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Association of efferent neurons to the compartmental architecture of the superior colliculus

(midbrain/multimodal integration/lamination/modular organization/dendritic morphology)

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ABSTRACT **The superior colliculus is a layered structure in the mammalian midbrain serving multimodal sensorimotor integration. Its intermediate layers are characterized by a compartmental architecture. These compartments are apparent through the clustering of terminals of major collicular afferents, which in many instances match the heterogeneous distribution of tissue components such as acetylcholinesterase, choline acetyltransferase, substance P, and parvalbumin. The present study was undertaken to determine whether efferent cells observe this compartmental architecture. It was found that subpopulations of both descending and ascending collicular efferents originate from perikarya situated in characteristic positions relative to the collicular compartments defined by elevated acetylcholinesterase activity and that their dendrites appear to be specifically coordinated with the heterogeneous environment. With the specific interlocking of afferent and efferent neurons through spatially distinguished neural networks, the compartmental architecture apparently constitutes an essential element for the determination of information flow in the superior colliculus.**

The nervous system is organized in functional units. Hierarchies of them comprise units on the synaptic, dendritic, and neuronal level, and functional units on the next higher level appear to exist as neuronal assemblies in the form of layers of neurons, local compartments, or modules within nuclei of the brain (1). In several vertebrate species, a compartmental organization of central nervous tissue has been discovered in various topographically organized brain areas (2). One of these areas is the superior colliculus in the roof of the mammalian midbrain. The colliculus receives, among other inputs, sensory afferents conveying various modalities (3), represents the sensory space in a stack of corresponding topographical maps (4, 5), and utilizes them for mediation of orientation responses, both overt (6) and covert (7). Structurally, the intermediate layers of the superior colliculus are characterized by several sets of periodically arranged, neurochemically defined compartmental domains (8–10). Various collicular afferents, and most notably those related to sensory systems, terminate in a discontinuous pattern such that their terminal fields lie largely in register with each other and with certain neurochemically defined patterns such as areas of low acetylcholinesterase (AChE) activity. By contrast, afferents associated with motor pathways reveal a complementary pattern of termination by preferentially innervating AChE-rich zones (Fig. 1). The surface between the compartments is profusely folded and, in the rat, their typical spacing in frontal sections is 100–200 μm. However, these compartments fall short of representing a modular organization in that they form intermingled meshworks rather than truly repetitive units.

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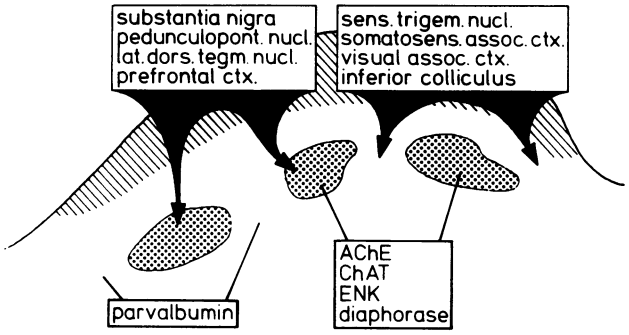

FIG. 1. Schematic drawing of a frontal section through the superior colliculus, summarizing data obtained in rat and cat; midline is to the right. Superficial (visual) layers are shown in shading. Intermediate (multimodal) layers are characterized by a periodic compartmental architecture (indicated as interdigitation of stippled and blank zones). This compartmentalization is apparent through both the geometry of terminal fields of collicular afferents and the distribution of certain biochemical constituents of the tissue. Stippled areas represent zones of high levels of AChE (rat, refs. 11 and 12; cat, refs. 8 and 13), choline acetyltransferase (ChAT) (rat, ref. 12; cat, ref. 8), the enkephalins (ENK) (cat, A. M. Graybiel and R.-B.I., unpublished data), and NADPH diaphorase (rat, ref. 9), and these zones are preferentially innervated by nigral afferents (rat, ref. 11; cat, refs. 13 and 14), by fibers from the pedunculopontine and lateral dorsal tegmental nucleus (rat, ref. 15; cat, ref. 16), and also receive prefrontal cortical input (cat, ref. 13). Conversely, the space surrounding these compartments is particularly rich in parvalbumin (rat, ref. 17) and receives innervation from the sensory trigeminal nuclear complex and the somatosensory and visual association cortices (cat, refs. 14 and 18) as well as the external and pericentral nuclei of the inferior colliculus (rat, unpublished observations).

