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Distribution of Neurons Projecting to the Superior Colliculus Correlates With Thick Cytochrome Oxidase Stripes in Macaque Visual Area V2

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ABSTRACT

In visual area V2 of macaque monkeys, cytochrome oxidase (CO) histochemistry reveals a pattern of alternating densely labeled thick and thin stripe compartments and lightly labeled interstripe compartments. This modular organization has been associated with functionally separate pathways in the visual system. We examined this idea further by comparing the pattern of CO stripes with the distribution of neurons in V2 that project to the superior colliculus. Visually evoked activity in the superior colliculus is known to be greatly reduced by blocking magnocellular but not parvocellular layers of the lateral geniculate nucleus (LGN). From previous evidence that V2 thick stripes are closely associated with the magnocellular LGN pathway, we predicted that a significant proportion of V2 neurons projecting to the superior colliculus would reside in the thick stripes.

To test this prediction, the tangential distribution of retrogradely labeled corticotectal cells in V2 was compared with the pattern of CO stripes. We found that neurons projecting to the superior colliculus accumulated preferentially into band-like clusters that were in alignment with alternate CO dense stripes. These stripes were identified as thick stripes on the basis of their physical appearance and/or by their affinity to the monoclonal antibody Cat-301. A significantly smaller proportion of labeled cells was observed in thin and interstripe compartments. These data provide further evidence that the spatial distribution of subcortically projecting neurons can correlate with the internal modular organization of visual areas. Moreover, they support the notion that CO compartments in V2 are associated with functionally different pathways. J. Comp. Neurol. 377:313–323, 1997.

Indexing terms: visual cortex; modular architecture; corticotectal pathway; parallel pathways; subcortical pathways

In visual area V2 of macaque monkeys, cytochrome oxidase (CO) histochemistry reveals a pattern of alternating densely labeled thick and thin stripes and lightly labeled interstripes (Horton and Hubel, 1981; Livingstone and Hubel, 1982; Tootell et al., 1983; Horton, 1984; Livingstone and Hubel, 1984; Wong-Riley and Carroll, 1984; Olavarria et al., 1989). These stripe-like compartments have been related to three parallel pathways emanating from subdivisions in V1. One pathway originates from layer 4B in V1 and projects to thick stripes in V2, area MT, and other areas within the parietal cortex (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985, 1989a,b; Livingstone and Hubel, 1987a; Felleman et al., 1988). A second pathway connects CO-dense blobs in V1 with thin stripes in V2, and a third pathway connects CO-pale interblobs in V1 with interstripes in V2 (Livingstone and Hubel, 1983, 1984). Thin stripes and interstripes project separately to V4 (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985; Zeki and Shipp, 1989; Van Essen et al., 1990; Nakamura et al., 1993; DeYoe et al., 1994), and from here these pathways project into inferotemporal cortex (Desimone et al., 1980; Nakamura et al., 1993; DeYoe et al., 1994).

In recent years, several views have emerged regarding the role of these pathways in vision. One of these views is that the pathway passing through thick stripes in V2 (dorsal stream) is mainly involved in visual motion analy-

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TABLE 1. Summary of Methods and Results

Monkey	SC	Histology	Blocks	CO Stripes	Period[1]	Fig.
5	Left FB	Operculum flattened.	5LD 5LV	Thick stripes identified in both blocks.[2]	4.7 mm in both blocks	2A–C & 3A
8	Left FB	Operculum flattened.	8LD	Thick stripes identified.[2]	3.6 mm	3B
2	Left and Right HRP	L-hem damaged. R-hem unfolded and flattened.	2RD 2RV	Thick stripes identified in both blocks.	2.8 mm in both blocks	4A, B & 5A
6	Left and Right HRP	L-hem damaged. R-hem unfolded and flattened.	6RD 6RV	Distinction between thick and thin stripes could not be made in either block.	3.8 mm in both blocks	5B, C
4	Left and Right HRP	Ventral blocks unfolded and flattened, opercula flattened in dorsal blocks.	4LV 4RV 4LD 4RD	Distinction between thick and thin stripes could not be made in ventral blocks. Dorsal blocks were analyzed in perpendicularly cut sections.		1A–C

[1]Values indicate the periods of significant frequencies in the distribution of labeled cells that cohered with frequencies in the distribution of cytochrome oxidase (CO).
[2]The identification of thick and thin stripes in these blocks was facilitated by immunostaining for cat-301 positive cells, which are found predominantly within CO dense thick stripe regions (Hendry et al., 1988; DeYoe et al., 1990).
HRP, horseradish peroxidase; FB, fast blue.

sis, whereas the pathway passing through thin and interstripes in V2 (ventral stream) is concerned with the analysis of form and color (Ungerleider and Mishkin, 1982; Mishkin et al., 1983; Hubel and Livingstone, 1987; Livingstone and Hubel, 1987b, 1988; Maunsell and Newsome, 1987; DeYoe and Van Essen, 1988; Zeki and Shipp, 1988). This hypothesis has been examined by many anatomical and physiological studies (see reviews by Felleman and Van Essen, 1991; Maunsell, 1992; Merigan and Maunsell, 1993). Some of the data support the idea that CO compartments in V2 are associated with different functional pathways, whereas other evidence points to a more subtle or complex relationship between modular architecture and specificity of visual functions (e.g., DeYoe and Van Essen, 1985; Martin, 1988; Merigan and Maunsell, 1990; Schiller et al., 1990; Van Essen et al., 1992; Peterhans and von der Heydt, 1993; Ferrera et al., 1994; Levitt et al., 1994a; Bullier and Nowak, 1995; Roe and Tso, 1995; Gegenfurtner et al., 1996).

Additional information about the relationship between CO stripes in V2 and specific functional pathways can come from studying the projections from V2 to subcortical centers that have been associated with functionally specific pathways. For instance, in the macaque, visual activity in the superior colliculus can be greatly reduced or abolished by blocking magnocellular layers but not parvocellular layers of the lateral geniculate nucleus (LGN; Schiller et al., 1979). Thick stripes in V2 receive significant input from magnocellular LGN layers via layer 4B in V1 (Livingstone and Hubel, 1987a) and project selectively to cortical areas associated with the "magnocellular" dorsal stream (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985, 1989b; Felleman et al., 1988). If CO compartments in V2 are associated with different functional pathways, then input from V2 to the superior colliculus should originate predominantly from cells within thick stripe compartments.

