of catecholaminergic projections to the amygdala from these regions would impact autonomic and limbic functions. These anatomical connections may have functional relevance for, for example, painful experiences.

Pain is often accompanied by motivational-affective and autonomic responses, including suffering, anxiety, increased attention and arousal, increased heart rate and blood pressure and endocrine changes. The neural structures that mediate these changes are likely to parallel those relaying information about somatic pain sensations, but are likely to include additional structures. In addition to contributing to the well-studied spinothalamic tract, which transmits information that helps in the discrimination and localization of pain, several groups have confirmed the existence of an ascending spinoreticular pathway. The pathway arising from cells in lamina I and deeper laminae has terminations in the brainstem, primarily in the parabrachial region and the periaqueductal gray (Cechetto et al. 1985; Hylden et al. 1986; Standaert et al. 1986; Menétrey and Basbaum 1987; Wiberg et al. 1987; Lima et al. 1991; Craig 1992, 1995; Kitamura et al. 1993; Slugg and Light 1994). Thus, lamina I projections to catecholaminergic regions which in turn project to higher brain regions, such as the amygdala, may contribute to the affective component of pain as well as to hierarchical autonomic control.

## Lamina I input to brainstem catecholamine systems with descending projections

Nociceptive, thermoreceptive and putative metaboreceptive lamina I input to catecholaminergic cell groups may influence the catecholaminergic descending control system, thereby forming a possible disynaptic spinobulbo-spinal feedback loop affecting sensory, motor and autonomic processing. In contrast to the newly recognized feedback loop via the medullary dorsal reticular region (SRD) discussed in a previous article (Craig 1995), the descending catecholaminergic system has been well studied with respect to nociception. For example, stimulation in the region of the Kölliker-Fuse nucleus can produce both a catecholaminergic and a non-adrenergic antinociception (Duggan and North 1984; Hodge et al. 1986; cf. Proudfit 1992). Noradrenergic brainstem projections to the spinal cord arise almost entirely from the pontine A5, A6 and A7 cell groups, with major input to the dorsal and ventral horns from the locus coeruleus, the subcoerulear region and the Kölliker-Fuse nucleus (Westlund and Coulter 1980; Westlund et al. 1983, 1984). Semi-quantitative studies in monkeys (Westlund et al. 1984), cats (Stevens et al. 1982) and rats (Proudfit 1992) indicate that the dorsolateral pons is a major contributor of spinal cord noradrenergic innervation. Retrograde horseradish peroxidase (HRP) studies have also demonstrated other non-noradrenergic spinally projecting neurons in the ventrolateral medulla that are scattered among and sometimes immediately adjacent to the noradrenergic cells of the A1 and A2 cell groups (Westlund et al. 1983, 1984; Tucker et al. 1987). Many textbooks citing nonspecific histofluorescence studies and preliminary work reported in an oft-cited abstract (Hudson et al. 1986) mistakenly describe noradrenergic spinally projecting neurons in the A1 cell group in the ventrolateral medulla. A more careful analy-

sis (Ross et al. 1984) demonstrated that catecholaminergic bulbospinal neurons in the ventrolateral medulla are actually in the more rostral C1 adrenergic cell group. Adrenergic spinally projecting neurons in the rostral ventrolateral medulla have also been identified in the monkey with HRP and an antibody to phenylethanolamine-*N*-methyltransferase (Carlton et al. 1989). This C1/C2 population may provide a small projection to the dorsal horn (Carlton et al. 1991), but the predominant projection of this region is to the thoracolumbar sympathetic preganglionic nuclei (Ross et al. 1984).

In monkeys and cats, bilateral dorsal horn projections from the locus coereleus and subcoerulear region were reported to be densest in laminae I, II and V contralaterally (Westlund and Coulter 1980; Holstege and Kuypers 1982). Recent PHA-L studies in rats and previous retrograde HRP studies in cats indicate that the densest input to the dorsal horn originates from ipsilateral Kölliker-Fuse nucleus (Stevens et al. 1982; Clark and Proudfit 1991). Noradrenergic terminals in the spinal cord have been demonstrated using immunocytochemical localization of noradrenaline in rats (Rajaofetra et al. 1992), cats (Lackner 1980) and monkeys (Westlund et al. 1984). Such terminals make direct contacts on dorsal horn neurons (Doyle and Maxwell 1991), including identified spinothalamic neurons in laminae I and V (Westlund et al. 1990).

