

# The Distribution of Tyrosine Hydroxylase-Immunoreactive Fibers in Primate Neocortex Is Widespread but Regionally Specific

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**An antiserum directed against tyrosine hydroxylase (TH), an enzyme involved in dopamine and norepinephrine synthesis, was used to visualize axons immunohistochemically in monkey neocortex. Labeled fibers were distributed throughout the entire neocortex, but they had striking patterns of regional and laminar specialization. For example, primary motor cortex contained the greatest density of TH-labeled fibers, whereas primary sensory regions were sparsely innervated. Marked heterogeneity of fiber density was also present among the association regions of the frontal, parietal, and temporal lobes. In addition, the laminar pattern of innervation in a given region was correlated with its fiber density. Sparsely innervated regions had labeled fibers only in layer I and sometimes layer VI. In regions of intermediate density, labeled fibers tended to be located in layers I–supercortical III and layers V–VI, whereas in densely innervated motor cortex TH-immunoreactive fibers were present in all cortical layers.**

Comparison of these distribution patterns with those produced by an antiserum directed against dopamine- $\beta$ -hydroxylase (DBH), a specific marker of neocortical noradrenergic axons, revealed marked differences. DBH-immunoreactive fibers were observed in some cortical locations where few or no TH-labeled fibers were present. In other regions, the density of TH-immunoreactive processes far exceeded that of DBH-labeled fibers. These findings indicate that nearly all of the immunoreactive fibers revealed by this anti-TH antiserum are dopaminergic. This interpretation was further supported by lesions of the ascending noradrenergic fibers in the brain stem, which reduced DBH immunoreactivity, but not TH immunoreactivity, in neocortex.

The distinctive innervation patterns of TH-immunoreactive fibers suggest a functional specialization of the dopaminergic projections to primate neocortex.

The expansion and differentiation of the neocortex is a distinguishing feature of the primate brain. These changes are par-

alleled by an elaboration and specialization of the noradrenergic and serotonergic projections to neocortex. Compared to rat, both of these systems in primates exhibit substantial regional heterogeneity in the density and laminar distribution of cortical fibers (Brown et al., 1979; Morrison et al., 1982a, b; Takeuchi and Sano, 1983). Midbrain dopaminergic neurons also project to neocortex, but little is known about the distribution of dopaminergic fibers in primate neocortex. Initial studies on the rat revealed that dopaminergic fibers were restricted to limited portions of the prefrontal, cingulate, and perirhinal cortical regions (Thierry et al., 1973; Berger et al., 1974, 1976; Fuxe et al., 1974; Hökfelt et al., 1974b; Lindvall et al., 1974). However, recent studies have suggested that the dopaminergic innervation of rat cortex is more extensive, with sparse projections extending to more posterior cortical regions (Berger et al., 1985; Martres et al., 1985). Biochemical studies in monkeys have revealed a heterogeneous distribution of dopamine in neocortex, with the greatest concentrations in frontal and temporal lobes (Bjorklund et al., 1978; Brown et al., 1979). In a study utilizing catecholamine histofluorescence, Levitt and colleagues (Levitt et al., 1984) also reported that almost all dopamine-like axons were confined to frontal and temporal regions. However, the biochemical studies were limited by the necessary use of relatively gross regional dissections, the inability to determine the laminar distribution of dopamine, and the uncertainty of the relative contributions of noradrenergic and dopaminergic fibers to the measured dopamine concentrations. In addition, interpretation of the histofluorescent findings was hindered by the difficulties involved in attempting to differentiate dopaminergic from noradrenergic axons on morphological grounds.

In the studies described here, we used immunohistochemical techniques to characterize the distribution of tyrosine hydroxylase (TH)- and dopamine- $\beta$ -hydroxylase (DBH)-containing axons and terminal fields in the neocortex of cynomolgus and squirrel monkeys. TH, the rate-limiting enzyme in the synthesis of all catecholamines, is contained in dopaminergic, noradrenergic, and adrenergic neurons, whereas DBH, the enzyme that converts dopamine to norepinephrine, is present only in noradrenergic and adrenergic neurons. Since no adrenergic fibers have been detected in neocortex (Hökfelt et al., 1974a), DBH is a specific marker for noradrenergic cortical fibers, whereas TH could be expected to be present in both dopaminergic and noradrenergic axons. However, previous studies in rats with an anti-TH antiserum suggested that TH is detectable immunohistochemically only in dopaminergic axons (Hökfelt et al., 1977). Our detailed comparisons of the regional and laminar distributions of TH- and DBH-immunoreactive fibers in monkeys also indicate that anti-TH and anti-DBH label distinct popu-

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lations of axons in *neocortex*, which presumably are dopaminergic and noradrenergic, respectively. Thus, the distribution of TH immunoreactivity we observed suggests that dopaminergic fibers are present throughout the entire primate neocortex, but they are distributed in a very heterogeneous fashion. The distinctive regional and laminar innervation patterns of these fibers may reveal a functional specialization of the dopaminergic projections to primate neocortex.

## Materials and Methods

Five male Old World cynomolgus monkeys (*Macaca fascicularis*) and 7 New World squirrel monkeys (*Saimiri sciureus*) were used in this study. Animals were perfused and brain tissue prepared for immunohistochemistry as previously described (Lewis et al., 1986). Animals were deeply anesthetized with ketamine hydrochloride (25 mg/kg, intramuscularly) and pentobarbital sodium (10 mg/kg, intraperitoneally). In cynomolgus monkeys, an endotracheal tube was inserted and mechanical ventilation with 100% oxygen was initiated. Squirrel monkeys were not ventilated. After the chest was opened, 1.0–2.0 ml of 1% aqueous sodium nitrite was injected into the left ventricle. Animals were then perfused transcardially with cold 1% paraformaldehyde in phosphate buffer (0.15 M) for 30–60 sec followed by perfusion with cold 4% paraformaldehyde in phosphate buffer. The latter solution was perfused for 8–10 min at a flow rate of 250–500 ml/min, depending upon the size of the animal. Immediately following the perfusion, the brain was removed and sliced into blocks, 3–5 mm thick, that were placed in cold fixative for an additional 6 hr. Tissue blocks were then washed in a series of cold, graded sucrose solutions and sectioned either coronally or sagittally in a cryostat at 40  $\mu$ m. Tissue sections were incubated with a rabbit antiserum directed against TH that had been purified from a clonal cell line of rat pheochromocytoma (Markey et al., 1980). The anti-TH antiserum was diluted 1:1500 in PBS, pH 7.4, containing 0.3% Triton X-100 and 0.5 mg/ml BSA. Sections were then processed by the avidin-biotin method of Hsu et al. (1981) using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine. Other sections were incubated with anti-DBH diluted 1:3000. Procedures for DBH immunohistochemistry were similar to those described for TH except that tissue blocks were postfixed for only 60–120 min (Morrison et al., 1982a).

