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Thalamic inputs to cytochrome oxidase-rich regions in monkey visual cortex

(lateral geniculate body/striate cortex/area 18/2-deoxyglucose)

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ABSTRACT **In primate primary visual cortex, staining for cytochrome oxidase reveals a regular array of blob-like structures, most prominent in layers II and III but also present in layers V and VI. In an attempt to learn more about the input to these blobs, we injected the lateral geniculate bodies of macaques and squirrel monkeys with [^{3}H]proline or horseradish peroxidase and looked in the cortex for transported label. As expected, label was present in layers IVa, IVcα, IVcβ, and VI. In addition, both methods revealed an array of puffs deep in layer III. Seen in tangential sections, the puffs precisely matched the cytochrome blobs. These results indicate a projection from the lateral geniculate body to the blob regions deep in layer II/III, either indirect via layer IV or more likely direct. In area 18 stained for cytochrome oxidase, we also observed complex banding patterns; these were remarkably similar to the pattern found after [^{3}H]proline or horseradish peroxidase injection and were also similar to the pattern produced with 2-deoxyglucose labeling after stimulation with vertical or horizontal stripes; the proline and peroxidase labels probably represent a projection from the pulvinar to area 18.**

The primate primary visual cortex (area 17, visual area 1 or V-1, striate cortex) is the first cortical receiving area for visual information from the thalamus. This area is probably engaged in analyzing or relaying most or all of the kinds of information handled by the visual system, including form, color, movement, and depth. The scatter of the systematic topographic map of the visual field onto the cortex is such that, in the upper layers, one must traverse roughly 2 mm of cortex to get from one region of the visual field into an entirely new region. Any 2 × 2 mm block of cortex must therefore contain a complete set of whatever machinery is needed for the analysis of a particular region of visual field; it must take care of all orientations, both eyes and all colors, for, if certain values of these parameters are omitted, there is no other region of cortex to do the job. One can thus think of the striate cortex as consisting of a large number of repeating modules, all with similar connections and organization (1). The apparatus for dealing with orientation and ocular dominance consists of vertical slabs of tissue extending through the cortex from surface to white matter: "orientation columns" and "ocular dominance columns." Columns were originally observed by physiological recording techniques (2). Classical methods such as Nissl or myelin stains failed to reveal any of these rich vertically running systems, whose anatomical demonstration has required special stains (3) or special experimental methods (4–6).

In 1978, studies of monkey striate cortex took a sudden spurt because of the discovery by Margaret Wong-Riley of a pattern of regularly repeating blob-like structures in the upper layers (II and III) of squirrel monkey striate cortex (M. Wong-Riley, personal communication). The pattern was revealed by staining for cytochrome oxidase, a mitochondrial enzyme. Wong-Riley noticed the similarity between these periodicities and structures that had previously been seen autoradiographically in the upper layers of squirrel monkey striate cortex after [^{3}H]proline injection into the lateral geniculate body (7, 8) or the eye (9). When the cytochrome blobs were viewed in sections tangential to layers II and III, they were seen to form a two-dimensional array of round or oval blobs spaced roughly 0.5 mm apart (10, 11). Similar patterns have been found in all primates examined including the macaque monkey, baboon, human, and galago (12). They are absent in a number of nonprimates, including rat, cat, and tree shrew. These structures have been observed by several other techniques, including stains for glutamic acid decarboxylase (13) and for acetyl and butyryl cholinesterase (14) and metabolic labeling with 2-deoxyglucose after diffuse-light stimulation (11).

In macaque monkeys the cytochrome blobs form parallel rows, which are aligned with and centered on the ocular dominance columns (10, 12, 13). In squirrel monkeys ocular dominance columns have not so far been revealed by anatomical methods, but swings in ocular dominance during recordings in tangential penetrations nevertheless indicate their existence and spacing, which is half that of macaque ocular dominance columns (9). The cytochrome blobs in squirrel monkey and macaque have about the same spacing, but in squirrel monkey there is no conspicuous tendency to form rows. Blobs in squirrel monkeys thus seem unrelated to ocular dominance.

Deoxyglucose studies have shown that activity in blobs can be increased by a variety of stimuli (10, 12, 15), suggesting that, in their response properties, cells in the blobs are much less specific than cells between blobs. We have compared responses from blob and nonblob cells in layers II and III (the upper layers) of striate cortex and find that cells in the blobs have poor orientation selectivity or none. Cells with no orientation selectivity have concentric, usually center-surround, receptive fields (16). Some blob cells closely resemble cells in the ventral (magnocellular) geniculate layers, responding over a broad range of wavelengths. Others are color coded (type 2 and double opponent), suggesting an input also from the dorsal (parvocellular) layers (17). Thus, cells within the blobs show properties that are utterly different from the properties of upper layer cells outside the blobs and more like cells in layer IV or the lateral geniculate body. We therefore set out to determine whether cells inside and outside the blobs have different inputs by examining patterns of orthograde transport of tracers injected into the thalamus. In the course of these studies, we observed a pattern of label in area 18 that closely matched a distinctive distribution of cytochrome oxidase staining.

