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Noradrenergic and serotonergic fibers innervate complementary layers in monkey primary visual cortex: An immunohistochemical study

(cortical organization/visual system/norepinephrine/serotonin/locus ceruleus)

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ABSTRACT **Antisera directed against human dopamine β-hydroxylase or serotonin were used to characterize the noradrenergic and serotonergic innervation patterns within the primary visual cortex of the squirrel monkey. The noradrenergic and serotonergic projections exhibit a high degree of laminar complementarity: layers V and VI receive a dense noradrenergic projection and a very sparse serotonergic projection, whereas layer IV receives a very dense serotonergic projection and is largely devoid of noradrenergic fibers. In addition, the noradrenergic fibers manifest a geometric order that is not so readily apparent in the distribution of serotonergic fibers. These patterns of innervation imply that the two transmitter systems affect different stages of cortical information processing—the raphe-cortical serotonergic projection preferentially innervates the spiny stellate cells of layers IVa and IVc, whereas the ceruleo-cortical noradrenergic projection innervates pyramidal cells.**

The noradrenergic (NA) and serotonergic (5-HT) projections to neocortex arise from brainstem nuclei that project monosynaptically to all major regions of neocortex (1, 2) in a highly divergent manner (3), without apparent topographic order (4, 5). Immunohistochemical (IHC) techniques for selective visualization of monoamine fibers (6–8) demonstrate that the monoamine innervation of rat cortex has a characteristic uniform laminar and tangential organization (3, 9–11), with significant regional variation only in the cingulate cortex (11–13). Although the general laminar and tangential features of NA axons are similar to those in the rat (3, 9, 10), the primate cortex exhibits far greater regional specificity in that many diverse neocortical areas possess specific patterns of laminar distribution and density of NA fibers (14–16).

Previously, we observed a distinct laminar pattern of innervation in the primary visual cortex (area 17) of squirrel monkey (15). The NA innervation of this cortical area is of special interest because area 17 is one of the most intensively studied and best understood neocortical regions with regard to function and to neuronal circuitry. Additionally, norepinephrine has been hypothesized to be essential for the "plasticity" seen in the development of ocular dominance (17, 18), although the possible anatomical substrate for this plasticity has been studied in only a limited way (19). There is evidence from biochemical studies that 5-HT levels may be higher in visual cortex and norepinephrine levels relatively low compared with more anterior areas of cortex (20, 21). Given previous examples of complementarity in the laminar innervation of cortical regions by monamines (12, 13), we felt it would be of interest to determine whether the NA and 5-HT projections to primate visual cortex exhibit distinctive organizational patterns.

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MATERIALS AND METHODS

Animals. Adult Guyanan squirrel monkeys (*Saimiri sciureus*) of either sex were the subjects of this study.

Preparation of Antisera. The antiserum directed against dopamine β-hydroxylase (DBH) was prepared by D. O'Connor and his colleagues (Department of Medicine, University of California at San Diego, School of Medicine) from human pheochromocytoma as described (15, 22).

The antiserum directed against serotonin was that prepared by Lidov *et al.* (11) as described (8, 11).

Tissue Preparation and Immunohistochemical Procedure. Ten to fifteen minutes prior to perfusion, animals were deeply anesthetized with ketamine hydrochloride (25 mg/kg, intramuscularly) and pentobarbital sodium (10 mg/kg, intraperitoneally). Some animals were treated with the monoamine oxidase (MAO)-inhibitor pargyline (25 mg/kg, intramuscularly) 24 hr prior to perfusion. Animals were perfused transcardially with either ice-cold phosphate buffer or 1% paraformaldehyde in phosphate buffer for 0.5–1 min followed by perfusion with cold 4% paraformaldehyde in phosphate buffer (0.15 M) for 8 min at 100 ml/min. After one to six additional hours in fixative, tissue blocks were washed in a series of cold, graded sucrose solutions and stored in 18% sucrose overnight.

