Projections from primary visual cortex to cytochrome oxidase thin stripes and interstripes of macaque visual area 2

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It has been controversial whether the cytochrome oxidase (CO)-dense blobs in primate primary visual cortex (V1) and CO-dense thin stripes in visual area 2 (V2) are parts of a cortical color-processing stream that is segregated from other functional streams. One of the key pieces of evidence for the segregated color stream is the previous report of specific connections between blobs and thin stripes, which is parallel to the connections between interblobs and interstripes. To study the degree of the segregation between the proposed different streams, in the current study, anatomical tracers were injected into different V2 compartments with the functional guidance of optical imaging. The spatial relationship between each labeled cell and the CO blobs in V1 were analyzed quantitatively. After tracer injections in the color-preferring modules in CO thin stripes, equal amounts of labeled cells were found in the blobs and interblobs. However, the density of the labeled cells was more than twice as high in the blobs as that in the interblobs, and most of the clusters of labeled cells partially overlapped with the blobs. Tracer injections in the interstripes labeled cells predominantly in the interblobs. Our results suggest that both the blobs and interblobs project to the thin stripes and call into question the proposition that different CO compartments in V1 and V2 are connected in parallel to form highly segregated functional streams.

The primate primary visual cortex (V1) and second visual area (V2) comprise regions of various cytochrome oxidase (CO) activity, which may subserve different functions (1). In V1, layers 2 and 3 are composed of CO-dense patches (blobs) and regions surrounding them (interblobs) (2–4). V2 is composed of alternating thin and thick CO-dense stripes and the pale interstripe regions between them (5, 6). Early studies have shown that most cells in the blobs and thin stripes were unoriented and selective for color, whereas most cells elsewhere were oriented and not selective for color (7, 8). Anatomically, thin stripes and interstripes receive V1 projections from blobs and interblobs in layers 2 and 3, respectively, whereas thick stripes received projections from layer 4B (8–10). Intrinsic horizontal connections in supragranular layers of V1 preferentially connect blob with blob, or interblob with interblob (11). Based on these findings, it has been proposed that color is processed in the blob–thin-stripe stream that is segregated from other functional streams (1, 9).

However, several subsequent studies have contradicted the one-to-one relationship between color processing and the stream originating in the blobs. In V1, Leventhal et al. (12) did not find differences in color and orientation selectivity between blob and interblob cells. Similarly, Lennie et al. (13) found no difference in chromatic selectivity between blob and interblob cells. Ts’o and Gilbert (14) reported cells near blob borders that were selective to both orientation and color. Using both optical and electrical recording, Landisman and Ts’o (15, 16) reported patches in V1 that were preferentially activated by chromatic stimulation and contained color-selective cells. These color patches were spatially associated with blobs in a somewhat loose sense and often enclosed interblob regions. Of their electrode penetrations that encountered color-selective cells, 29% were located outside blobs.

In V2, color-selective cells and orientation-selective cells were found in all three types of CO compartments (7, 17–22). In these studies, the percentage of color-selective cells in thin stripes ranged from 27% to 86%, whereas the percentage of orientation-selective cells ranged from 20% to 73%. In almost every study, however, the percentages of the color-selective cells and unoriented cells were higher in thin stripes than in other CO compartments. Studies by using optical imaging or the 2-deoxyglucose technique revealed V2 modules that preferred chromatic to achromatic stimulation or were more activated by diffuse color stimuli than other V2 regions (19, 23–25). These V2 color-prefering modules were centered in regions that were devoid of orientation-selective columns as revealed by optical imaging. They were colocalized with CO thin stripes but were not registered with the latter exactly (19). More recently, we found a spatially organized representation of color in and beyond the color-prefering modules in V2 (25). Isoluminant gratings of different colors elicited V2 responses that peaked at different locations in or near the color-prefering modules. Significantly, the peak locations were arranged across the cortical surface in the same order as their activating colors were organized in perceptual color space, suggesting an important role of the color-prefering module in color processing.

More recent studies on the intrinsic horizontal connections in V1 also called into question the strict segregation of the blob and interblob streams. Although the intrinsic connections between similar CO compartments predominate, there are significant connections between blobs and interblobs (26, 27). So far, studies of the connections between V1 and V2 have suggested a segregated stream from blobs to thin stripes (9, 10, 28), although the more recent study found no laminar difference in the origin of the V1 projections to different V2 compartments (28). However, the conclusions of both studies were based on the distributions of the densely labeled patches after nonphysiologically guided tracer injections into V2. In the current study, we injected tracers in thin CO stripes or interstripes after visualizing the color-prefering modules with optical imaging and examined the spatial relationship between each labeled cell and blobs. We found that both blobs and interblobs in V1 contain cells that project to the thin stripes in V2, and these results thus challenge the existence of a highly segregated stream from blobs to thin stripes.