We tested this prediction by comparing the distribution of corticotectal projection neurons in V2 with the pattern of CO stripes. We found that neurons that projected to the superior colliculus accumulated preferentially into band-like clusters that were in alignment with thick CO stripes. These results support the notion that CO compartments in V2 are associated with functionally separate pathways. A brief report of this study has been presented previously (Abel et al., 1994).

MATERIALS AND METHODS

Data in this report come from five adult monkeys (*Macaca fascicularis*) weighing 3–7 kg. Surgery was performed aseptically under general anesthesia, which was induced with ketamine (0.10 mg/kg, i.m.) and maintained with Halothane (1.5–3.0% in oxygen). Following a midline craniotomy over the central sulcus, the position of the superior colliculus was determined by recording evoked responses to stroboscopic illumination with low-impedance (<2 MΩ) tungsten electrodes (A-M Systems). The stereotaxic coordinates at which evoked potentials were recorded were used to guide subsequent penetrations with pipettes containing anatomical tracers.

In three monkeys, the superior colliculi were injected bilaterally with horseradish peroxidase (HRP; 30% HRP Sigma type VI in saline), and in two additional monkeys, the superior colliculus on one side was injected with Fast Blue (FB; 10% FB in dH_2O; see Table 1). In each superior colliculus, total volumes of 2.0–3.0 μl were delivered in 10–20 pressure injections distributed evenly across the surface of the nucleus. At each site, the anatomical tracer was injected along tracks that extended about 2 mm below the tectal surface.

After a 3- or 5-day survival period, to allow for retrograde transport of HRP or FB, respectively, animals were deeply anesthetized with pentobarbital (0.40 mg/kg, i.v.) and perfused transcardially with 0.9% saline, followed by a fixative solution containing 2% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M phosphate buffer. All surgical procedures were performed in accordance with NIH guidelines (NIH publications 85-23 and 91-3207) and according to protocols approved by the University of Washington Animal Care Committee (IACUC).

Histochemical processing

Data were analyzed in cortical sections cut tangentially to the surface of the cortex in all but two of the tissue blocks (see Table 1). In the two animals injected with FB, the occipital opercula containing portions of dorsal and ventral V2 were separated from the rest of the brain and flattened between glass slides. In two of three animals injected with HRP, the hemispheres were physically unfolded and flattened according to procedures described previously (Olavarria and Van Sluyters, 1985). In the remaining animal injected with HRP, both ventral opercula were unfolded and flattened, and the dorsal opercula were separated and sectioned perpendicular to the cortical surface. The extent of the injection sites was analyzed in coronal or parasagittal sections through the superior colliculus.

The flattened tissue was kept between glass slides for about 12 hours at 4°C in 0.1 M phosphate buffer. The glass slides were then removed, and the block of tissue was

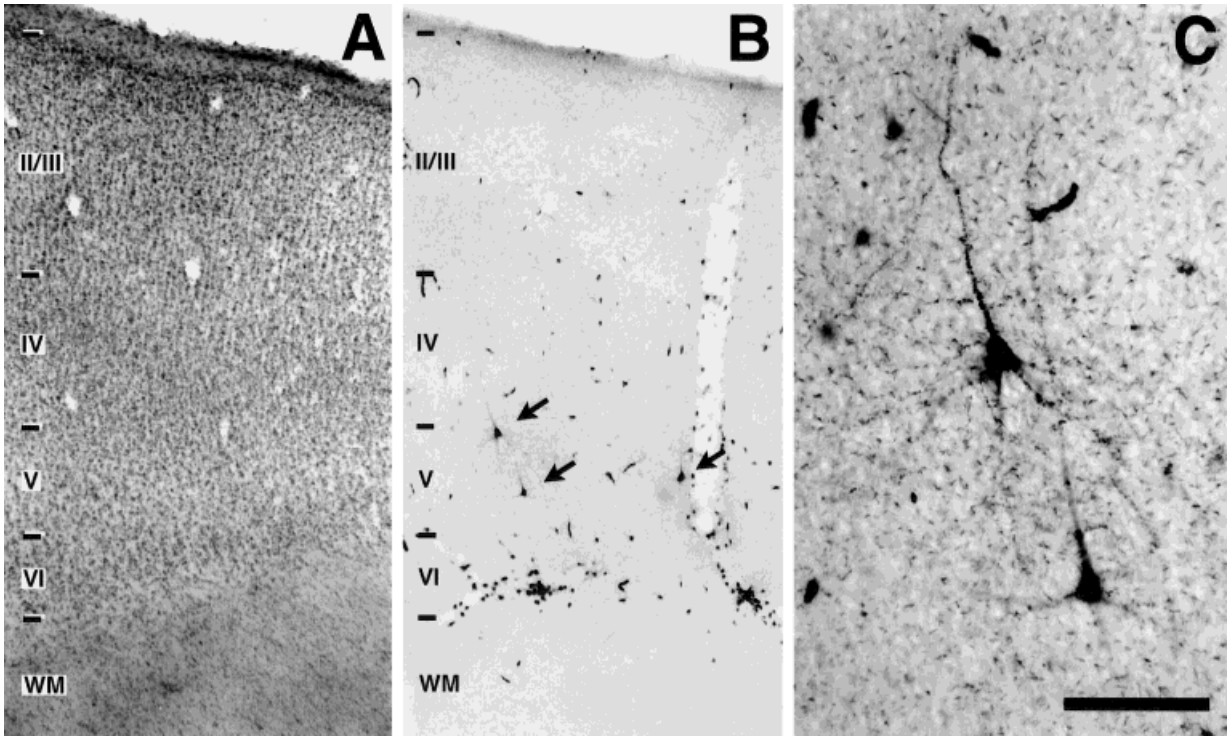

Fig. 1. **A:** Nissl-stained section cut perpendicular to the cortical surface of the dorsal operculum (block 4RD). **B:** Adjacent horseradish peroxidase (HRP)-stained section shows three labeled pyramidal cells (arrows) within layer V. **C:** Higher power view of HRP-labeled cells shown to the left in B. Scale bar = 500 μm in A,B, 100 μm in C.

placed in 20% sucrose in fixative for approximately 30 minutes, after which time it was transferred to a solution of 20% sucrose in phosphate buffer at 4°C until it sank. The brainstems were kept in 20% sucrose in fixative at 4°C until they sank. All tissue blocks were sectioned at 40–60 μm on a freezing microtome.