The IHC has been described in detail in a separate report (15). Briefly, the tissue blocks were frozen and sectioned in a cryostat at 40 or 50 μm. Freely floating sections in cold phosphate-buffered saline were then processed for IHC with either anti-DBH or anti-serotonin as the primary antiserum. The anti-DBH and anti-serotonin were commonly used at dilutions of 1:4,000 and 1:2,000, respectively. After incubation in the secondary antiserum, peroxidase-conjugated anti-rabbit IgG (1:1,000), the sections were developed for peroxidase reactivity with 3,3′-diaminobenzidine, mounted on gelatin-coated slides, and dried in a stream of warm air. To enhance visualization of the fine 5-HT fibers, some of the mounted sections were briefly exposed to osmium tetroxide vapor. Several of the sections that were processed for DBH or 5-HT IHC and several unincubated sections were counterstained with cresyl violet.

RESULTS

NA Innervation. As is the case in other regions of primate neocortex, the DBH-positive fibers in area 17 are highly varicose and of relatively small caliber, although the overall density of NA innervation is low in area 17 compared to other primary sensory regions of squirrel monkey cortex. There are very few DBH-positive fibers present in the subcortical white matter.

Abbreviations: NA, noradrenergic; 5-HT, serotonergic (5-hydroxytryptaminergic); IHC, immunohistochemical procedure; DBH, dopamine β-hydroxylase; MAO, monoamine oxidase.