Methods

Experiments were conducted in 15 juvenile macaque monkeys (Macaca fascicularis). All procedures were approved by the local

Abbreviations: CO, cytochrome oxidase; BDA, biotinylated dextran amine; FR, rhodamine-conjugated dextran amine; V1, primary visual cortex; V2, second visual area; c/s, cycle per degree; c/s, cycle per sec.

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The intrinsic optical signal, derived from 630 (± 15) nm light reflected from the exposed cortical surface, was recorded during various visual stimulation conditions. The cortical surface was imaged by using a slow-scan CCD array camera (Photometrics, Tucson, AZ) through a tandem lens system (29).

The data consist of a series of 8–10 frames (two frames per sec) of the cortical surface (focused 300–600 µm below the surface), beginning before stimulus presentation and continuing 3 sec into the stimulation period. Each stimulus lasted 3 sec, and an interstimulus interval of 10 sec allowed cortical activity to return to baseline conditions. These image sequences were averaged quantitatively by gray scale image processing and binary thresholding of the scanned image so that the blob regions occupied 27–30% of the total area. Before the thresholding, the image was processed by the following procedures. First, to remove the noise of low spatial frequency caused by uneven staining and illumination, the image was subtracted by its low-pass filtered version (Gaussian, σ = 196 µm). Then, the image was filtered by a medium filter (radius = 49 µm) to remove the blood vessels and other noise of high frequency. The image processing and thresholding procedures were accomplished by using NIH IMAGE. In the raw CO image, the darkest spot within each blob was identified as the blob center. Custom software running on a UNIX workstation (INDIGO2 R10000 Silicon Graphics, Mountain View, CA) allowed interactive alignment of scored sections to each other or to images of stained sections. Radially aligned blood vessels and other fine edge landmarks were used to align sections.

Results

In each hemisphere, the CO thin stripes were identified in vivo based on three groups of differential images. First, in the differential images derived from isoluminant chromatic stimuli vs. achromatic luminance stimuli, the dark regions in V2 were identified as the putative color-prefering modules. Some of these modules could be in the CO thick stripes that responded to a residual low-luminance contrast in the chromatic stimuli. To address this concern, two additional groups of differential images were obtained: images derived from stimuli of orthogonal orientations and images derived from low-contrast (7%) vs. high-contrast (100%) stimuli. Because the CO thin stripes do not contain orientation-selective columns (19, 33, 34), we excluded those putative color-prefering modules that had orientation selectivity or responded to low-contrast stimuli and used the remaining color-prefering modules as guides for tracer injections in the CO thin stripes. An example of the color-prefering modules in thin stripes is shown in Fig. 1A.

Small injections (0.3–1.3 mm in diameter) of anatomical tracers were then made either within or at least 1 mm away from the color-prefering modules to study the connections associated with the CO thin or other stripes. After tissue processing, the location...
and size of each injection was determined by carefully comparing the sections stained for CO with those stained for the tracer, or with the unstained sections for a fluorescent tracer (Fig. 1 B–D). The identification of CO-dense stripes as thin or thick was based on the width of the stripes and/or on the density of immunoreactivity to the Ab CAT-301 (31). Eleven of 14 injections targeted at color-preferring modules hit the CO thin stripes. However, because of the cortical curvature near the lunate sulcus, and the narrowness of the CO thin stripes, eight injections straddled the border between a thin stripe and an interstripe. Only the remaining three injections (<0.4 mm in diameter), which were clearly restricted to thin stripes, were used to study the projection from V1 to the thin stripes. Three additional injections restricted to interstripes were used to study the V1 projections to V2 interstripes. All injections were made in the cortical regions representing parafoveal visual field (2.5–5° eccentricity). They were centered at layer 4 but spanned all layers.