Ever since the discovery of the compartmental organization of the superior colliculus, it has been an open issue whether there might be a corresponding geometry in the distribution of efferent neurons. There have been several studies reporting a clustering of efferent cell populations in the deeper collicular layers (11, 19, 20). Although these observations seemed to favor participation of the efferent cells in the compartmental organization, it has not been known what spatial relation these cell clusters assume with respect to the various types of compartments.

It is not *a priori* certain that there should be a close spatial relation between the terminal fields of collicular afferents and the perikarya of efferent neurons. An argument that cautioned against this assumption has been that the width of the dendritic tree of representative collicular neurons on the level of the compartments extends beyond 200 μm (21). This geometry may suggest that neurons collect their input indiscriminately from patch and nonpatch areas alike. If integration takes place locally between dendritic segments and presynaptic elements (22), such an arrangement could still be

Abbreviation: AChE, acetylcholinesterase.

compatible with differential integrative mechanisms inside different compartments. Such a mode of neuronal integration is likely to involve presynaptic dendrites (23), which have been seen in the deep layers of the feline colliculus, where they probably belong to interneurons (24, 25). Alternatively, signal flow through the colliculus may be determined by the specific position of efferent neurons relative to the compartmental matrix. With the present study, I attempted to answer the question of the existence of an architectural matrix shared between afferent and efferent collicular systems. I chose to relate the position of efferent cells of three collicular projections to the cholinergic domain, a matrix characterized by a near-perfect spatial correlation of elevated AChE activity and choline acetyltransferase-like immunoreactivity (8, 12).

MATERIALS AND METHODS

Twenty-two albino Wistar rats weighing 220–330 g were anesthetized with a mixture of Ketanest (100 mg/kg) (Parke–Davis) and Rompun (5 mg/kg) (Bayer Leverkusen), i.p., and received injections of 50–100 nl of 4% Fluoro-Gold (Fluorochrome, Englewood, CO) in either of three areas of the brain: the intralaminar thalamic nuclei (20), the lateral pons (26), or the tractus tectoreticulospinalis (predorsal bundle) (27) at the level of the inferior colliculus. The latter two of these target areas were approached along an oblique tract through the cerebellum. After survival times of 3–4 days, the animals were transcardially perfused with 4% paraformaldehyde and 0.02% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were cut in the frontal plane on a cryostat and 30-μm-thick sections of the colliculi were stained for AChE activity (28). In the material thus produced, neuronal perikarya and their proximal dendrites, retrogradely labeled with Fluoro-Gold, were seen under UV illumination, and the pattern of AChE staining could separately or simultaneously be visualized through dark-field microscopy. Since heavily labeled neurons were observed both within and outside AChE-rich areas in various parts of the brainstem, interferences between these two histochemical procedures could be excluded.

In selected experiments, the fixed colliculi were cut on a Vibratome in 50-μm-thick sections and backfilled cells were injected with the fluorescent dye Lucifer yellow (29) to visualize their dendritic morphology more completely. Subsequent staining for AChE activity allowed us to evaluate the pattern of dendritic arborization relative to the cholinergic landscape in more detail.

RESULTS

Depending on their size, patches rich in AChE cut across several subtiers of the intermediate gray and white layers of the rat colliculus. After injections of Fluoro-Gold into the thalamus, centered to cover the nuclei centralis lateralis, paracentralis, and parafascicularis (Fig. 2*A*), a rich population of retrogradely labeled cells was found in the superior colliculus, and this population was particularly dense in the intermediate gray layer. These neurons represented a comparatively homogeneous population of intermediate cell body size (mean diameter, 14 μm) and, confirming earlier observations (20), they were often arranged in clusters. The simultaneous visualization of these cells with the pattern of AChE staining revealed that the great majority of the perikarya of these cells resided in AChE-poor territory, populating spaces between, occasionally above, but mostly below AChE-rich compartments. Within this latitude, labeled neurons were mainly found in proximity to the AChE-rich domain by populating its borders (Fig. 3 *A–C*).