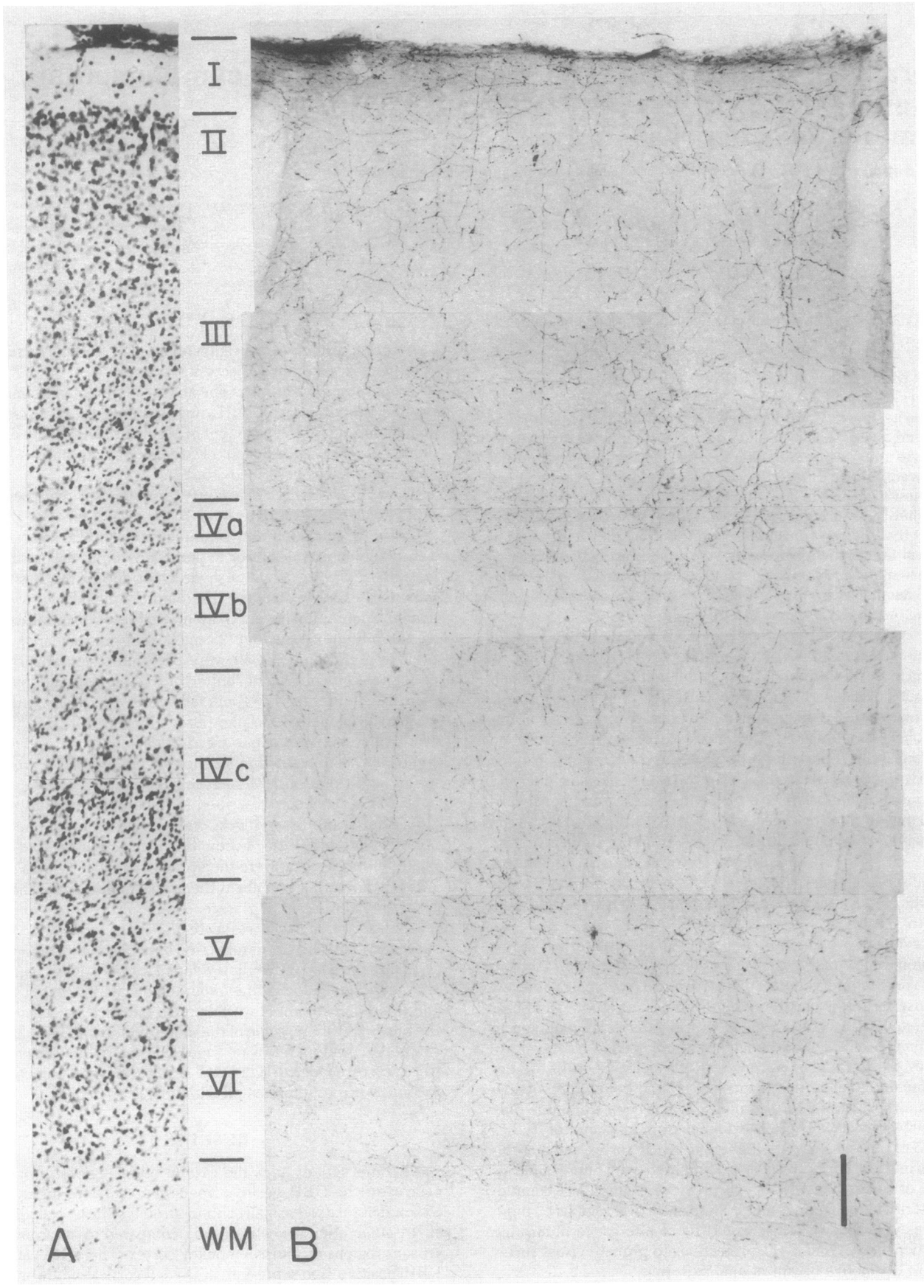

FIG. 1. NA innervation of area 17. (*A*) Cresyl violet-stained section through primary visual cortex. Section adjacent to *B*. (*B*) DBH-positive fibers in primary visual cortex. Roman numerals refer to cortical layers. WM denotes white matter. Bar = 100 μm.