In the hemisphere illustrated in Fig. 1 (hemisphere 1), FR was injected into a color-prefering module, whereas BDA was injected 1 mm away (Fig. 1A). The CO-stained sections suggest that the injection sites of FR and BDA were centered at a thin stripe and an adjacent interstripe, respectively (Fig. 1B). The FR-injection site was ~350 μm in diameter and was restricted entirely to the thin stripe without diffusion into the adjacent interstripes (Fig. 1C). The BDA-injection site was ~500 μm in diameter and avoided both nearby thin and thick stripes (Fig. 1D). In Fig. 1E, the locations of FR- or BDA-labeled cells (red or green dots, respectively) from two sections in layers 2 and 3 of V1 are overlaid on the raw image of the adjacent CO-stained section, along with the blob borders. Although the vast majority of BDA-labeled cells were located in the interblobs, the FR-labeled cells were found in both blobs and interblobs. Almost all clusters of FR-labeled cells partially overlapped with blobs (e.g., the arrowhead), with some scattered FR-labeled cells in the interblobs (e.g., the arrow). Blue dot, mark of blood vessels on scored sections; purple line, outline of analyzed region. (Fig. 1A, B, and E) A CO-stained section confirming that the FR and BDA injections were at the centers of a thin stripe and an interstripe, respectively. (Fig. 1C) The fluorescent picture of the FR-injection site (diameter ~350 μm). (Fig. 1D) A BDA-stained section showing the BDA-injection site (~500 μm). (Fig. 1F) Marks of the FR- and BDA-labeled cells (red and green dots, respectively) overlaid on a CO section with outlines of blobs (black lines). Most clusters of FR-labeled cells partially overlapped with blobs (e.g., the arrowhead), with some scattered FR-labeled cells in the interblobs (e.g., the arrow). Blue dot, mark of blood vessels on scored sections; purple line, outline of analyzed region. (Fig. 1A, B, and E) A CO-stained section confirming that the FR and BDA injections were at the centers of a thin stripe and an interstripe, respectively. (Fig. 1C) The fluorescent picture of the FR-injection site (diameter ~350 μm). (Fig. 1D) A BDA-stained section showing the BDA-injection site (~500 μm). (Fig. 1F) Marks of the FR- and BDA-labeled cells (red and green dots, respectively) overlaid on a CO section with outlines of blobs (black lines). Most clusters of FR-labeled cells partially overlapped with blobs (e.g., the arrowhead), with some scattered FR-labeled cells in the interblobs (e.g., the arrow). Blue dot, mark of blood vessels on scored sections; purple line, outline of analyzed region. (Fig. 1A, B, and E) A CO-stained section confirming that the FR and BDA injections were at the centers of a thin stripe and an interstripe, respectively. (Fig. 1C) The fluorescent picture of the FR-injection site (diameter ~350 μm). (Fig. 1D) A BDA-stained section showing the BDA-injection site (~500 μm). (Fig. 1F) Marks of the FR- and BDA-labeled cells (red and green dots, respectively) overlaid on a CO section with outlines of blobs (black lines). Most clusters of FR-labeled cells partially overlapped with blobs (e.g., the arrowhead), with some scattered FR-labeled cells in the interblobs (e.g., the arrow). Blue dot, mark of blood vessels on scored sections; purple line, outline of analyzed region. (Fig. 1A, B, and E) A CO-stained section confirming that the FR and BDA injections were at the centers of a thin stripe and an interstripe, respectively. (Fig. 1C) The fluorescent picture of the FR-injection site (diameter ~350 μm). (Fig. 1D) A BDA-stained section showing the BDA-injection site (~500 μm). (Fig. 1F) Marks of the FR- and BDA-labeled cells (red and green dots, respectively) overlaid on a CO section with outlines of blobs (black lines). Most clusters of FR-labeled cells partially overlapped with blobs (e.g., the arrowhead), with some scattered FR-labeled cells in the interblobs (e.g., the arrow). Blue dot, mark of blood vessels on scored sections; purple line, outline of analyzed region.

To ensure that the above intercompartment distribution of labeled cells was not caused by errors in identifying the blob borders, we measured the distance from each labeled cell to its closest blob center. A blob center was defined as the spot with the densest CO staining within a blob. Fig. 1F illustrates the percentage of labeled cells as a function of the distance to blob centers. Consistent with the high percentage of the FR-labeled cells outside the blobs, 67% of the total FR-labeled cells were located at least 150 μm away from blob centers. Similar results were obtained in two additional hemispheres that had tracer injections that were restricted to the CO thin stripes (Fig. 2). The labeled cells in V1 were located in both blobs and interblobs (Fig. 2A and C). On average, across three tracer injections in thin stripes, 52.5% of the labeled cells in V1 were located in the blobs, whereas 47.5% were located in interblobs (SE = 1.9%, Fig. 3A, left bars). There was no significant difference between the
percentage of the labeled cells in the blobs and that in the interblobs ($P > 0.31$, paired $t$ test). Consistently, $55\%$ of the labeled cells were located >150 μm away from the blob centers ($SE = 10.4\%$, $n = 3$).