Regional and laminar distribution patterns of TH and DBH immunoreactivity were determined by comparison of immunohistochemical sections with adjacent Nissl-stained sections and by Nissl counterstaining of some immunohistochemical sections. Cortical cytoarchitectonic areas were identified according to published criteria (Walker, 1940; Von Bonin and Bailey, 1947; Rosabal, 1967; Jones and Burton, 1976). The relative density of TH-immunoreactive fibers in different cortical regions was confirmed by 3 neuroanatomists who were “blind” to the findings of the primary investigator. Inter-rater reliability was evaluated by having each of the 3 investigators independently make 21 pairwise comparisons of the density of TH-immunoreactive fibers in 7 different cytoarchitectonic areas. In 60 of the resulting 63 comparisons, these ratings agreed with those of the primary investigator (95.2% concordance).

In 3 squirrel monkeys, the ascending noradrenergic projections of the locus ceruleus were lesioned. Anesthetized animals were placed in a stereotaxic instrument, and single-neuron activity was recorded through a glass micropipette. These recordings were used to locate locus ceruleus neurons as previously described (Aston-Jones et al., 1985). In 1 monkey, a metal electrode was then introduced at the anterior pole of the nucleus, its placement in locus ceruleus being verified electrophysiologically. An electrolytic lesion was created by passing 0.5 mA of cathodal current through the electrode tip for 15 sec. The electrode was then lowered 1.0 mm, and a knife cut was created by moving the electrode 750  $\mu$ m in each direction in the mediolateral dimension. In the other 2 monkeys, the glass micropipette was reintroduced 500  $\mu$ m anterior to the locus ceruleus and a knife cut was created as described above. In each monkey, the same procedure was repeated in the other hemisphere to create bilateral lesions. Four to 6 weeks later the animals were sacrificed for immunohistochemical studies. Adjacent sagittal sections through the brain stem, treated with anti-DBH or stained with cresyl violet, were examined in order to determine the location and extent of the lesions.

## Results

**Regional distribution of TH-immunoreactive fibers.** TH-immunoreactive fibers were present in every cortical region examined. In addition to this widespread distribution of TH-immunoreactive fibers, there was striking regional heterogeneity in both fiber density and laminar distribution in both monkey species. In cynomolgus monkey (Fig. 1), primary motor cortex (area 4) contained the greatest density of TH-labeled fibers. Other motor regions such as premotor cortex (area 6) were also densely innervated. Fiber density decreased in the more rostral prefrontal cortex. Among these regions, dorsomedial prefrontal cortex (area 9) and anterior cingulate cortex (area 24) had the greatest density of TH-labeled fibers. Dorsolateral prefrontal cortex (area 46) and the frontal pole (area 10) were relatively sparsely innervated, whereas the orbital regions (areas 11 and 12) tended to have an intermediate density of labeled fibers compared to other prefrontal regions.

The density of immunoreactive fibers decreased dramatically across the central sulcus from motor to primary somatosensory cortex. However, the density increased again caudally such that portions of the inferior parietal lobule (area 7) had a density of TH-labeled fibers similar to that of medial prefrontal cortex.

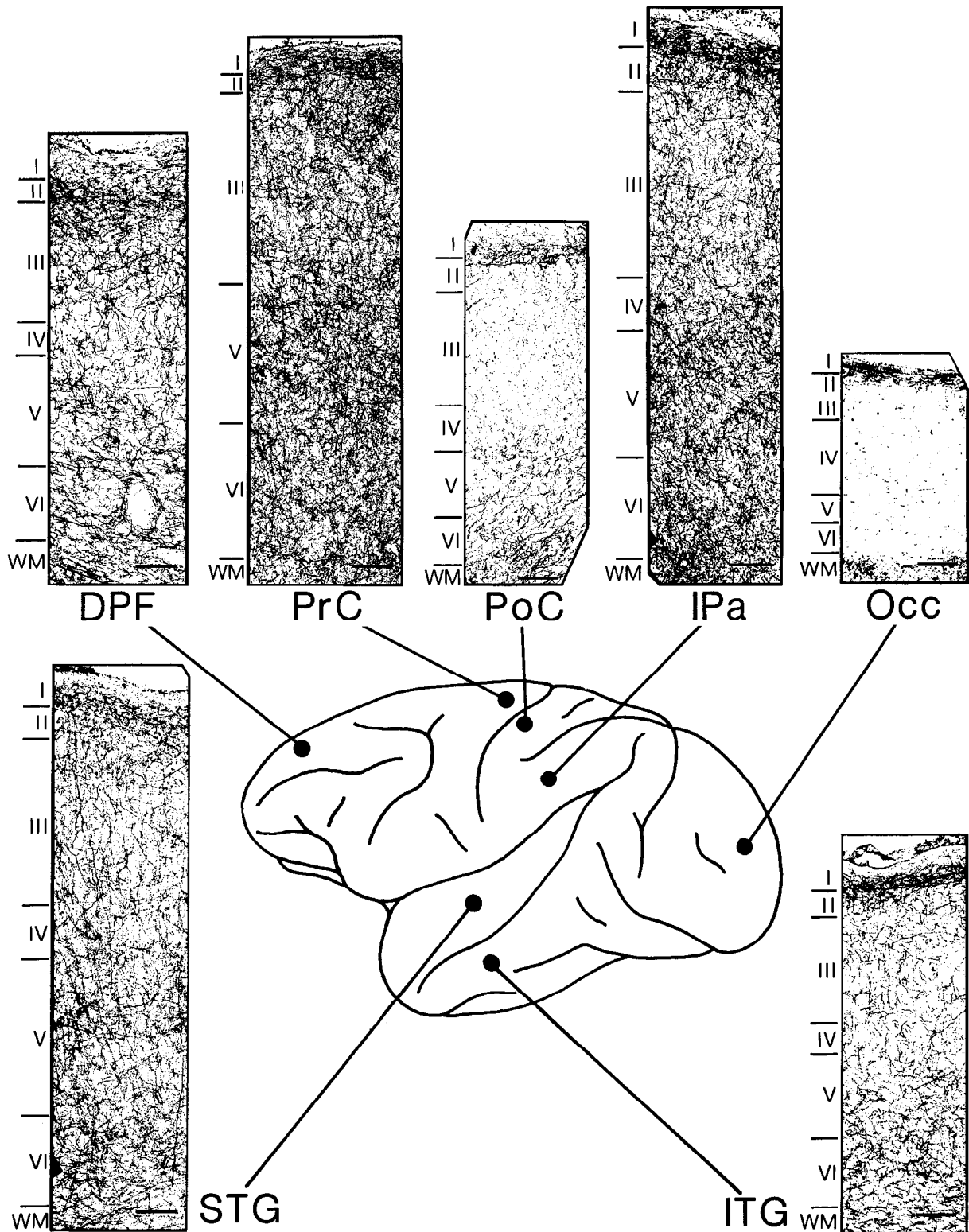
Within the temporal lobe, caudal portions of the superior temporal gyrus (including primary auditory cortex) contained the lowest density of immunoreactive fibers, whereas the rostral portion of the superior temporal gyrus (auditory association cortex) had the greatest density of TH-labeled fibers. By comparison, all regions of the inferior temporal gyrus (visual association cortex) had an intermediate density of labeled fibers, although a rostral-to-caudal gradient of increasing density was present in this gyrus.