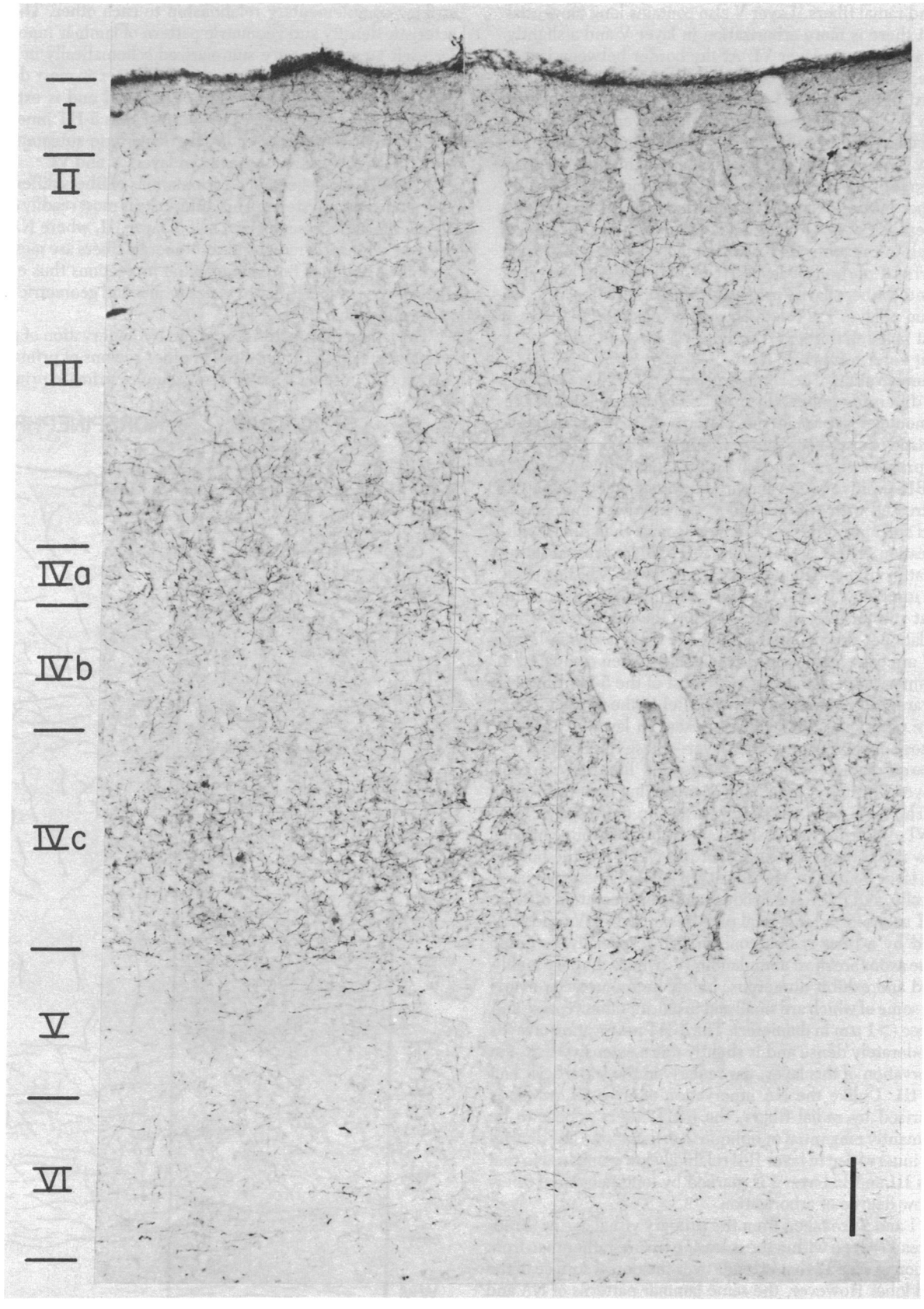

FIG. 2. 5-HT innervation of area 17. Serotonin-positive fibers in primary visual cortex. Roman numerals refer to cortical layers. Bar = 100 μm.

A photomontage depicting the pattern of NA innervation characteristic of area 17 is shown in Fig. 1. There is a moderately dense horizontal plexus of NA axons deep and superficial to layer IV. The laminae receiving the densest NA innervation are deep III, V, and VI. Layer VI contains many long fibers that are oriented parallel to the subcortical white matter as well as

oblique and radial fibers. Layer V also contains long tangential fibers, but there is more arborization in layer V and a slightly higher density than in layer VI. At the border between layers V and IVc, the pattern changes abruptly, such that layer IVc contains extremely few DBH-positive fibers. The fibers that are present in IVc generally extend across the layer radially, with a minimal degree of arborization. Layers IVb and IVa also have a very low density of NA innervation but slightly higher than that present in IVc. The density of NA axons increases abruptly at the upper border of layer IVa, and fibers arborize copiously in the deep half of layer III. The deep portion of III contains fibers of all orientations and is more densely innervated than the superficial portion of layer III or of layer II, where the DBH-positive fibers have a primarily radial orientation. The NA innervation of layer I is relatively sparse, consisting largely of occasional tangential fibers traveling within layer I and radial fibers that enter from layer II.

5-HT Innervation. The laminar pattern of 5-HT innervation of area 17 differs fundamentally from that of the NA innervation. A photomontage depicting the pattern of 5-HT innervation characteristic of area 17 is shown in Fig. 2. The 5-HT-positive fibers in primary visual cortex are not as distinctively varicose as the DBH-positive fibers and are of even smaller caliber. In sections treated with osmium vapor, the fibers are more easily visualized and appear denser and more coarse than fibers without osmication. There are very few 5-HT fibers present in the white matter immediately adjacent to layer VI; however, 200–500 μm below layer VI, bands of 5-HT fibers are encountered that run for long distances within the subcortical white matter (data not shown; see Fig. 3). The 5-HT axons in layers VI and V are extremely sparse, particularly when compared to the NA innervation of these layers. Most of the 5-HT fibers in layer VI are unbranched and run parallel to the white matter. There are occasional 5-HT fibers present in layer VI that are unlike those present in any other layer—these fibers are of relatively large diameter and run for several hundred microns within layer VI without branching or modifying their horizontal course. The 5-HT axons in layer V are even more sparse than in layer VI, consisting of occasional short oblique axon segments and a few radial fibers extending across layer V. At the border between layer V and IVc, the density of labeled fibers increases dramatically, and there is a striking laminar innervation of layer IV. 5-HT axons form tangential plexuses in layers IVa and IVc, separated by a fiber-sparse zone in IVb (especially its outer half). The axons are most abundant in IVc where they are highly arborized and exhibit numerous, highly immunoreactive varicosities, some of which are small and fusiform, others coarse and quite large (>1 μm in diameter). The 5-HT innervation of layer III is moderately dense and is slightly more extensive than the NA innervation of this layer, particularly in the superficial half of layer III. Unlike the NA innervation of layer III, which is characterized by radial fibers, the 5-HT fibers appear to be predominantly tangential or oblique in orientation. The density of 5-HT innervation in layer II is relatively low compared to that of layers III and I. Layer I is marked by long tangential fibers with a low degree of arborization.