To compare the densities of the labeled cells in different CO compartments in each hemisphere, the two densities, one for each compartment, were normalized by the highest among them to generate two relative densities. After each thin-stripe injection, the relative density in the blobs was 1, whereas the relative density in the interblobs was 0.36 on average across three hemispheres (Fig. 3B, left bars). Consequently, the density of the thin-stripe-projecting cells was significantly higher in the blobs than in the interblobs ($P < 0.0025$, Student’s $t$ test, $n = 3$).

Previous studies have examined the spatial relationship between the CO compartments and the densely labeled patches in V1 after tracer injections in V2 (9, 28). To compare our results with those studies more directly, we outlined (under a low-magnification lens) the densely labeled patches in V1 in one hemisphere with the tracer BDA injected in a thin stripe (Fig. 2C). All of these patches overlapped with the blobs, but some of them extended into interblob regions.

The BDA injection in hemisphere 1 (Fig. 1) was restricted to an interstripe. The majority of the BDA-labeled cells (81%) in V1 were in the interblobs. On average across three interstripe injections, 87.5% of the labeled cells in V1 were located in the interblobs, a significantly higher percentage than located in the blobs ($P < 0.01$, paired $t$ test; Fig. 3A, right bars). After each of the three interstripe injections, the relative density of the labeled cells in the interblobs was 1, whereas the average density in the blobs was 0.36 (Fig. 3B, right bars).

**Discussion**

Our results suggest an asymmetrical segregation between the projections from the various CO compartments in V1 to those in V2. Interstripes in V2 receive V1 inputs predominantly from interblobs, as had been reported by previous studies (9, 10). However, thin CO stripes receive V1 inputs from both blobs and interblobs, a finding that is inconsistent with the earlier studies. This discrepancy could have resulted from differences in the method of analysis or from differences in the subcompartments of V2 thin stripes injected.

Previous studies have examined the spatial relationship between the CO compartments and the densely labeled patches in V1 after tracer injections in different CO compartments in V2. They did so by superimposing the CO-stained sections on either the sections stained for tracers (9, 10), or the contours of the labeled patches (28). They observed that, after tracer injections in the V2 thin stripes, the labeled patches in V1 overlapped the CO blobs. Based on this observation, they concluded that V2 thin stripes receive V1 projection from blobs. By superimposing the contours of blobs and contours of labeled patches drawn under a low magnification lens, we also observed the overlap between the blobs and the labeled patches after injections in thin stripes (Fig. 2C). Our observations of the labeled patches were thus consistent with those of previous studies.

The densely labeled patches were composed of clusters of labeled cells and a plexus of labeled dendrites. The visibility of these patches under low magnification is attributed to the high density of the labeled cells in them relative to that in the surrounding regions. The labeled cells scattered in the surround-
After each tracer injection in a thin stripe, the density of the labeled cells was significantly higher in blobs than in interblobs ($P < 0.0025$, Fig. 3B, left bars). This result is consistent with the overlap between the densely labeled patches and the blobs found in previous and current studies (9, 10, 28). However, because the interblobs occupy more than twice as large an area as the blobs (in current and previous studies, e.g., refs. 9, 35, and 36), the interblobs were all in the V2 parts representing the parafocal visual field where the functional segregation is shown to be strong (19, 25, 37). Therefore, the apparent contradiction between the current and previous studies as regarding to the anatomical segregation is unlikely to be explained by potential difference in eccentricity of the tracer injections. The significant amount of the labeled interblob cells after thin-stripe injections were unlikely to be the result of the potential tracer spillage into the adjacent interstripes. To minimize this contamination, if any, are likely to be neglected in a patch-based analysis. To study the distribution of the individual labeled cells, we marked each labeled cell under high magnification. We then analyzed quantitatively the density and number of labeled cells in the various CO compartments, as well as the distance between each labeled cell and its nearest blob center.

The results of the 11 injections that hit the thin stripe strongly suggest that functional differences between different CO compartments are eccentricity-dependent. This eccentricity dependency could explain the contradiction among previous physiological studies as regarding to the functional segregation between different CO compartments. However, our tracer injections were all in the V2 parts representing the parafocal visual field where the functional segregation is shown to be strong (19, 25, 37). Therefore, the apparent contradiction between the current and previous studies as regarding to the anatomical segregation is unlikely to be explained by potential difference in eccentricity of the tracer injections.

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