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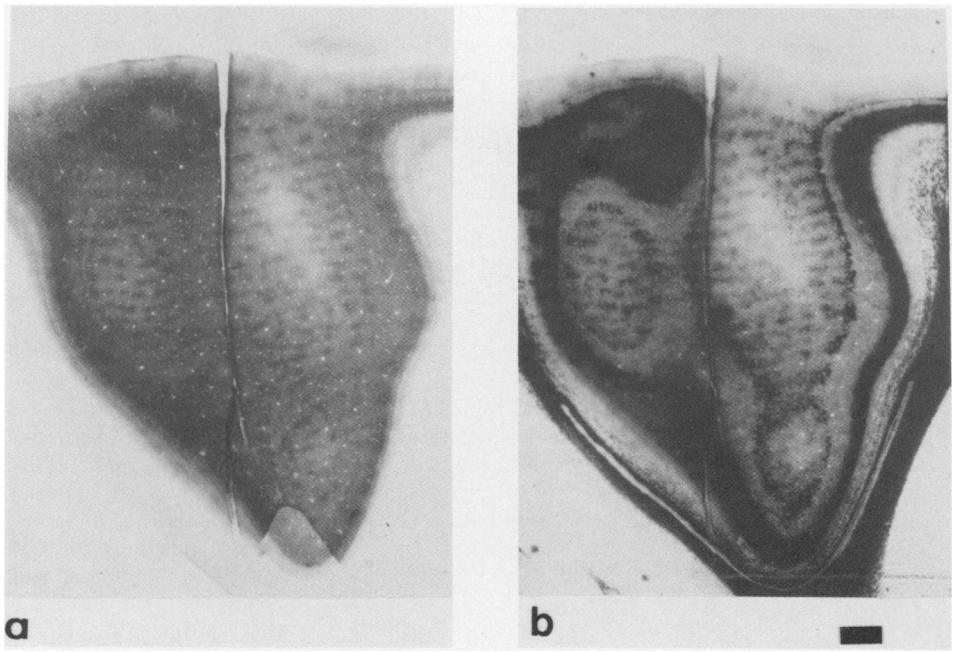

FIG. 1. (*a*) Cytochrome oxidase pattern in macaque monkey striate cortex. Films of two adjacent sections were overlaid by lining up radial blood vessels to accentuate the pattern. (*b*) Autoradiogram of two overlaid adjacent cortical sections. This monkey had been injected with 0.2 mCi of [^{3}H]proline in the ipsilateral lateral geniculate nucleus. The photograph is a negative dark field, so the silver grains appear dark. Overlaying films of the cytochrome oxidase pattern and the autoradiograms shows that the two blob patterns are in register. These sections are tangent to buried cortex in the calcarine roof, so the upper layers are in the three coalescing central regions surrounded by deeper layers. Bar = 1 mm.

MATERIALS AND METHODS

Ten adult monkeys [five *Macaca fascicularis* (crab-eating macaque) and five *Saimiri sciureus* (squirrel monkey)] were used. The animals were anesthetized and the position of the lateral geniculate nucleus or the pulvinar was determined by recording neuronal activity with a tungsten microelectrode (18), which was subsequently replaced by a glass micropipette in precisely the same position. The pipette (tip diameter, 10–40 μm) was filled with either [^{3}H]proline (New England Nuclear; 0.1 mCi/μl; 1 Ci = 3.7×10^{10} becquerels) or 15% horseradish peroxidase (Boehringer Mannheim) in 2 M NaCl. We injected 0.1–1 μl using pressure. All animals were injected bilaterally.

Survival time was 10–14 days for ^{3}H injections and 2 days for peroxidase injections. In several animals four injections were made, two with each tracer. The animal was anesthetized and perfused transcardially with 0.5 liter of normal saline followed by 1 liter of fixative (1.5% formaldehyde/1.5% glutaraldehyde/0.9% NaCl/0.1 M phosphate buffer, pH 7). The occipital lobes were sectioned on a freezing microtome (60-μm sections). Alternate sections were stained for cytochrome oxidase (19), tested with tetramethylbenzidine for horseradish peroxidase (20), or dipped in emulsion for autoradiography.