To stain a major subpopulation of uncrossed descending collicular neurons, tracer injections were placed in the pons

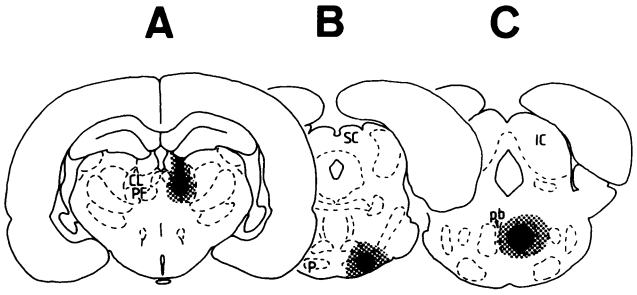

FIG. 2. Representative injection sites of the three types of experiments performed drawn into outlines of frontal sections (30). CL, centrolateral nucleus; IC, inferior colliculus; PF, parafascicular nucleus; P, pontine nuclei; SC, superior colliculus; pb, predorsal bundle.

(Fig. 2*B*). Retrogradely labeled perikarya of intermediate size were found to reside in AChE-poor zones of the intermediate gray and white layers, but again there was a conspicuous tendency of them to reside close to the AChE-rich neuropil. The distribution of these cells differed from that of the ascending neurons in that many of them resided close to the border of the AChE-rich patches at a slightly more ventral level in the lower part of the intermediate gray layer.

In the third set of experiments, tracer injections were placed close to the midline into the predorsal bundle (Fig. 2*C*), labeling the cells of origin of a variety of crossed and uncrossed descending tectal projections (Fig. 3 *D–F*), some of which comprise axonal collaterals of neurons also projecting to thalamic nuclei (31). Accordingly, these injections led to labeling of tectal cells of widely different morphologies throughout the deep collicular layers. For instance, perikarya diameters ranged from <10 to almost 40 μm. Despite this heterogeneity, most of the backfilled perikarya shared the preference for AChE-poor zones. Occasionally, however, clusters of rather small cell bodies provided exceptions by populating AChE-rich patches, even in central to medial parts of the intermediate layers in the caudal colliculus where these patches are fairly compact. For the cell bodies residing outside these patches, the larger ones were mostly found a few perikaryal diameters below the AChE-rich zones, whereas many of the perikarya of intermediate size were again found to reside close to border regions of the cholinergic domain (Fig. 3 *D–F*).

The retrogradely labeled perikarya of either of the three projectional systems studied were rarely seen to be completely surrounded by the cholinergic domain. This arrangement was most evident in the caudomedial four-ninths of the intermediate collicular layers, where the AChE-rich and AChE-poor domains are rather distinctly delineated. The extended, but loose and heterogeneous texture of the cholinergic domain rostrally and laterally in the colliculus appears to guarantee that always being in contact with the noncholinergic domain holds true for efferent perikarya even there.

Seeking statistical confirmation for the seemingly specific distribution of all three populations of backfilled neurons relative to the AChE-rich domain, a numerical analysis has been made relying on the evaluation of microscopic fields covering areas where there were backfilled neurons in the vicinity of comparatively well-delineated borders between AChE-poor and AChE-rich neuropil. In a first step of statistical analysis, the cross-sectional areas of the AChE-rich and AChE-poor domains was determined (32). In 21 sample fields from four animals (with an equal number of samples from the three types of experiments), 395 backfilled neurons were encountered and classified as residing in either one or the other domain. The hypothesis that the neurons fall in each domain with a probability proportional to the area assumed