In each of the unfolded and flattened cortical blocks from animals injected with HRP, 4–5 superficial sections were processed for CO according to the protocol of Wong-Riley (1979). The remaining sections were reacted for HRP, with tetramethyl benzidine as the chromogen (Mesulam, 1978). In the flattened cortical blocks from animals injected with FB, CO histochemistry was performed on alternate sections. The remaining sections were immediately mounted on subbed slides, and after they had been scored for fluorescent FB labeling, they were processed for Cat-301 immunohistochemistry to aid in the identification of CO stripes (Hendry et al., 1988; DeYoe et al., 1990). A series of sections from the dorsal opercula that had been sectioned perpendicular to the cortical surface and a series from each brainstem were stained for Nissl substance.

Data acquisition

Histological sections were scored by using a microscope equipped with a motorized stage controlled by the graphic system Neurolucida (MicroBrightField). Reconstructions of the distribution of retrogradely labeled cells and Cat-301-positive cells were prepared by superimposing all tangential sections that contained labeled cells. These sections were carefully aligned with each other and with CO-stained sections by using blood vessels and tissue contours as landmarks.

Statistical analysis

To correlate the patterns of labeled cells and CO staining quantitatively, we determined the density of labeled cells and measured the density of CO staining in regions 8.0–16.5 mm long, which were measured parallel to the V1/V2 border. Each region was subdivided into bins that were either 0.25 or 0.50 mm wide, oriented roughly parallel to the CO stripes. In each bin, tracer-labeled cells and Cat-301-positive cells were counted and the density of CO staining was measured by using digitized images of neighboring CO-stained sections. The measurements of CO staining density were done with the public-domain NIH Image program (written by Wayne Rasband, National Institutes of Health).

Time-series analysis was used to quantitatively evaluate the degree of correlation between the distributions of tracer-labeled neurons and CO stripes. To investigate whether neurons projecting to the superior colliculus were distributed randomly, we used spectral analysis to search for frequencies in the distribution of tracer-labeled cells that were significant ($P < 0.05$). We then performed a cross-spectral analysis of coherence and phase to determine whether the distribution of tracer-labeled cells and the CO density profile were significantly coherent ($P < 0.05$) at these frequencies. Phase values not significantly different from zero ($P > 0.05$) for a particular frequency indicated that the distributions of labeled cells and CO