RESULTS AND DISCUSSION

Area 17. In area 17 of normal macaque and squirrel monkeys, cytochrome oxidase stain is most intense in layers IVc and IVa (Fig. 1*a*; see also Fig. 3*a*). Layer IVa has the form of a fine-mesh net that is best seen in tangential section (Fig. 2*a*) and seems identical to the autoradiographic pattern seen after eye or geniculate injection with tritiated proline or methionine (see, e.g., ref. 8). Layer I shows a very thin dark line at about its middepth and is otherwise lightly stained. The blobs are best seen in layers II and III, where they form roughly cylindrical pillars extending from IVa, with which they seem to merge, to the top of II. They are present also in V and VI but are much fainter in these layers (Fig. 3*a*). The two sets, above and below layer IV, lie in precise register. In layer IVb, the blobs can occasionally be seen faintly against a very light background.

Injections of [^{3}H]proline into the lateral geniculate body produced the expected very dense labeling of layer IVcα and IVcβ over a large but variable extent depending on the site of the injection and the usual reticulated pattern in IVa. In the squirrel monkey the labeling was uniform along the length of the layer; in the macaque it was usually uniform but sometimes showed typical ocular dominance columns, especially at the edges of the

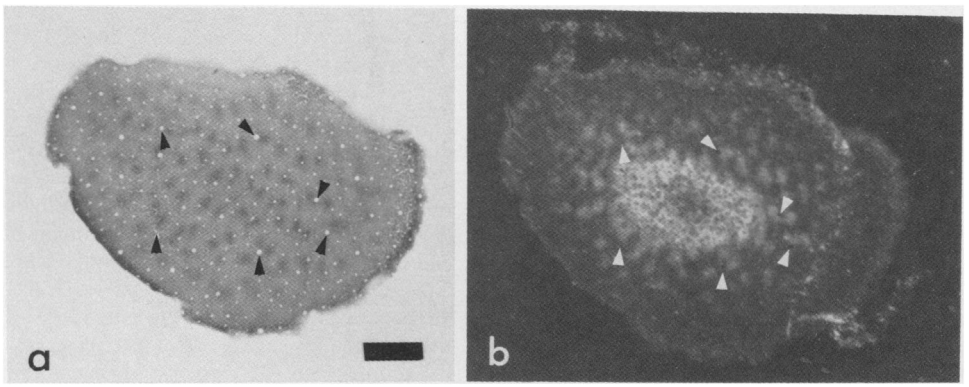

FIG. 2. Two adjacent tangential sections from area 17 of a macaque monkey that had been injected with 0.5 μl of 15% horseradish peroxidase in the ipsilateral lateral geniculate nucleus. (*a*) Section stained for cytochrome oxidase and passing tangentially to deep layer III. (*b*) The adjacent section grazing layer IVb, tested for peroxidase with the tetramethylbenzidine method, is shown on the right. The peroxidase section was photographed by using dark field and crossed polarizers, so the reaction product appears light. Small arrowheads indicate corresponding points on each pair of sections. Layer IVa is the reticulated region near the center of *b*. Bar = 1 mm.