Figs. 1 and 2 are taken from the primary visual cortex (Brodmann area 17) deep within the calcarine fissure rather than from the portion of area 17 that extends over the dorsal surface of the occipital lobe. However, the same laminar patterns of NA and 5-HT innervation were found in all regions of primary visual cortex.

DISCUSSION

Both the NA and 5-HT innervation patterns of primary visual cortex exhibit profound laminar variations in density, with a striking complementary relationship to each other. The characteristic density and geometric pattern of laminar innervation for each monoamine are summarized schematically in Fig. 3. The NA innervation of primary visual cortex is most dense in layers V, VI, and the deep half of layer III and is extremely sparse throughout layer IV. In contrast, the 5-HT innervation is extremely dense in layer IV, particularly in sublaminae IVa and IVc, and is nearly absent from layers V and VI.

In addition, the geometric orientations of fibers differ for the two monoamine systems. This difference is most readily evident in layer II and the superficial half of layer III, where NA fibers are predominantly radial and serotonergic fibers are mostly tangential or oblique. Both monoamine projections thus exhibit a high degree of specificity in their laminar and geometric pattern of termination.

The laminar characteristics of the NA innervation of primary visual cortex differ from those of other regions of primate neocortex (15) in several ways. For example, primate primary so-

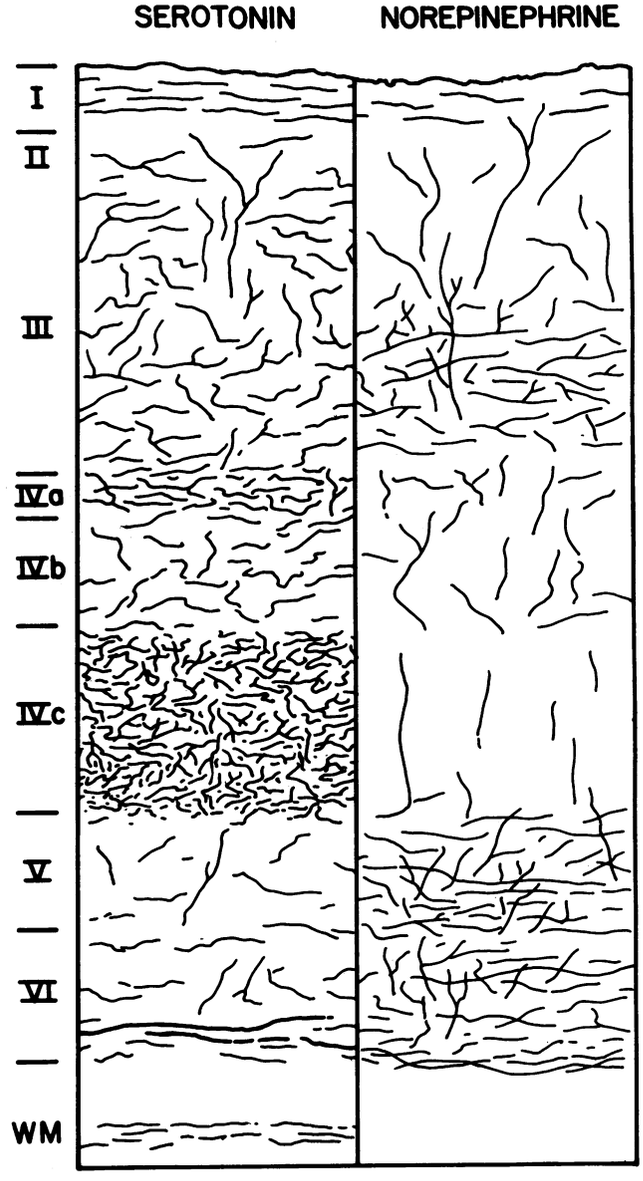

FIG. 3. Schematic diagram showing laminar patterns of NA and 5-HT innervation of primary visual cortex. Note laminar complementarity in layers IV–VI and predominant orientation of each type of fiber in each layer. Also, note presence of 5-HT fibers in subcortical white matter. WM denotes white matter.