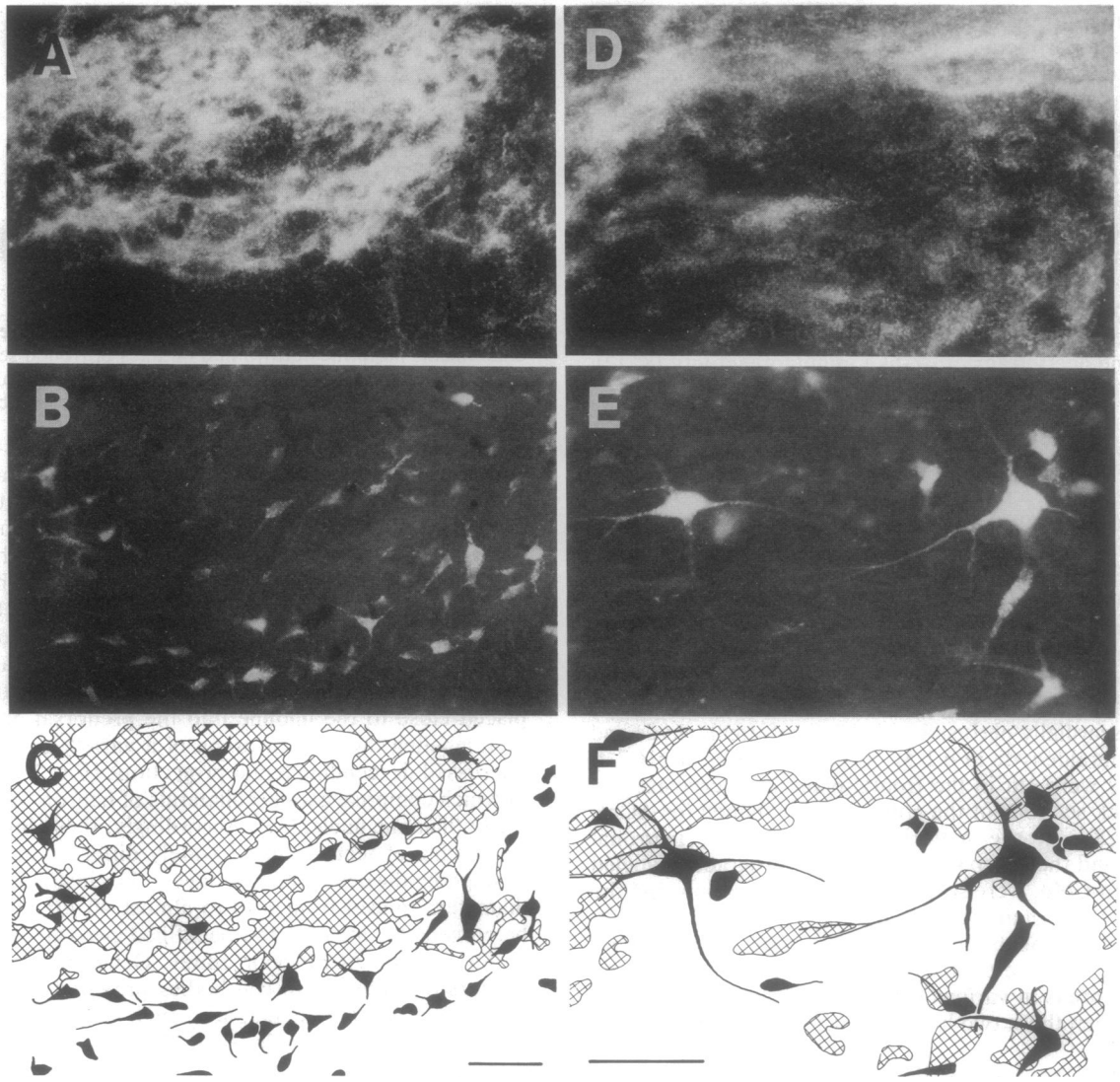

FIG. 3. Spatial correlation of the AChE-rich patches of the intermediate collicular layers and efferent cell bodies with their proximal dendrites. (*A–C*) Correlation of the cholinergic landscape to neurons sending their axons to the intralaminar thalamus. (*D–F*) Correlation of the mosaic of AChE-rich and -poor zones with cells sending their axons through the contralateral predorsal bundle. (*A* and *D*) Sections under dark-field illumination to visualize the mosaic of AChE-rich (bright) and AChE-poor territory. (*B* and *E*) Same sections shown under UV illumination to visualize retrogradely labeled cells. (*C* and *F*) Overlay drawings of AChE-rich area (cross-hatched) and perikarya with proximal dendrites to show their relative position to the cholinergic domain. Most of the ascending and descending efferent cell bodies reside in AChE-poor zones, but, where longer segments of dendrites are labeled, several of them can be traced toward and into AChE-rich zones. Note that many cell bodies reside at the border to AChE-rich patches. (Bars = 50 μm.)

by the domains had to be rejected: 339 cells resided in 68% of the analyzed area covered by the AChE-poor domain and only 56 neurons were found in 32% of the area taken by the AChE-rich domain ($P < 0.00001$; Yates-corrected χ^2 test).

In a second step of evaluation, an attempt was made to quantify the impression that many of the efferent neurons projecting to either of the three injection sites resided close to the border of the cholinergic domain. This had to be based on lines drawn to separate the AChE-rich from the AChE-poor domain on sample photographies visualizing the cholinergic landscape only. Realizing that the border between these domains shows remarkable parallels to a fractal coast line (33), decisions for drawing it were made on the scale of neuronal cell bodies—i.e., ignoring radii smaller than those of the smallest labeled perikarya (Fig. 3 *C* and *F*). The length l of the borderline between the two domains was determined according to Gundersen *et al.* (32) and a band of AChE-poor area along the borderline one average perikaryal diameter $w = 14$ μm wide was defined. The area of this borderline band is $l \times w$, provided that there is an overall balance of concave and convex profiles, a criterion largely met in the material analyzed, and provided that borders of adjacent spits of high histochemical activity are not closer than $2w$. Violation of this latter presupposition did happen and, together with sharp turns of the border, must have led to an overestimation of