Primary visual cortex (area 17) possessed the lowest density of labeled fibers of any region examined. The adjacent area 18 of the occipital lobe showed a small but definite increase in fiber density but did not approach the density of the inferior temporal gyrus.

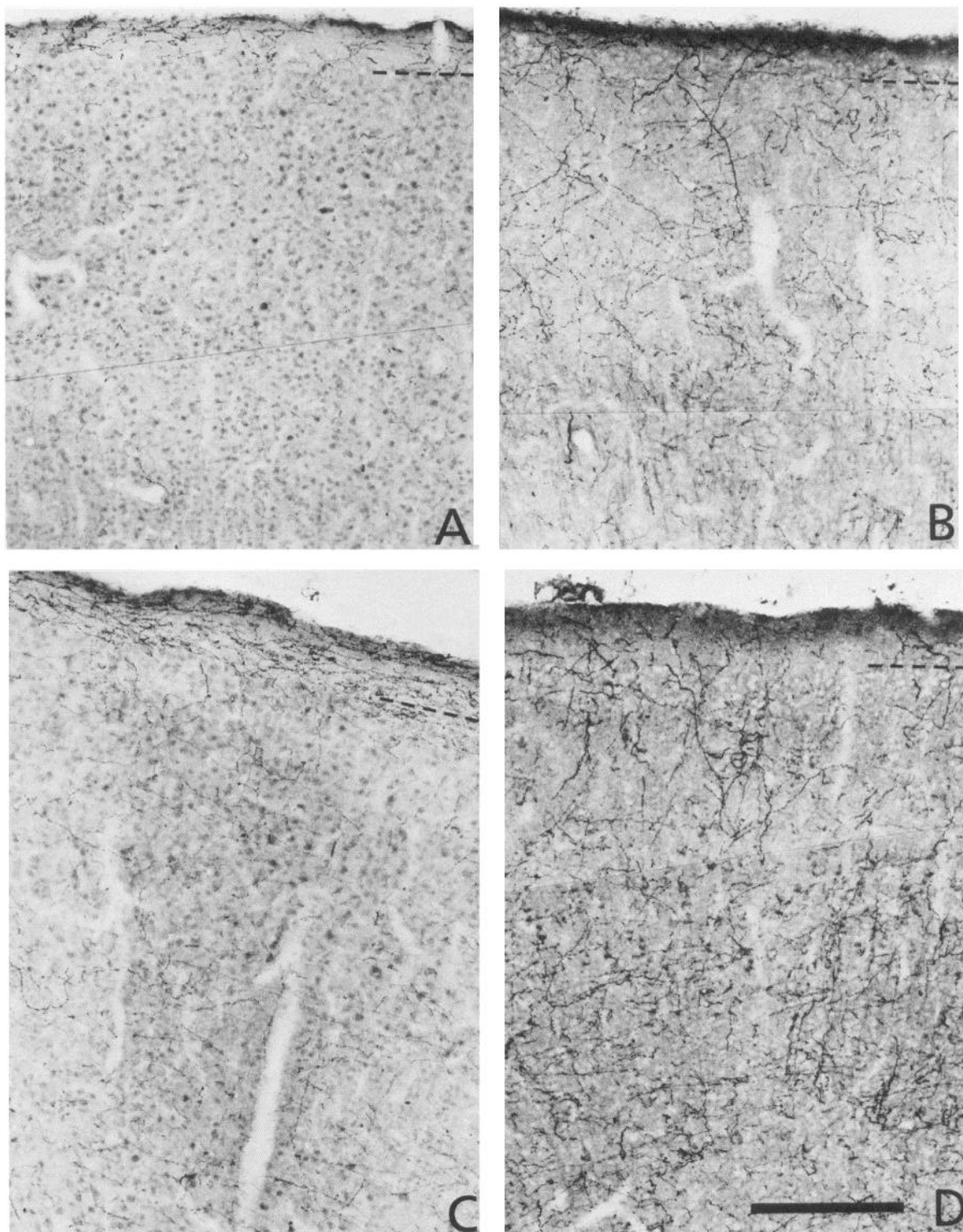
A similar regional distribution of labeled fibers was present in squirrel monkey cortex (Figs. 2, 4, 5).

**Laminar distribution of TH-immunoreactive fibers.** The laminar pattern of innervation in a given region of cynomolgus cortex was correlated with its fiber density (Fig. 1). The sparsely innervated primary visual cortex had labeled fibers only in layer I, whereas the slight increase in fiber density seen in area 18 and primary auditory cortex was associated with the presence of immunoreactive fibers in both layers I and VI. The appearance of labeled fibers in layer V of primary somatosensory cortex was correlated with the greater fiber density of this region as compared to primary auditory or visual cortex. In regions of intermediate fiber density, such as the association cortices of the frontal, temporal, and parietal lobes, labeled fibers tended to be located in layers I–superficial III and layers V–VI. In densely innervated motor cortex, TH-immunoreactive fibers were present in all cortical layers.

Despite these regional variations in the laminar distribution of fibers, there was a fairly characteristic innervation pattern in each cortical layer. Fibers in layer I were primarily tangential in orientation, whereas those in layers II–superficial III were oriented in a variety of directions. In layers deep III–IV, labeled fibers were primarily radial in orientation; arborization was less



*Figure 1.* TH immunoreactivity in 7 cytoarchitectonic areas of cynomolgus cortex. Note the extensive regional heterogeneity in the density and laminar distribution of labeled fibers. Photographs are reversed images of dark-field photomontages. *DPF*, dorsomedial prefrontal cortex (area 9); *PrC*, precentral (primary motor cortex); *PoC*, postcentral (primary somatosensory cortex); *IPa*, inferior parietal cortex (area 7); *Occ*, occipital (primary visual cortex); *STG*, rostral superior temporal gyrus (auditory association cortex); *ITG*, rostral inferior temporal gyrus (visual association cortex). Calibration bars, 200  $\mu$ m.