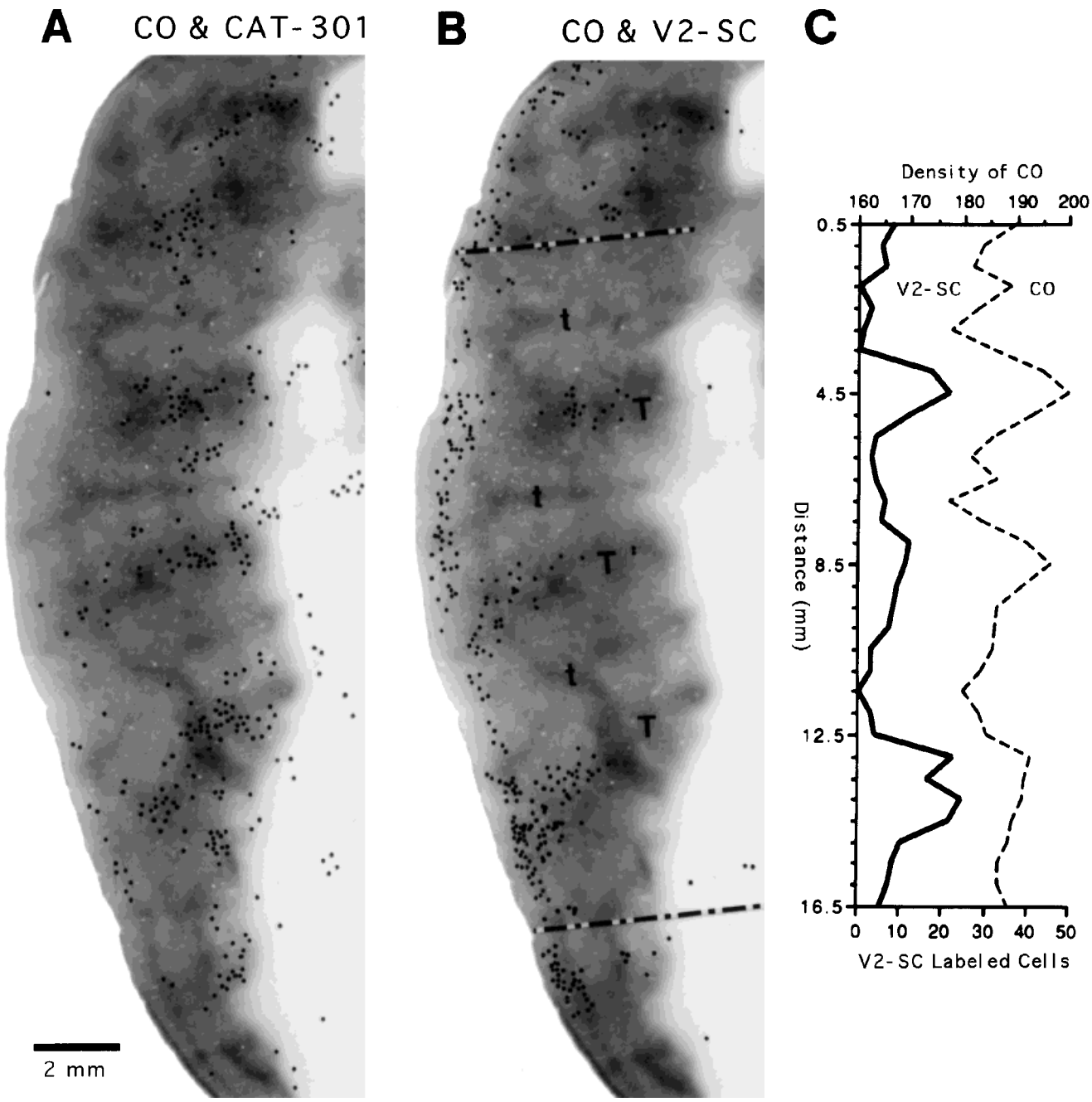

Fig. 2. **A:** Distribution of Cat-301-positive cells (black dots) superimposed on the pattern of cytochrome oxidase (CO) staining in dorsal V2 (block 5LD). Posterior is to the left, dorsal is up. **B:** Distribution of V2 cells (black dots) projecting to the superior colliculus (V2-SC). This population was retrogradely labeled by injections of Fast Blue (FB) into the tectum. The distribution of FB-labeled cells is shown superimposed on the pattern of CO staining. T, thick stripes; t, thin stripes. These sections come from a flattened operculum that contains portions of V2 on one side and portions of V1 on the other. The apparent superficial location of labeled layer 5 cells at the edge of the reconstruction in B is an artifact resulting from not unfolding the opercular lip prior to flattening the tissue block. The area analyzed quantitatively (bounded by the segmented lines) measured 16.5 mm in the direction parallel to the V1/V2 border. Labeled cells lying on white areas of the section shown in A and B were scored in more superficial sections. **C:** Quantitative analysis of area located between segmented lines in B. The solid curve (V2-SC) represents the distribution of FB-labeled cells and the segmented curve (CO) indicates the density profile for CO staining.

staining were aligned at that frequency. These techniques are specially suited for analyzing cyclic data series and have the advantage that they do not require the delineation of the boundaries of CO stripes, which can be difficult to determine unambiguously. Similar methods were used by Olavarria and Abel (1996) in their analysis of the

correlation between callosal connections and CO stripes in V2. Detailed descriptions of the techniques and software used are provided elsewhere (Gottman, 1981; Williams and Gottman, 1982).

RESULTS

In all five monkeys studied, the region infiltrated with HRP or FB extended virtually throughout the superior colliculus. This region included the superficial (I–III) and intermediate (IV–V) layers of the nucleus, where afferents from V2 terminate (Kuypers and Lawrence 1967; Wilson and Toyne, 1970; Lund, 1972; Graham et al., 1979; Tigges and Tigges, 1981; Cusick, 1988; Lui et al., 1995).

Retrogradely labeled cells studied in sections cut perpendicular to the cortical surface (blocks 4LD and 4RD) were pyramidal in shape and located in layer 5 (Fig. 1), in agreement with previous studies (Lund et al., 1981; Fries, 1984; Fries et al., 1985). In sections cut tangentially to the cortical surface, we found that labeled cells in V2 were not homogeneously distributed in the tangential plane. Instead, they formed band-like clusters that bore a consistent spatial relationship to the pattern of CO labeling.

Figure 2 shows the data from the dorsal portion of V2 from an animal injected with FB (block 5LD). This figure illustrates that in CO-stained sections, an alternating sequence of CO-dense thick (T) and thin (t) stripes could clearly be recognized (Fig. 2A,B). In addition, the preferential accumulation of Cat-301-immunopositive cells (black dots in Fig. 2A) in the thick stripes confirmed the identity of the CO stripes derived by their physical appearance (Hendry et al., 1988; DeYoe et al., 1990).

The reconstructed distribution of FB-labeled cells is superimposed with the pattern of CO staining in Figure 2B. Inspection of this figure shows that FB-labeled cells (black dots) form bandlike clusters that correlate very closely with the thick CO stripes. This impression was borne out by our quantitative analyses of the area bounded by the segmented lines in Figure 2B. The spectral analysis of the distribution of FB-labeled cells (solid line curve in Fig. 2C) revealed a significant frequency whose period was approximately 5 mm. The cross-spectral analysis showed that the spatial coherence between the distributions of labeled cells and CO staining was significant at this frequency and that these data series were in phase.

Similar results were obtained from the ventral portion of V2 (block 5LV) in the same animal (Fig. 3A). Large fluctuations in the number of FB-labeled cells occurred at 5 mm intervals. Peaks in cell labeling coincided with alternate increases in CO density and with increases in the number of Cat-301-positive cells (Fig. 3A). This result was borne out by our quantitative analyses of these data (see Table 1).

Figure 3B shows data from the dorsal portion of V2 (block 8LD) in the second monkey studied with injections of FB into the superior colliculus. As in the case shown in Figure 3A, fluctuations in the number of FB-labeled cells were in close correspondence with increases in the number of Cat-301-positive cells and with alternate peaks in CO density. The quantitative analyses of these data indicated that there was a significant spatial correspondence between accumulations of FB-labeled cells and thick CO stripes. The increases in the number of FB-labeled cells occurred approximately every 4 mm.

In the animals injected with FB (Figs. 2, 3), we were able to reveal the distribution of Cat-301-positive cells in the