the borderline area. The borderline area so defined constituted 47% of the AChE-poor domain in the samples, and, as seen after matching its geometry to an overlay drawing of exactly the same frame but depicting labeled perikarya rather than the cholinergic landscape, it accommodated the center of gravity of 59% of the backfilled cells found in AChE-poor territory. This distribution significantly deviates from the expectation based on the assumption of a random distribution of labeled neurons outside the cholinergic domain ($P < 0.01$; Yates-corrected χ^2 test). This deviation becomes even more significant when areal overestimations are taken into account. The statistical analysis therefore indicates that the cells of origin of the three projections surrounding the cholinergic domain are accumulated in close vicinity to it.

The proximal dendrites of descending cells could in many instances be traced for considerable distances, occasionally

up to 100 μm. They gave an indication of the environment from which the neurons collected their input (Fig. 3 *D–F*). Dendritic geometries differently organized with respect to the cholinergic landscape were seen. As far as their branches were visible, several dendritic trees appeared to be confined to the AChE-poor domain. In some instances, dendrites were seen to follow compartment borders, but in most cases neurons close to the compartmental border extended major dendritic branches across the border into the cholinergic domain. This observation could be confirmed after filling retrogradely labeled cells intracellularly with Lucifer yellow (Fig. 4). Given the heterogeneity of efferent collicular cells, particularly those descending in the predorsal bundle, these observations would be consistent with a specific dendritic geometry of several subpopulations among them.

DISCUSSION

The cells of origin of major efferent projections have been found to superimpose the cholinergic landscape of the multimodal collicular layers in a distinctly nonrandom manner. Having acknowledged the heterogeneous distribution of transmitter-related markers as well as of terminal fields of numerous collicular afferents, one could still have argued that this structural periodicity is merely an expression of the developmental dynamics of the system but irrelevant for its function. However, with the evidence presented here that efferent neurons themselves observe the periodic architecture, one can no longer escape the conclusion that these compartments are an essential element in orchestrating the interface for sensorimotor integration in the superior colliculus.