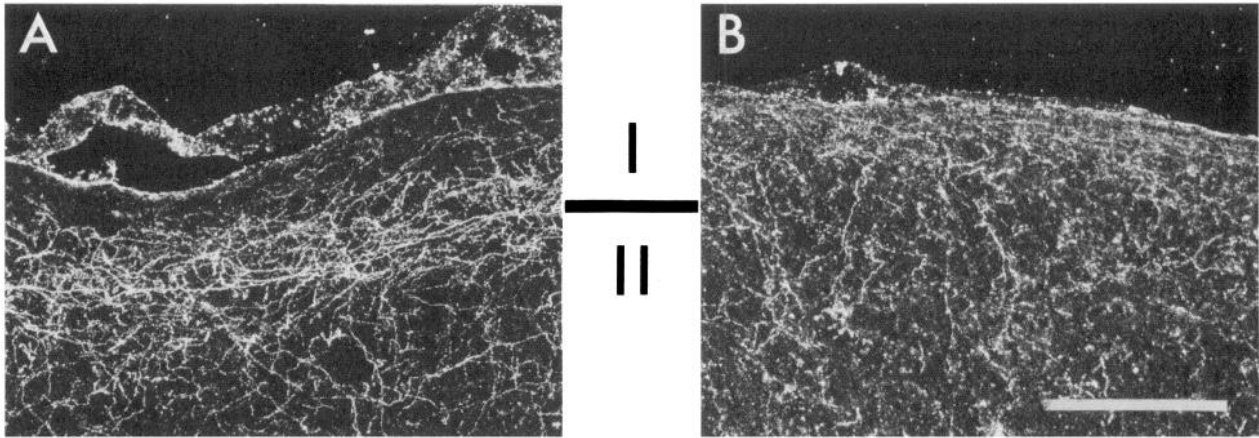
**Figure 2.** Bright-field photomicrographs of TH (*left*) and DBH (*right*) immunoreactivity in the supragranular layers of areas 17 (*top*) and 18 (*bottom*) of squirrel monkey visual cortex. *A*, In area 17, TH-labeled fibers are strongly immunoreactive in layer I but only sparse, weakly immunoreactive fibers are present in other layers. *B*, In contrast, DBH-labeled fibers are present in all cortical layers and are especially dense in layer III. In area 18, layer I contains a band of TH-immunoreactive fibers (*C*), but very few DBH-labeled fibers (*D*). In contrast, layers II and III have a much greater density of DBH- than TH-immunoreactive fibers. Note also the differences in fiber size and morphology revealed by each antiserum. *Dashed lines* indicate the border of layers I and II. Calibration bar (*A–D*), 200  $\mu\text{m}$ .

evident in these layers, except in the more densely innervated regions. Layers V–VI contained both radially and obliquely oriented fibers. A band of tangentially oriented fibers was present in the white matter immediately subjacent to layer VI.

With a few exceptions, the laminar distribution of TH-im-

munoreactive fibers was similar in squirrel monkey cortex.

*Comparison of TH- and DBH-immunoreactivity.* In order to assess the degree to which these innervation patterns reflected the projections of dopaminergic axons, we compared the regional and laminar distributions of TH- and DBH-immuno-



**Figure 3.** Dark-field photomicrographs of TH (*A*) and DBH (*B*) immunoreactivity in the superficial layers of prefrontal cortex of cynomolgus monkey. Note the dense band of TH-labeled fibers in deep layer I and in layer II and the relative paucity of fibers in the superficial portion of layer I. In contrast, DBH-labeled fibers are most dense in superficial layer I. Calibration bar, 200  $\mu$ m.

reactive processes. Although many differences were found, those most relevant to this issue involved the presence of DBH-labeled fibers in areas or layers where TH immunoreactivity was absent. For example, in primary visual cortex, DBH-labeled fibers were most dense in layers III, V, and VI, but TH-immunoreactive fibers were present almost exclusively in layer I (Fig. 2, *A, B*). Similarly, in caudal entorhinal cortex, DBH-labeled fibers traversed all cortical layers, whereas TH-labeled fibers were very sparse, and those present were usually restricted to layer I. Within layer I of some cortical areas, DBH-immunoreactive fibers were restricted to the superficial portion, whereas TH-labeled fibers were more concentrated in the deep portion of layer I (Fig. 3). In layer III of area 18, DBH-labeled fibers were much denser than TH-immunoreactive processes, whereas the opposite was true in layer I of this region (Fig. 2, *D, C*). In addition, the density of DBH-labeled fibers in the middle layers of primary somatosensory cortex exceeded that of TH-labeled fibers (Fig. 4). These comparisons revealed the existence of DBH-containing, presumably noradrenergic, fibers that were not visualized by the anti-TH antiserum.

There were also many regions where TH immunoreactivity was substantially greater than DBH immunoreactivity. For example, the density of TH-labeled fibers in primary motor cortex (Fig. 5), dorsomedial prefrontal cortex, and rostral inferior parietal cortex far exceeded the density of DBH-labeled fibers in those regions.

In addition, TH- and DBH-labeled fibers were different morphologically. In general, TH-immunoreactive axons tended to be smooth and very thin (Fig. 6, *A, C*), whereas DBH-immunoreactive fibers were usually more varicose and much thicker (Fig. 6, *B, D*).

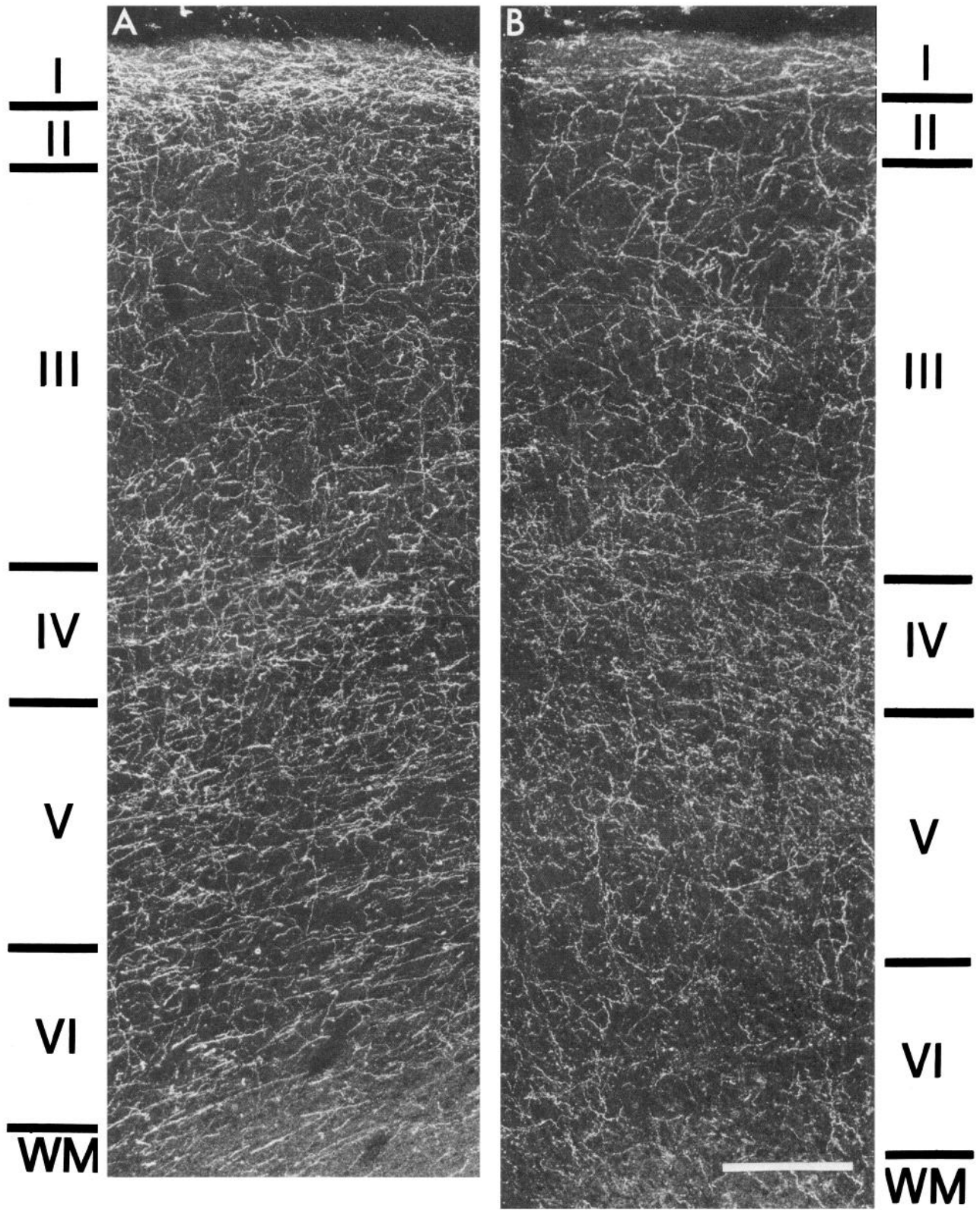
**Lesion studies.** Histological examination of the brain stem confirmed the presence of lesions of at least a substantial portion of the ascending projections of the locus ceruleus in each of the 3 squirrel monkeys in which this manipulation was attempted. In the animal (SM12) that received both the electrolytic lesions and knife cuts, extensive gliosis was present in the anterior pole of the locus ceruleus (Fig. 7*A*). In addition, there was a loss of DBH immunoreactivity in the anterior pole of the nucleus (Fig. 7*B*) and in the noradrenergic fibers of the ascending dorsal bundle. In contrast, the noradrenergic fibers of the ventral bundle could still be visualized by the anti-DBH antiserum. In the 2