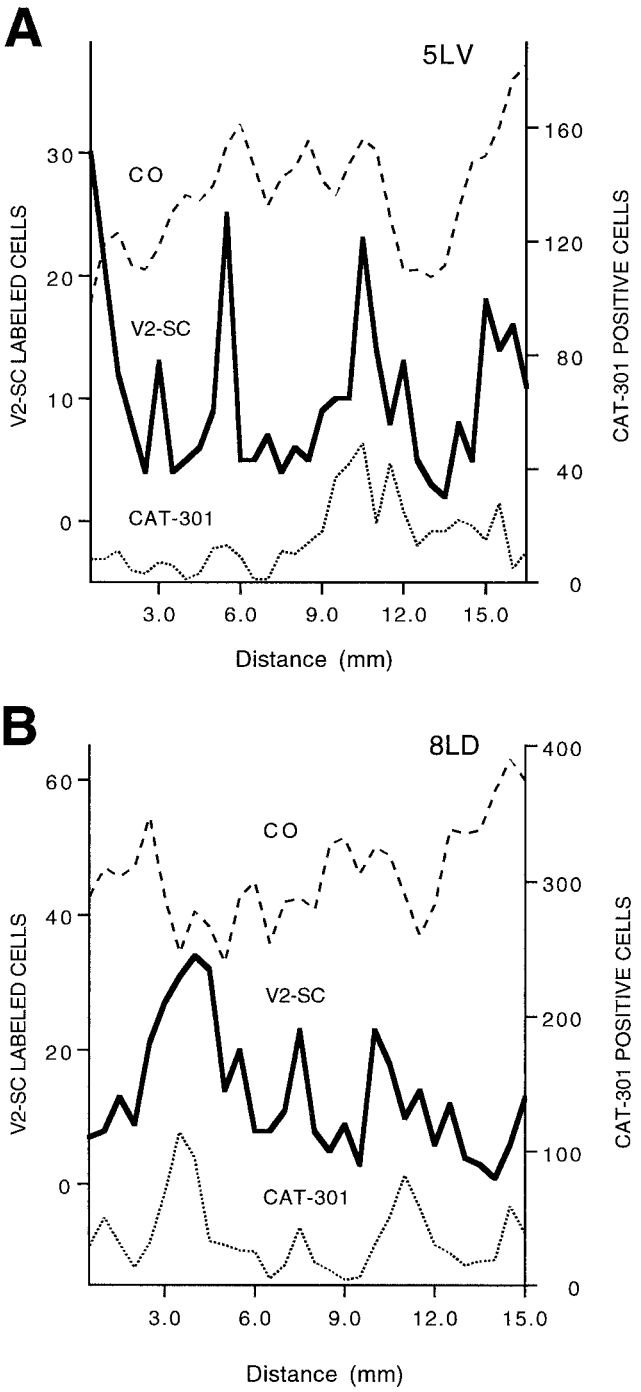

Fig. 3. Data from ventral V2 in monkey 5 (**A**) and from dorsal V2 in monkey 8 (**B**). The solid curves (V2-SC) represent the distribution of FB-labeled cells, the dashed curves (CO) indicate the density profile for CO staining (ordinate scale is not shown), and the dotted curves represent the distribution of Cat-301-positive cells. In both graphs, the number of FB-labeled cells peaks in close correspondence with increases in the number of Cat-301-positive cells and alternate CO dense regions (thick stripes).

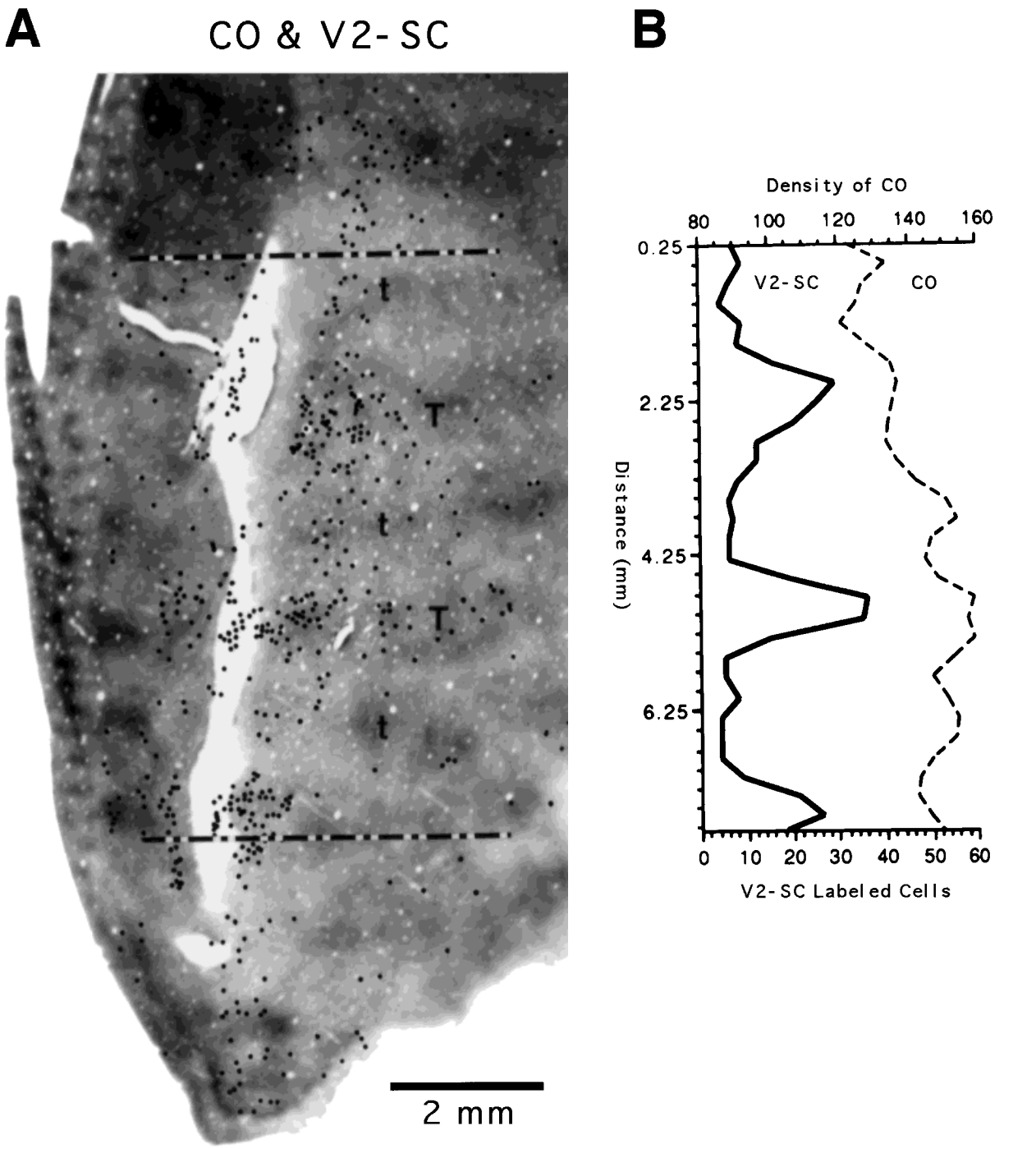

Fig. 4. Data from dorsal V2 in monkey 2 studied following injections of HRP into the tectum. **A:** Distribution of HRP-labeled cells (black dots) superimposed on the pattern of CO staining. T, thick stripes; t, thin stripes. Tangential sections came from unfolded and flattened tissue. Labeled cells lying in the region of tissue discontinuity were scored in sections that were continuous in this region. Posterior is to the left, dorsal is up. At left, the CO-stained section includes a portion of V1, which can be recognized by the presence of CO blobs. The segmented lines indicate area analyzed quantitatively. **B:** Distribution of HRP-labeled cells (V2-SC) and density profile for CO staining (CO).

same cortical sections that had been scored for fluorescent labeling. This approach was not possible in the cortical sections processed for HRP. To reconstruct the distributions of HRP-labeled cells and CO staining as completely as possible, we did not stain a separate series of sections for Cat-301 in these tissue blocks.