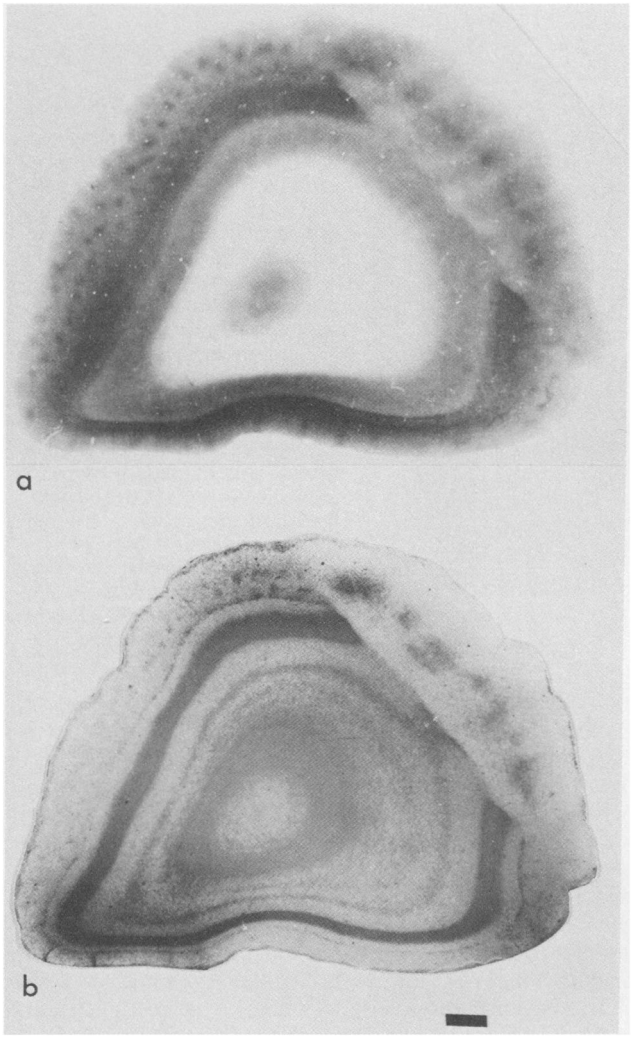

FIG. 3. Cytochrome oxidase pattern (*a*) and a negative dark-field photograph (silver grains appear dark) of an autoradiogram of an adjacent section (*b*) from a squirrel monkey that had been injected with 0.5 mCi of [^{3}H]proline into the ipsilateral lateral geniculate nucleus. The 17/18 border cuts diagonally across the upper right part of the section. The injection site was large enough to include the pulvinar, so both area 18 and layer I in area 17 show label. Faint blobs are visible in layers V and VI in area 17 in the cytochrome section, especially near the 17/18 border. Bar = 1 mm.

labeled areas. In both macaque and squirrel monkeys, in the layers above IV there were also puffs of label that resembled the cytochrome blobs in their pattern and spacing (Fig. 1); these were much fainter than the labeling in IVc and at low power could usually be seen only with dark-field illumination. They were most obvious in the deep part of layer III, again appearing as blobs, sitting on and merging with layer IVa.

When two adjacent tangential sections, one stained for cytochrome oxidase and the other autoradiographed, were compared by bringing the hundreds of radial blood vessels into exact alignment, it was clear that the cytochrome blobs and the autoradiographically labeled puffs lay in precise register.

In both species the labeling between the puffs was above background levels. We saw no hint of any periodic labeling in layers IVb, V, or VI.

Layer VI contained a uniformly labeled band, which over much of its length was split into two, with a space between, like a pair of railroad tracks. The more superficial of these leaflets lay in the uppermost part of layer VI; the deeper was at the very bottom of VI. The middle part of VI, in which the cell bodies are very densely packed, was relatively free of label. Like layer IVa, this band or pair of bands in VI was seen only when IVcβ was labeled, suggesting that the input was from cells in the parvocellular geniculate layers (21).

These results confirm previous findings (7–9) and add the fact that the puffs lie in register with the cytochrome-oxidase blobs. They leave open the question of whether the label is transported transneuronally via layer IVc or represents a direct geniculocortical projection to layer III. To help decide this question, we injected horseradish peroxidase into the geniculate. The results (Fig. 2) were very similar to what was seen after injection of proline: we saw continuous label over large stretches of layers IVa and IVc, puffs that were densest in the lower part of layers II/III and coincided with the cytochrome blobs, and a leaflet of label, sometimes single but usually double, in VI. In regions of densest orthograde label, stained cell bodies indicating a corticogeniculate projection were found in layer VI; these lay in two neat and slender rows that coincided precisely with the leaflets of orthograde label. In the largest injections a few large pyramidal cells were labeled in layer V, probably the result of spread of the enzyme from the injection site to the pulvinar (22).

Horseradish peroxidase (not wheat-germ conjugated) is not generally thought to be transported transneuronally (20); (see, however, ref. 23). The peroxidase labeling of the puffs thus suggests that the input is direct, from the geniculate, rather than involving a synapse in layer IV. We do not, however, consider this to be fully established. The only difference between the labeling seen after peroxidase injection and that seen after proline injection was that the peroxidase injection did not produce diffuse labeling between the blobs in layers II/III. We suspect that the faint diffuse tritium labeling of the interblob regions of II/III is transneuronal via layer IV.

Fitzpatrick *et al.* (24) have suggested that the input to the blobs may come from the intercalated layer of small cells between the parvo- and magnocellular layers of the geniculate (25–27). Our results are consistent with this suggestion. When our geniculate injections were confined to either magno- or parvocellular layers, we did not see the puffs labeled but, whenever our injection site spanned the division between magno- and parvocellular layers, we did see puffs labeled.

The fact that the blobs in striate cortex are both anatomically and physiologically distinct structures suggests that they perform a special function. Therefore every module, in addition to covering all orientations, all colors and both eyes, must contain one blob (or, in the macaque, a blob for each eye). Previously one could conceive of the striate cortex as subserving a number of independent interlacing functions, and it was possible that a module was merely a theoretical entity with arbitrary boundaries, covering, for example, all orientations but beginning at any orientation. The existence of the blobs makes it more likely that a module is a physical structure with at least some defined boundaries.