animals that had received knife cuts only, the location of the lesions, as revealed by the presence of gliosis, was just anterior to the locus ceruleus in 1 case (SM14) and through the anterior pole of the nucleus in the other (SM16). In both cases there was also a loss of DBH immunoreactivity in the dorsal, as compared to the ventral, noradrenergic bundle. The loss of DBH-labeled fibers in the dorsal bundle was greater in SM14 than in SM16.

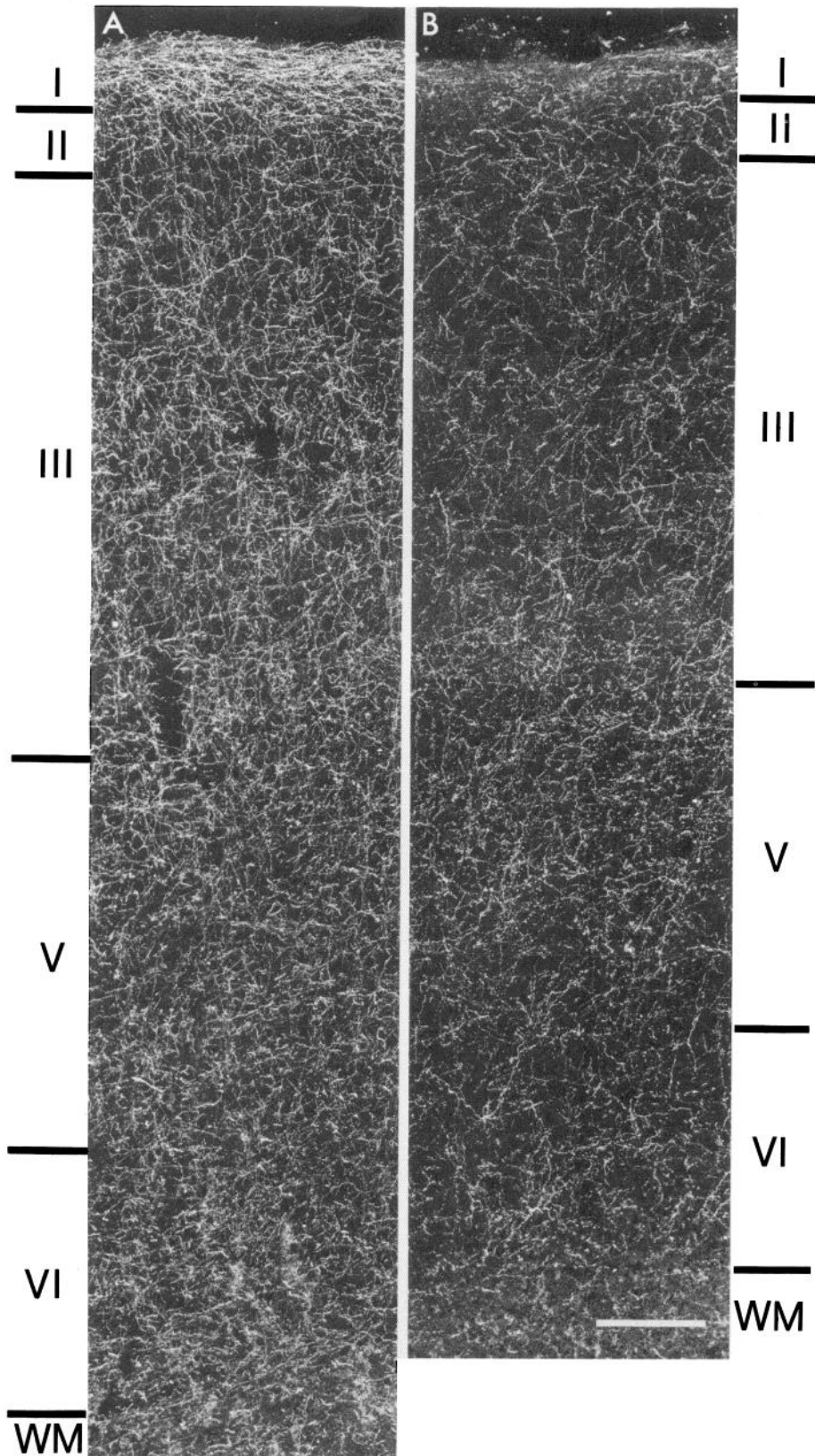
All 3 of the squirrel monkeys showed a substantial decrease in the density of cortical DBH-immunoreactive fibers compared to control animals (Fig. 8, *A, B*). The loss of DBH-labeled fibers was greatest in SM12, the monkey that had received the electrolytic lesions. In contrast, cortical TH immunoreactivity in all 3 experimental monkeys was unchanged compared to controls (Fig. 8, *C, D*).