Electrical or chemical stimulation of the dorsolateral pons produces analgesic effects mediated by $\alpha_2$ adrenoreceptors that can be differentiated from cardiovascular effects, and such stimulation causes inhibition of nociceptive neurons in the deep dorsal horn (Hodge et al. 1986; Jones and Gebhart 1988; Zhao and Duggan 1988; Proudfit 1992; Yeomans and Proudfit 1992). Norepinephrine and $\alpha_2$ agonists applied to the spinal cord inhibit the responses of superficial and deep dorsal horn cells to noxious heat, including primate spinothalamic tract neurons (Headley et al. 1978; Fleetwood-Walker et al. 1983; Willcockson et al. 1984). These agents also reduce the stimulation-induced release of substance P in the dorsal horn (Kuraishi et al. 1985). Intrathecal administration of norepinephrine produces antinociception, measurable as increased latencies in hot plate and tail flick tests (Yaksh 1986).

Thus, lamina I input to the dorsolateral pons could activate the descending noradrenergic system. The actions of such a feedback loop on the ascending lamina I spinobulbar cells, on other lamina I projection cells, and on other dorsal horn and ventral horn cells remain to be determined. One possibility is activation of an anti-nociceptive system in the dorsal horn (Yeomans and Proudfit 1992). Spinobulbar lamina I input to A7 cells may cause inhibition of dorsal horn cells that project to the thalamus, resulting in an indirect effect on nociception. Certainly, anti-nociception is just one aspect of the function of this pathway. The predominant association of Kölliker-Fuse nucleus with respiration suggests the possibility of a role in the descending homeostatic control of spinal excitability levels and responses to environmental demands (see discussion in Craig 1995). A role for the descending catecholaminergic system in motor processing has been reviewed previously (Fung et al. 1991). Identification of the physiological types of lamina I spinobulbar cells involved in the spino-bulbo-spinal loop will help determine the functional role(s) of this loop and its integration with sensory, motor and autonomic processing.

## Conclusions

A spinal dorsal horn projection to the parabrachial and periaqueductal gray regions have been well documented. Use of the PHA-L tracing technique has enabled demonstration of the innervation of a number of other specific regions in the brainstem by the projections of spinal lamina I neurons as they course through the brainstem (Craig 1995). Many of these regions are characterized by the presence of catecholaminergic cells and are the origins of substantial ascending or descending catecholamine projections that are important for coordinated integration of sensory input to autonomic, hypothalamic and limbic centers. The present observations reveal close appositions of lamina I projections on elements of the contralateral catecholaminergic cell column extending through the medulla and pons. Examples of synaptic contacts of spinobulbar lamina I terminations onto cells of the A7 catecholamine cell group of the pons were demonstrated by preliminary electron microscopy. These projections potentially provide spinal input to both ascending bulbo-hypothalamic/thalamic/limbic and descending bulbo-spinal somatic and autonomic systems. These anatomical findings also provide a potential basis for feedback onto spinal nociceptive systems from the brainstem catecholaminergic system, which coordinates somatic and visceral motor activity and influences sensory processing. Lamina I projections relaying nociceptive, thermoreceptive and metaboreceptive input into catecholaminergic cell regions with ascending projections could help explain the strong effect that pain, temperature and inflammation have on hypothalamic and stress responses, arousal, motivation, affect and memory formation. Nociceptive lamina I inputs to pontine catecholaminergic cell regions that have descending projections to the spinal cord could provide an anatomical substrate to drive the descending control that these groups have on spinal motor and sensory systems. The lamina I projection thus provides a major anatomical conduit for transmission of information about nociceptive, thermoreceptive and metaboreceptive activity to key brainstem sites. The findings from the present study support the general conclusion that the lamina I spinobulbar projection provides sensory input important for the physiological maintenance of the organism, as well as its changing response to environmental demands. In this manner, sensory input can impact both homeostatic and dynamic responses to internal and external environmental challenges.

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