Area 18. In four monkeys we injected [^{3}H]proline or peroxidase into the inferior and lateral pulvinar; also some of the larger geniculate injection sites included the inferior pulvinar. Whenever our injection site included the pulvinar, there was label in layer I of striate cortex, which we did not see labeled in injections confined to the geniculate. We did not see the patchy labeling in layer II of area 17 previously seen after pulvinar injections (28), perhaps because our injections did not extend as far medially. After most pulvinar injections and many of the larger injections of the geniculate, area 18 showed a characteristic pattern of parallel alternating wide and narrow bands running at right angles to the 17/18 border (Fig. 3*b*). The wider bands measured about 1 mm, the narrower, 0.12–0.4 mm, and

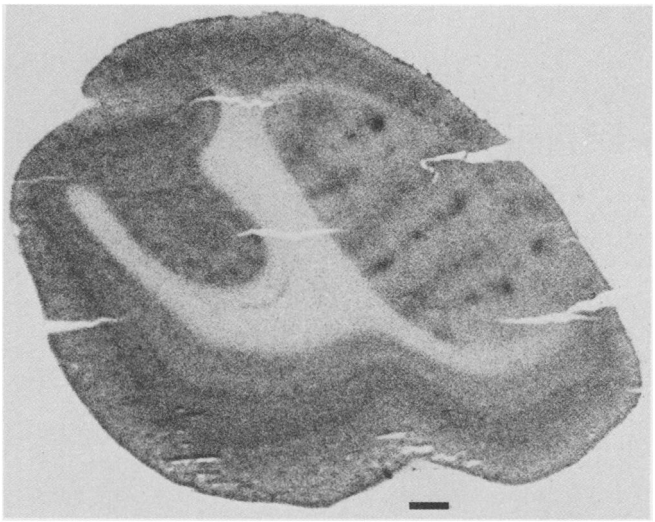

FIG. 4. Autoradiogram of a section from a macaque monkey that had been injected with 100 μCi of deoxy[^{3}H]glucose and stimulated with moving vertical stripes for 45 min (6). Area 18 (the oval region in the upper right) shows alternating thick and thin stripes and both types of stripes show cross striations. The cytochrome oxidase pattern of an adjacent section showed faint stripes that lay in precise register with the stripes on the autoradiogram. Bar = 1 mm.

the separation between the two, 0.2 mm, giving a repeat distance of just under 2 mm. The wider bands of label extended from layer III to layer V and were cleft horizontally by a region of reduced label density centered on layer IV. The narrow bands of label were confined to the deep part of III, lacking the infragranular component seen in the wide bands. A similar pattern of bands in area 18 after pulvinar injection has been reported by Curcio and Harting (29), who make no comment on the alternation of wide and narrow, although their figure 2 shows one clear example.

Whenever our injections were confined to the geniculate we saw no label in area 18, or at most a single puff of label just on the 18 side of the 17/18 border, at the same depth as layer IVa of 17. When we did see label both in 17 and in 18, there was always label in layer I of 17. Taken together, these results suggest that the bands of label in 18 represent a pulvinar input. A very similar pattern of alternating wide and narrow bands was seen with cytochrome oxidase staining and, when photographs of adjacent cytochrome and proline or peroxidase sections were overlaid, the two patterns were in precise register (Fig. 3).

Finally, in area 18 both squirrel and macaque monkeys showed similar patterns of labeling with 2-deoxyglucose after stimulation with black and white stripes in a single orientation (either vertical or horizontal). In each case the deoxyglucose-labeled stripes lay in register with cytochrome oxidase stripes. In Fig. 4, from a macaque, the thinner bands are denser than the thicker ones and show a regular beaded pattern of heightened density along their length, spaced about 0.7 mm apart. There are also occasional regular periodicities faintly visible in the broader bands, about 0.5 mm apart. One thus has the impression of a complex, very regular two-dimensional array made up of at least two kinds of interlaced structures. In preliminary recordings in macaque, cells in the cytochrome oxidase-rich regions behaved very much like cells in the area-17 blobs (unpublished results), whereas cells between bands were orientation selective.

In conclusion, in areas 17 and 18 of monkey cortex a set of periodically recurring cytochrome oxidase-rich structures differ from the more lightly staining background in having a distinct, probably more direct, thalamic input. Indeed, there seems to be a remarkable correspondence between cytochrome-rich structures and structures receiving direct thalamic input.

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