## Discussion

Using immunohistochemical techniques, we found that TH-containing axons have a widespread distribution in primate neocortex such that labeled fibers are present in every cortical region. In addition, substantial regional heterogeneity exists in both the density and distribution of TH-labeled fibers. Clearly, interpretation of the significance of these findings depends upon knowledge of the relative degree to which dopaminergic and noradrenergic axons are visualized by the anti-TH antiserum. If the anti-TH antiserum visualizes both dopaminergic and noradrenergic fibers equally, comparison of TH and DBH immunoreactivity still indicates that the distributions of dopaminergic and noradrenergic fibers differ substantially in primate neocortex. For example, the greatest density of DBH-labeled fibers in primate neocortex is in the pre- and postcentral regions (Morrison et al., 1982a), both of which have a similar density of fibers (Figs. 4*B, 5B*). However, TH-labeled fibers are much more dense than DBH-immunoreactive fibers in precentral cortex (Fig. 5) and somewhat less dense in postcentral cortex (Fig. 4). Within the parietal lobe, the density of DBH-immunoreactive fibers decreases in a rostral to caudal direction (Morrison et al., 1982a), whereas the density of TH-labeled fibers increases across the same rostral-caudal extent. In addition, within both the prefrontal and temporal cortices, the density of DBH-immunoreactive fibers is low and relatively homogeneous across cytoarchitectonic regions, but the density of TH-labeled fibers



*Figure 4.* Dark-field photomicrographs of TH (*A*) and DBH (*B*) immunoreactivity in squirrel monkey primary somatosensory cortex. Note the slightly greater density of DBH- than TH-immunoreactive fibers in layer III and the much greater density of TH- than DBH-labeled fibers in layer I. Calibration bar, 200  $\mu\text{m}$ .

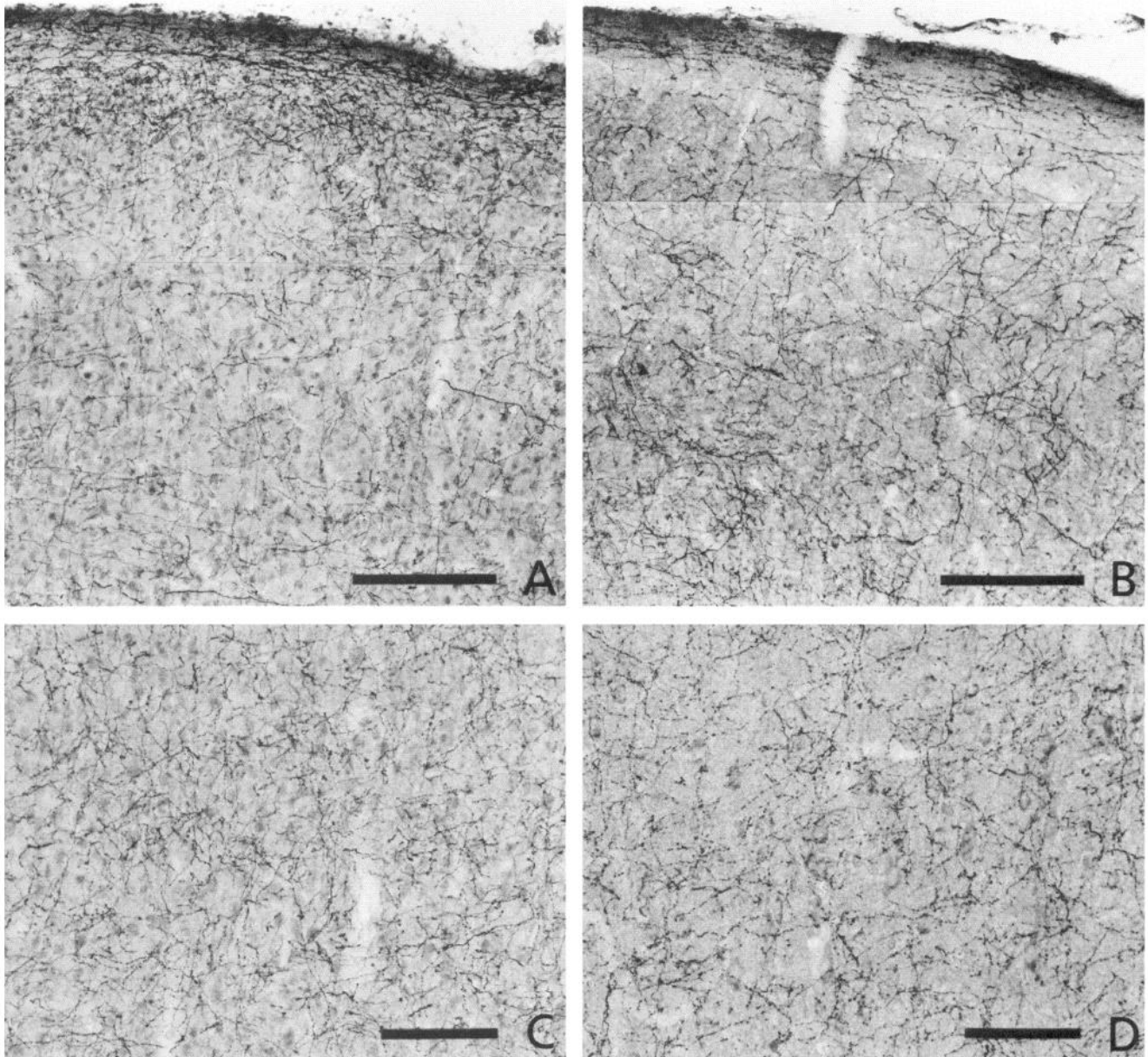


*Figure 5.* Dark-field photomicrographs of TH (*A*) and DBH (*B*) immunoreactivity in squirrel monkey primary motor cortex. Note the much greater density of TH- than DBH-labeled fibers. The density of TH-immunoreactive fibers is substantially greater in primary motor cortex than in primary somatosensory cortex (compare Figs. 5*A* and 4*A*). In contrast, the density of DBH-labeled fibers is similar in both regions (compare Figs. 5*B* and 4*B*). Calibration bar, 200  $\mu$ m.

varies substantially and is very dense in some regions (Lewis et al., 1985).

However, several lines of evidence suggest that our anti-TH antiserum labels predominately dopaminergic fibers. Both bio-

chemical (Emson and Koob, 1978; Schmidt and Bhatnagar, 1979) and immunocytochemical (Pickel et al., 1975; Hökfelt et al., 1977) studies in rodents have demonstrated that dopaminergic fibers contain a greater amount of TH than do norad-



**Figure 6.** Bright-field photomicrographs of TH (*left*) and DBH (*right*) immunoreactivity in the supragranular layers of primary somatosensory cortex (*top*) and layer V of primary motor cortex (*bottom*) in squirrel monkey. Note the smooth, fine morphology of the TH-labeled fibers (*A, C*) as compared to the thicker, more varicose DBH-labeled fibers (*B, D*). Calibration bars, 200  $\mu\text{m}$  (*A, B*) and 100  $\mu\text{m}$  (*C, D*).

renergic fibers. In addition, previous immunocytochemical studies with an anti-TH antiserum strongly suggested that TH was detectable only in dopaminergic axons in rat neocortex (Hökfelt et al., 1977).

Second, as described above, our studies in primates have revealed the presence of DBH-labeled fibers in cortical areas and layers where little or no TH immunoreactivity was observed. These findings indicate the existence of noradrenergic axons in which TH is not detectable immunohistochemically. It is of interest in this regard that we occasionally observed faint TH-immunoreactive structures in layers III and V of primary visual cortex (Fig. 2*A*). This observation might indicate that the anti-TH antiserum weakly recognizes a minority of noradrenergic fibers in layers III and V of an area where it clearly demonstrates dopaminergic fibers in layer I. However, these fibers

could represent a minor dopaminergic projection to layers III and V of primary visual cortex.