Animals injected with HRP yielded results similar to those described above. Figure 4A,B (block 2RD) shows that

HRP-labeled cells in dorsal V2 accumulated preferentially in alternate CO-dense stripes that were identified as thick based on their appearance. Data from ventral V2 in the same animal (block 2RV) are shown in Figure 5A. In both data sets, the spectral analysis revealed that the distribution of HRP-labeled cells had a significant frequency with a period of approximately 3 mm. At this frequency, the cross-spectral analyses showed that the distributions of labeled cells and CO labeling cohered and were in phase.

In a second monkey studied with HRP, peaks in the numbers of HRP-labeled cells from both dorsal (block 6RD; Fig. 5B) and ventral (block 6RV; Fig. 5C) V2 occurred about every 4 mm. Although the distinction between thick and thin CO stripes was less clear in this animal, the data obtained were consistent with those from the other cases; increases in the number of HRP-labeled cells coincided with alternate CO dense stripes.

In ventral V2 from both hemispheres of animal 4 (block 4LV and 4RV), the labeled regions were restricted to areas representing central visual fields (Gattass et al., 1981). The small size of the labeled regions precluded statistical analyses, but visual inspection of the data showed that two clusters of HRP-labeled cells were present in each block and that these were aligned with increases in the density of CO staining (data not shown).

DISCUSSION

We found that corticotectal neurons in V2 were located preferentially in regions that were in spatial register with thick CO stripes. Our finding is based on the direct correlation of the distributions of labeled corticotectal cells and CO staining and on the use of spectral analysis techniques to assess statistically the significance of this correlation. The latter techniques were chosen because they circumvent the need to delineate the boundaries of CO stripes, which can be difficult to identify unambiguously. The analyses revealed that periodic increases in the numbers of neurons projecting to the superior colliculus occurred about every 3–5 mm, which is approximately the distance between two CO stripes of the same class (Olavarria et al., 1989; Shipp and Zeki, 1989b; Tootell and Hamilton, 1989; Van Essen et al., 1990). Because these increases in the number of labeled cells were spatially aligned with alternate peaks in the CO density profile, it could be inferred that periodic increases in the density of superior colliculus projection neurons were in register with one class of CO dense stripes. From analyzing cases in which thick and thin CO stripes were easily identified either by their physical appearance (e.g., Figs. 2, 4A) and/or by their affinity to the Cat-301 monoclonal antibody (Figs. 2A, 3), we concluded that periodic increases in the density of corticotectal cells in V2 were in register with thick CO stripes.

Our results extend previous reports on the distribution of corticotectal cells in macaque V2. Fries (1984) and Fries et al. (1985) analyzed the overall distribution of retrogradely labeled corticotectal cells in horizontally cut sections. Although these investigators did not describe the detailed tangential distribution of cells in V2, it is possible to see clusters of labeled cells in V2 in some of their data (see Figs. 3, 6, 9 in Fries, 1984; Fig. 2 in Fries et al., 1985).

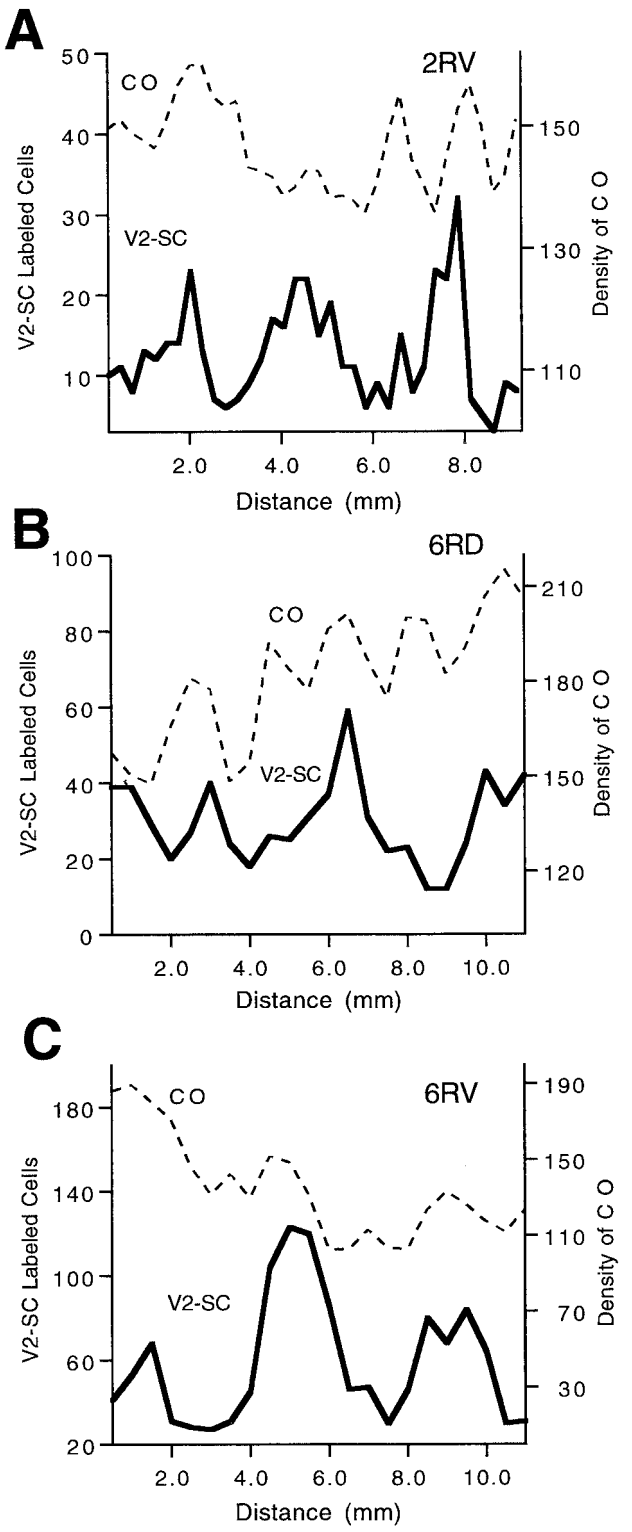

Fig. 5. **A:** Data from ventral V2 in monkey 2. **B,C:** Data from dorsal V2 (B) and ventral V2 (C) from monkey 6. Monkeys 2 and 6 were studied following injections of HRP into the tectum. In each graph, increases in the number of HRP-labeled cells (V2-SC) correspond closely with alternate peaks in the CO density profile (CO).