A common denominator of all three pathways studied was perikaryal residency mostly outside the cholinergic domain. The observation that many of these neuronal perikarya reside close to the AChE-rich neuropil may turn out to be a key to understanding the functional logic behind the compartmental architecture of the colliculus. Given that the surface between the two domains is profusely folded, this borderline population must be quite substantial. Since numerous neurons extend major dendrites into the cholinergic domain, both domains must be interconnected by an extensive dendritic plexus. A neuron so positioned between these differently characterized neuronal spaces could collect afferents with its dendrites from a particular environment and receive input to its soma of a different nature. This input may then shunt, modulate, or override the dendritic input (34). The compartments may therefore be considered expressions of a particular type of periodic order on the synaptic level.

It was reported earlier (17) that another type of collicular compartment is defined by an elevated level of parvalbumin-like immunoreactivity present in perikarya, dendrites, and neuropil, and that these elements are located mostly outside, but usually immediately adjacent to, the AChE-rich domain. This is the space into which pathways related to sensory systems project; by contrast, the AChE-rich domain has been shown to be the major target of afferents associated with motor pathways (cf. Fig. 1). The efferents described in this study therefore appear to arise from neurons with their perikarya situated inside the terminal fields of the sensory-related afferents in parvalbumin-rich territory while seeking motor-related input from the cholinergic domain with their dendrites. Preliminary findings suggest that a similar arrangement exists in the feline colliculus.

An intriguing implication of the observations made seems to be that the arrangement of efferent neuronal perikarya alongside the borders of domains defined biochemically or by the pattern of afferentation emerges as a folded derivative of a laminated organization, forced upon this part of the colliculus through spatial constraints. The major constraint responsible for the folding could be that, despite newly acquired assignments of sensorimotor processing to which the colliculus had to respond with local increases in neuronal mass, the topographic representations had to be kept aligned across collicular layers (4, 35). If this interpretation is valid, the compartmental architecture present in the superior colliculus may naturally fall between a simple laminar and a truly modular organization of nervous tissue.