Differences in morphology between TH- and DBH-immunoreactive fibers also suggest that these 2 antisera selectively label distinct populations of axons in neocortex. The morphological features of TH- (smooth, thin) and DBH-labeled (varicose, thick) fibers that we observed (Fig. 6) are similar to the descriptions of dopaminergic and noradrenergic axons in histofluorescent studies (Lindvall and Bjorklund, 1974; Berger et al., 1976; Levitt and Moore, 1979; Levitt et al., 1984).

A fourth line of evidence suggesting that dopaminergic fibers are selectively visualized by the anti-TH antiserum comes from our studies of squirrel monkeys with histologically confirmed ablations of the ascending noradrenergic projections of the locus ceruleus. In these animals, cortical DBH immunoreactivity was



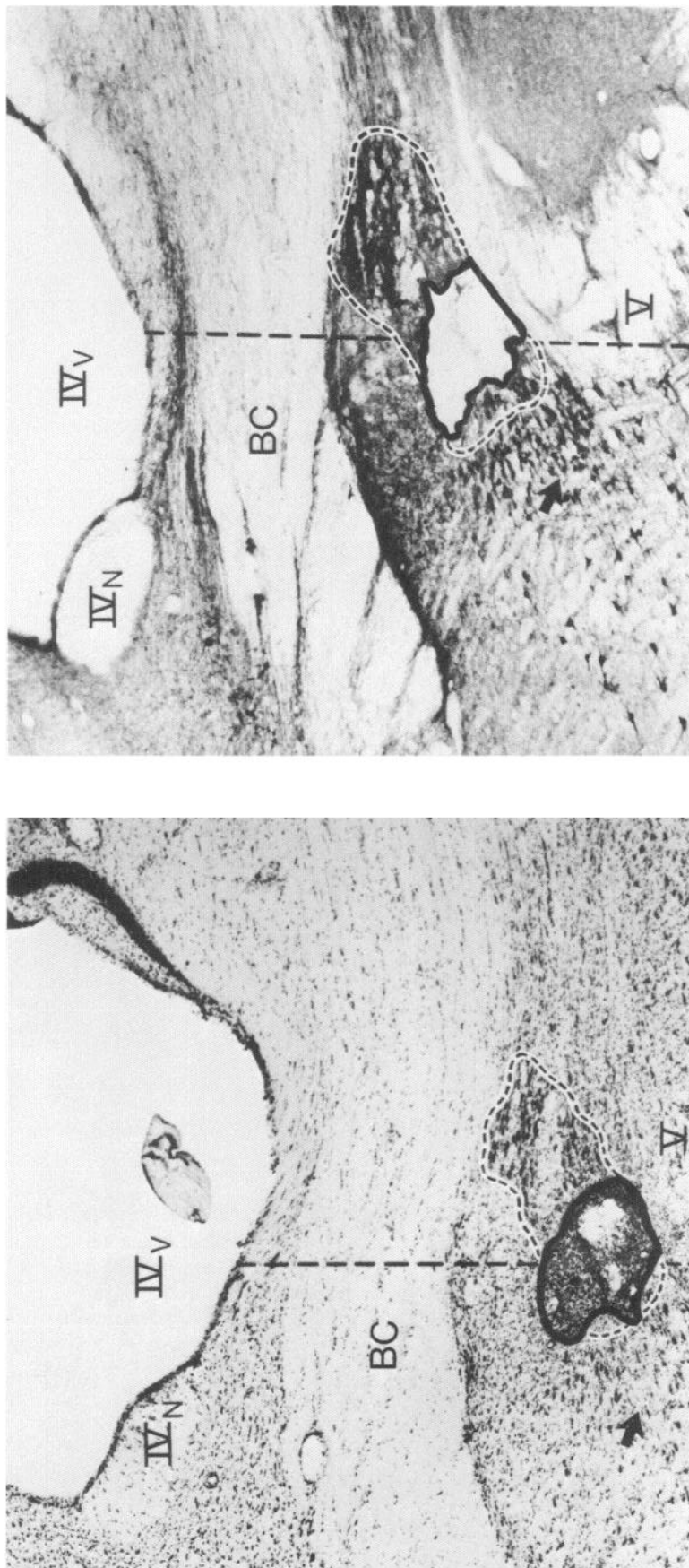
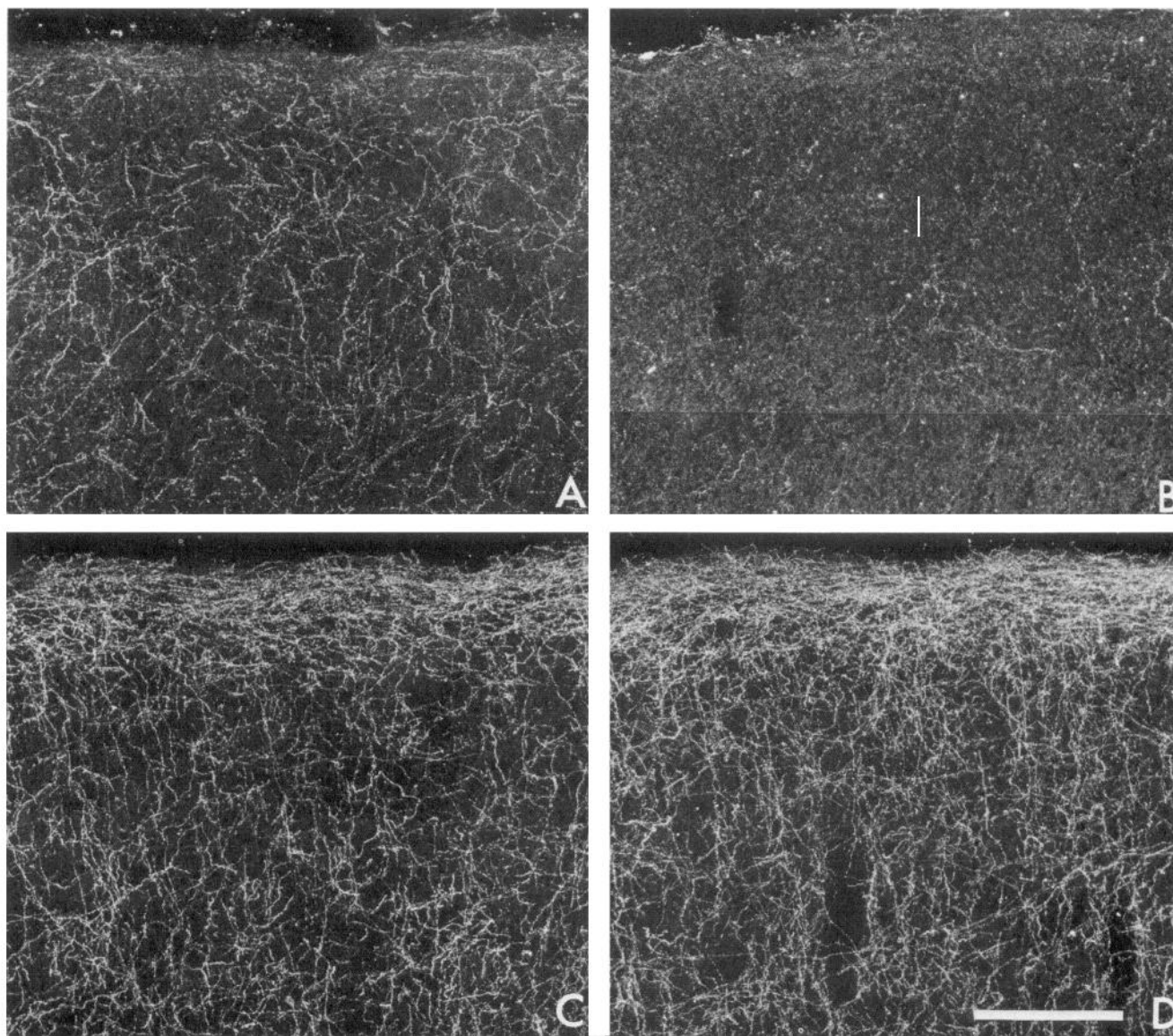