matosensory cortex possesses a far more uniform density of innervation across the six cortical laminae and, in particular, contains a fairly dense plexus of fine fibers in layer IV (15). These data, as well as previous data concerning the monoamine innervation of primate and rat cortex (3, 9–16, 23), indicate that the monoamine afferents are highly specific in their mode of termination, especially in primate where regional differences are a striking feature of the monoamine innervation pattern (13–15).

Primary visual cortex is the most rigidly laminated of neocortical areas. Primarily, the layers are a reflection of systematic variation in the size and density of the neuronal cell bodies; however, each layer also is characterized by a specific and discrete combination of cell types, dendritic branching patterns, and extrinsic afferents (see ref. 24 for review). In the case of layer IV, spiny stellate neurons in layers IVa and IVc are the primary targets of the geniculo-cortical projection (25), and these cells are rarely encountered in other layers of visual cortex (26). Moreover, the dendrites of the spiny stellate cells are primarily restricted to layer IV (26). Thus, their entire receptive surface is largely within that layer that receives the geniculocortical projection, the most dense 5-HT input, and virtually no noradrenergic input. Thus, it would appear that within the primary visual cortex there is a convergence of raphe-cortical (5-HT) and geniculo-cortical input on the spiny stellate cells within layer IV, where the NA projection is conspicuously sparse.

The pyramidal cell is the most distinctive cell class within layers VI, V, and III (26–28). In addition, these laminae contain most of the dendritic branches from the basal and apical dendrites of these neurons (27, 28). These pyramidal cells furnish the major efferents from the primary visual cortex, such as the projections to areas 18 and 19 (primarily from III; refs. 29, 30) and the lateral geniculate nucleus (primarily from VI; refs. 31, 32). Many of the pyramidal cells within layers V and VI possess basal dendrites that branch profusely within these layers and an apical dendrite that branches extensively in layers III and V but does not emit branches as it ascends through layer IV (26–28). The deep portion of layer III also contains extensive dendritic arborization from the basal dendrites within layer III (27, 28). Thus, the NA projection appears to be directed at precisely those laminae that contain a dense plexus of pyramidal cell dendrites, namely deep III, V, and VI. In addition, the orientation of the NA fibers within each cortical layer bears a striking resemblence to that of the pyramidal cell dendrites as seen in the Golgi studies of Lund and her colleagues (26–28). In contrast, the 5-HT projection is extremely sparse in layers V and VI.

We interpret these fiber patterns as indications that the NA projection to primary visual cortex may preferentially innervate pyramidal cells, whereas the 5-HT projection may exert its primary influence on the spiny stellate cells of layers IVa and IVc. The 5-HT projection is in a position to modulate neuronal activity at the initial stage of signal processing in cortex. Layer IV cells are closely linked to the thalamic input and as such generally do not exhibit any orientation specificity and are monocular (33). The principal targets of the NA system, the pyramidal cells superficial and deep to layer IV, exhibit higher order information processing in that they are binocular and possess orientation specificity (ref. 33; see ref. 34 for review). Thus, the fact that the ceruleo- and raphe-cortical projections have complementary patterns of termination suggesting separate postsynaptic targets indicates that these two projections from the brain stem occupy distinct and separable roles in the regulation of signal processing in the visual cortex. The validity of these hypotheses can be determined only by ultrastructural and electrophysiological studies that clearly demonstrate the nature of the NA and 5-HT neuronal contacts and identify their postsynaptic targets.

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