Methodological considerations

It is unlikely that periodic distributions of neurons projecting to the superior colliculus resulted from uneven uptake of the tracer in the tectum. In all cases, inspection of histological sections through the tectum indicated that the injections had impregnated virtually the entire nucleus, from the superficial through the deep layers, including all layers receiving projections from V2 (Kuypers and Lawrence 1967; Wilson and Toyne, 1970; Lund, 1972; Graham et al., 1979; Tigges and Tigges, 1981; Cusick, 1988; Lui et al., 1995). In addition, our results did not depend on the type of tracer used because we obtained the same results with HRP and the fluorescent tracer FB. The observation that labeled cells were located in layer 5 also indicates that the tracers did not spread to axons of the neighboring interhemispheric pathway because the laminar and tangential distribution of neurons in this pathway is different (Lund et al., 1981; Van Essen et al., 1982; Kennedy et al., 1986; Olavarria and Abel, 1996).

Relationship of CO architecture and functional pathways

CO compartments in V2 may identify pathways that are anatomically and functionally segregated (Hubel and Livingstone, 1987; Livingstone and Hubel, 1987b, 1988; DeYoe and Van Essen, 1988; Shipp and Zeki, 1988). This hypothesis has been examined in numerous anatomical and physiological studies (for reviews, see Felleman and Van Essen, 1991; Maunsell, 1992; Merigan and Maunsell, 1993). Although some studies support the idea that CO compartments in V2 represent different functional pathways, evidence from other studies is more ambiguous.

Perhaps the most compelling suggestion that V2 stripes are associated with separate functional pathways comes from anatomical studies of cortical afferent projections to V2 and efferent projections from V2. Each stripe compartment receives input from separate compartments in V1 (Livingstone and Hubel, 1983, 1984, 1987a) and projects differentially to other cortical areas (DeYoe and Van Essen, 1985; Shipp and Zeki, 1985, 1989b; Felleman et al., 1988; Zeki and Shipp 1989; Van Essen et al., 1990; Nakamura et al., 1993; DeYoe et al., 1994). Moreover, analysis of the intrinsic connections in V2 reveals that connectivity patterns can reflect the striped architecture of V2, even though interconnections between stripes of different compartments appear to exist (Livingstone and Hubel, 1984; Rockland, 1985; Levitt et al., 1994b; Malach et al., 1994; Olavarria and O'Brien, unpublished observations).

On the other hand, inferences about the functional segregation of V2 stripes based on physiological data have been somewhat mixed. A recent analysis of the fine visual topography in V2 revealed that each class of V2 compartments contains a continuous representation of the visual field (Roe and Tso, 1995). In addition, there is general agreement that neurons with selective responses to some visual attributes, such as color, motion, and disparity, tend to cluster in different CO compartments (DeYoe and Van Essen, 1985; Hubel and Livingstone, 1987; Peterhans and von der Heydt, 1993; Levitt et al., 1994a; Gegenfurtner et al., 1996). However, responses to other visual attributes appear to be more homogeneously distributed in V2, without an obvious relationship to CO architecture (Peterhans and von der Heydt, 1993; Levitt et al., 1994a; Gegenfurtner et al., 1996). The latter results may in part reflect the mixing of physiological and anatomical pathways that appears to occur as early as striate cortex (Malpeli and Schiller, 1981; Lachica et al., 1992; Nealey and Maunsell, 1994; Yoshioka et al., 1994; Sawatari and Callaway, 1996; for reviews, see Merigan and Maunsell, 1993; Casagrande and Kaas, 1994).

Corticosubcortical projections: Area V2

The present study stems from the idea that correlating the distribution of subcortically projecting neurons with the pattern of CO stripes may yield additional information about the relationship of V2 stripes with functional pathways. We studied the corticotectal pathway because neurons in the superior colliculus depend on "broadband" input passing through the magnocellular layers of the LGN (Schiller, 1981). Using cooling and ablation procedures, Schiller et al. (1974, 1979) found that visual responses of collicular cells located preferentially outside the retinorecipient layers depend on cortical input. Moreover, by inactivating either magnocellular or parvocellular LGN layers, they showed that the responses of this neuronal population depend on the magnocellular but not the parvocellular pathway (Schiller et al., 1979). Because V2 thick stripes receive significant input from magnocellular LGN layers through layer 4B in V1 (Livingstone and Hubel, 1987a), we predicted that projections to the superior colliculus from V2 would originate preferentially from cells within thick stripes.

Our findings bear out this prediction, thereby supporting the notion that CO compartments in V2 are associated with functionally separate pathways. Our observation that a small proportion of labeled cells were located in thin and interstripe regions suggests that these compartments are less closely associated with broadband functions than thick stripes are.

It is important to note that V2 thick stripes may not receive only magnocellular input. Parvocellular input can potentially reach thick stripes through intrinsic V2 projections (Livingstone and Hubel, 1984; Rockland, 1985; Levitt et al., 1994b; Malach et al., 1994). In addition, layer 5 cells in V2 can have extensive dendritic trees (Valverde, 1978; Lund et al., 1981), making it possible for dendrites of corticotectal cells in V2 thick stripes to sample information from neighboring stripes. Dendritic sampling across processing streams has been investigated for some cell populations in V1 (Hübener and Bolz, 1992; Malach, 1992), but data on this issue are not yet available for corticotectal cells in V2. Moreover, the recent report (Sawatari and Callaway, 1996) that layer 4B in striate cortex also receives parvocellular input opens the possibility that this input can be directly relayed to thick CO stripes in V2 (Livingstone and Hubel, 1987a). However, the dependance of collicular cells on magnocellular input shown by Schiller et al. (1979) implies that even if substantial parvocellular input reaches V2 thick stripes either from layer 4B or other sources this input does not appear capable of driving collicular activity during blockade of the magnocellular LGN layers.