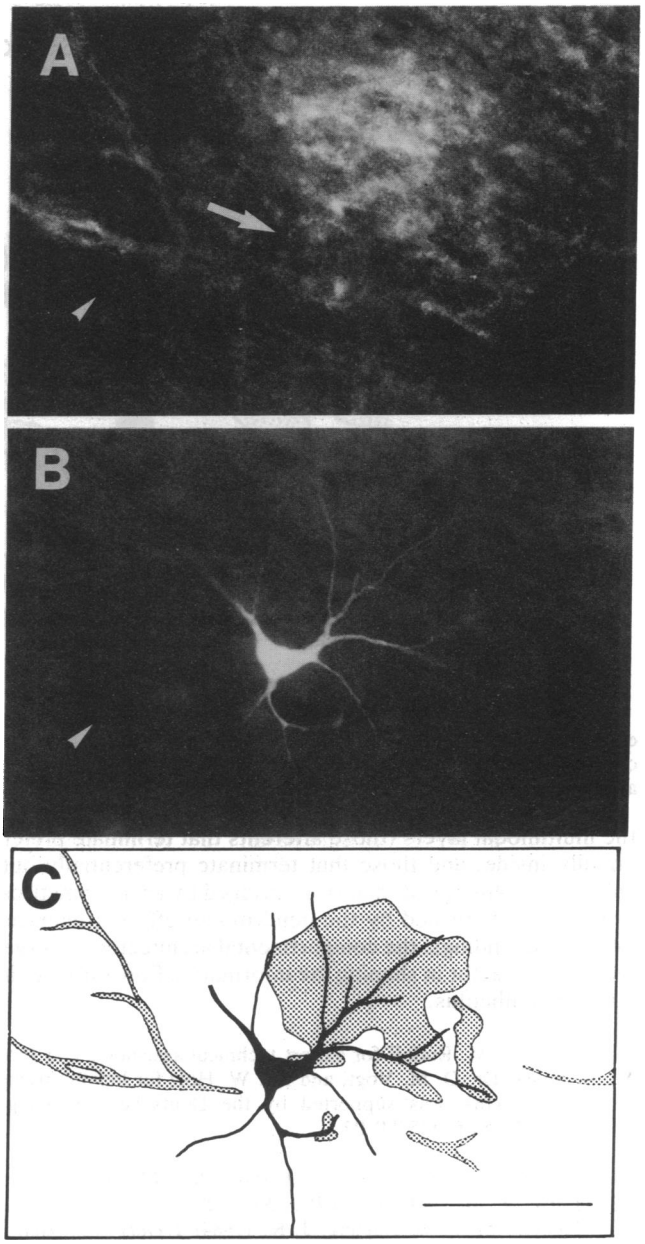

FIG. 4. Spatial relationship between dendritic arborization of a neuron in the intermediate collicular layers projecting through the predorsal bundle and its cholinergic environment. (*A*) Geometry of zones of low (dark) and high (bright) AChE activity; arrow points to position of cell body shown in *B*. (*B*) Pattern of dendritic arborization of a neuron filled with Lucifer yellow in the same field; arrowhead points to landmark indicated in *A*. (*C*) Overlay drawing showing perikaryon residing in AChE-poor area, while several of its dendrites apparently collected input from AChE-rich area (stippled). (Bar = 100 μm.)

The major conclusion to be drawn from this study is 2-fold (Fig. 5). First, the compartmental (and apparently fractal) architecture of the multimodal collicular layers provides a structural frame for the specific interlocking of its afferents and efferents. Second, the two principle afferent streams into

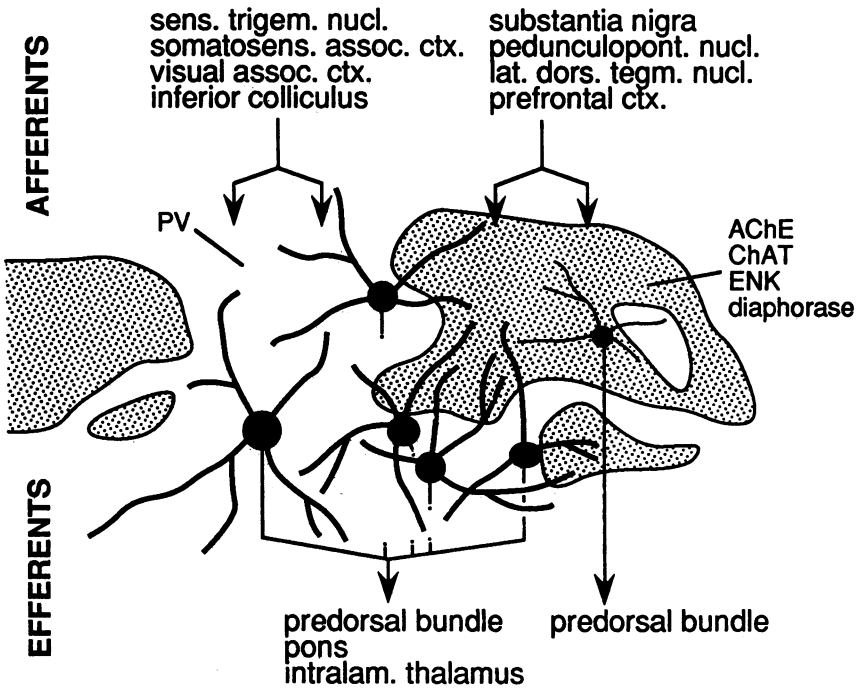

FIG. 5. Diagram summarizing spatial relationship between afferents and efferents in the multimodal layers of superior colliculus. Large efferent perikarya resided generally outside, with small ones occasionally inside the cholinergic domain (stippled areas), while many neurons of intermediate size were found close to the cholinergic domain, extending major dendritic branches into it. PV, parvalbumin; other abbreviations are as in Fig. 1.

the multimodal layers (those afferents that terminate preferentially inside, and those that terminate preferentially outside, the cholinergic domain) are bridged by an asymmetrical cross-link established by the dendrites of efferent neurons. With these findings, the compartmental architecture emerges as a crucial factor in shaping the information flow through the superior colliculus.

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