Figure 7. Sagittal sections through the brain stem of a squirrel monkey (SM12) with bilateral ablations of the locus ceruleus. *Left*, Nissl-stained section. *Right*, Section treated with anti-DBH. The dashed black-and-white lines indicate the borders of the locus ceruleus and the solid black lines demarcate the boundaries of the electrolytic lesion. The dashed black lines show the location of the knife cut. The dorsal and rostral portions of the sections are to the top and left, respectively. The large cells rostral and ventral to the locus ceruleus are noradrenergic neurons of the subceruleus, which do not project to cortex. BC, brachium conjunctivum; IV<sub>V</sub>, fourth ventricle; IV<sub>N</sub>, decussation of trochlear nerve; V, tract of mesencephalic nucleus of trigeminus.



**Figure 8.** Dark-field photomicrographs of DBH (*top*) and TH (*bottom*) immunoreactivity in the superficial layers of squirrel monkey primary motor cortex. *Left*, control animal; *right*, animal (SM12) with a lesion of the locus ceruleus. Following ablation of the locus ceruleus, DBH immunoreactivity (*B*) was substantially reduced compared to control (*A*). In contrast, TH immunoreactivity was unchanged in lesioned animals (*D*) versus controls (*C*). Calibration bar, 200  $\mu\text{m}$ .

substantially reduced, whereas TH immunoreactivity was unchanged compared to controls.

Thus, these findings strongly suggest that our anti-TH antiserum predominantly labels dopaminergic axons in monkey neocortex. Although anti-TH clearly labels the noradrenergic cell bodies of the locus ceruleus, there are several possible explanations for the apparent selectivity of the antiserum for dopaminergic fibers in neocortex. First, since dopaminergic fibers contain a greater amount of TH than do noradrenergic fibers (Emson and Koob, 1978; Schmidt and Bhatnagar, 1979), it may be that the quantity of TH in cortical noradrenergic fibers is insufficient to be recognizable with immunohistochemical techniques. This may be particularly true in primate neocortex, where noradrenergic fibers and terminal fields are quite distant from their cell bodies of origin. Second, TH appears to be associated with synaptosomal plasma membranes only in dopaminergic neurons (Hwang et al., 1985). This association may

be necessary to create immunohistochemically detectable concentrations of TH in axons and terminal fields. Third, TH has been reported to exist in different states of aggregation in dopaminergic and noradrenergic neurons (Joh and Reis, 1975). Anti-TH might have a greater affinity for the lower-molecular-weight form of TH present in the dopaminergic system than the higher-molecular-weight form found in noradrenergic neurons (Pickel et al., 1975). Finally, it may be that our antibody recognizes a portion of the TH molecule that is accessible to the antiserum only in dopaminergic fibers. Each of these hypotheses could imply a fortuitous attribute of our antiserum that might not generalize to other anti-TH antisera.

#### *Functional implications*

Biochemical studies suggested that the greatest concentrations of dopamine in primate cortex were in prefrontal and temporal regions and that the density of dopaminergic innervation de-

creased in a rostral-caudal fashion from these areas (Bjorklund et al., 1978; Brown et al., 1979). In a histofluorescence study, Levitt and colleagues (Levitt et al., 1984) reported that they rarely saw dopamine-like axons in cortical regions outside of the frontal and temporal lobes. However, our findings suggest that the dopaminergic innervation of primate cortex is global, such that labeled fibers are present in every cortical region. There also appears to be substantial regional heterogeneity in dopaminergic innervation density, which does not follow a simple rostral-caudal gradient. For example, primary motor cortex, a preferred target for TH-immunoreactive fibers, is more densely innervated than any frontal region rostral to it (Fig. 1, *A, B*). Although the density of labeled fibers decreases immediately caudal to motor cortex (primary somatosensory cortex), fiber density increases again further caudally, revealing a preference for somatosensory association cortex over primary somatosensory cortex (Fig. 1, *C, D*). This predilection for association over primary sensory regions is also apparent in both the visual and auditory systems. In addition, the density of labeled fibers differs markedly across cortical regions previously reported to have a relatively homogeneous dopaminergic innervation (Bjorklund et al., 1978; Brown et al., 1979; Levitt et al., 1984). For example, the rostral superior temporal gyrus (auditory association cortex) has a substantially greater density of TH-immunoreactive fibers than does the rostral inferior temporal gyrus (visual association cortex) (Fig. 1, *F, G*). These distribution patterns suggest a functional specialization of the dopaminergic cortical projections such that dopaminergic fibers preferentially innervate motor over sensory regions, sensory association over primary sensory regions, and auditory association over visual association regions.

The laminar distribution of TH-immunoreactive fibers may also reflect a functional role of cortical dopaminergic projections. Despite the regional heterogeneity in the laminar distribution of TH-labeled fibers, layer IV in cynomolgus monkey consistently contained the lowest density of immunoreactive fibers. In addition, those cortical regions in which layer IV is most prominent (primary sensory areas) had the lowest density of labeled fibers, whereas agranular cortical regions, such as anterior cingulate cortex, were densely innervated. In other cortical regions, the density of TH-immunoreactive fibers also tended to be inversely related to the prominence of layer IV. These findings suggest that dopamine is more likely to modulate the activity of the cells of origin of corticocortical, corticostriate, or corticobulbar projections than that of cells receiving primarily thalamic afferents or the feed-forward corticocortical projections (Jones, 1981, 1984; Van Essen, 1985).

In conclusion, our findings suggest that the dopaminergic projections to primate cortex are both more widespread and more regionally heterogeneous than previously reported. These distinctive innervation patterns imply a functional specialization of the dopaminergic cortical projections that may provide insight into their role in normal brain function and certain human disease states.

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