If the segregation of V2 corticotectal projections does reflect functional differences between CO compartments, then the analysis of the distribution of corticotectal projections may provide a useful criterion for delineating functional differences among visual areas beyond V2. The potential of this approach is illustrated by recent studies

reporting that parietal and temporal cortices (Baizer et al., 1993) and areas within parietal cortex (Lynch et al., 1985; Lui et al., 1995) differ in the extent of their projections to the superior colliculus.

Corticosubcortical projections: Area V1

Segregation of corticotectal projection neurons in relation to CO architecture has also been described for V1. In this area, corticotectal cells are distributed preferentially in the interblob compartment in infragranular cortex (Lia and Olavarria, 1996). A similar relationship with the CO architecture has been reported for cytologically identified Meynert cells in V1 (Fries, 1986; Payne and Peters, 1989), many of which project to both the superior colliculus and area MT (Fries et al., 1985).

Interestingly, segregation of neuronal populations in relation to CO architecture does not appear to be a general rule for all cortical layers. For instance, in contrast to the infragranular corticotectal population, the magnocellular-recipient supragranular layer 4B population projecting to the thick stripes in V2 (Livingstone and Hubel, 1987a) and to MT (Maunsell and Van Essen, 1983; Shipp and Zeki, 1989a) is not segregated in relation to CO architecture (Shipp and Zeki, 1989a). In addition, Fitzpatrick et al. (1994) reported that there are sublaminar differences between the populations of layer 6 neurons projecting to either the magnocellular or parvocellular LGN layers, but no overt patchiness in the tangential plane has yet been described for this neuronal population (Horton, 1984).

Segregation of V2 subcortical loops

In addition to the superior colliculus, another major subcortical target of V2 is the pulvinar complex, and a recent study suggests that this descending projection also correlates with the modular organization of this area. Levitt et al. (1995) reported that the distribution of pulvinar projecting neurons in V2 fluctuates within layer 5A in vertical registration with thick and thin CO stripes. Moreover, pulvinar afferents to V2 are segregated into stripe-like domains (Ogren and Hendrickson, 1977; Curcio and Harting, 1978) in vertical alignment with thick and thin CO stripes (Livingstone and Hubel, 1982; Levitt et al., 1995). These data open the possibility that the exchange of visual information between V2 and the pulvinar complex flows along functionally segregated loops.

Input from V2 may also reach the pulvinar complex through superior colliculus–pulvinar projections (Benevento and Fallon, 1975; Partlow et al., 1977; Harting et al., 1980; Benevento and Standage, 1983). Tectal-recipient regions in the pulvinar appear to overlap with cortically projecting regions (Benevento and Rezak, 1976), but the specificity of the tectopulvinofugal projections with respect to thick and thin stripes in V2 and with other visual areas of the dorsal or ventral streams remains to be determined. Based on physiological data the hypothesis has been put forth that the tectofugal pathways are associated preferentially with cortical areas of the dorsal stream (Bruce et al., 1986; Girard et al., 1991; Gross, 1991). This proposal raises the intriguing possibility that corticotectal projections, including those from thick stripes described in the present study, may be part of a superior colliculus–pulvinar–cortical loop that preferentially processes broadband information. Additional studies are necessary to characterize the different components of such tectofugal circuits further and the extent to which they relate to specific visual functions.

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LITERATURE CITED

Abel, P.L., B.J. O'Brien, B. Lia, and J.F. Olavarria (1994) The distribution of cortico-collicular projection neurons correlates with thick cytochrome oxidase stripes in visual area V2 of macaque monkey. Soc. Neurosci. Abstr. *20:*710.6.

Baizer, J.S., R. Desimone, and L.G. Ungerleider (1993) Comparison of subcortical connections of inferior temporal and posterior parietal cortex in monkeys. Vis. Neurosci. *10:*59–72.

Benevento, L.A., and J.H. Fallon (1975) The ascending projections of the superior colliculus in the rhesus monkey (*Macaca mulatta*). J. Comp. Neurol. *160:*339–362.

Benevento, L.A., and M. Rezak (1976) The cortical projections of the inferior pulvinar and adjacent lateral pulvinar in the rhesus monkey (*Macaca mulatta*): An autoradiographic study. Brain Res. *108:*1–24.

Benevento, L.A., and G.P. Standage (1983) The organization of projections of the retinorecipient and nonretinorecipient nuclei of the pretectal complex and layers of the superior colliculus to the lateral pulvinar and medial pulvinar in the macaque monkey. J. Comp. Neurol. *217:*307–336.

Bruce, C.J., R. Desimone, and C.G. Gross (1986) Both striate cortex and superior colliculus contribute to the visual properties of neurons in superior temporal polysensory area of macaque monkey. J. Neurophysiol. *55:*1057–1075.

Bullier, J., and L.G. Nowak (1995) Parallel versus serial processing: new vistas on the distributed organization of the visual system. Curr. Opin. Neurobiol. *5:*497–503.

Casagrande, V.A., and J.H. Kaas (1994) The afferent, intrinsic, and efferent connections of primary visual cortex in primates. In A. Peters and K. Rockland (eds): Cerebral Cortex, vol. 10. New York: Plenum Press, pp. 201–259.

Curcio, C.A., and J.K. Harting (1978) Organization of pulvinar afferents to area 18 in the squirrel monkey: Evidence for stripes. Brain Res. *143:*155–161.

Cusick, C.G. (1988) Anatomical organization of the superior colliculus in monkeys: Corticotectal pathways for visual and visuomotor functions. Prog. Brain Res. *75:*1–15.

Desimone, R., R.J. Fleming, and C.G. Gross (1980) Prestriate afferents to inferior temporal cortex: An HRP study. Brain Res. *184:*41–55.

DeYoe, E.A., and D.C. Van Essen (1985) Segregation of efferent connections and receptive field properties in visual area V2 of the macaque. Nature *317:*58–61.

DeYoe, E.A., and D.C. Van Essen (1988) Concurrent processing streams in monkey visual cortex. Trends Neurosci. *11:*219–226.

DeYoe, E.A., S. Hockfield, H. Garren, and D.C. Van Essen (1990) Antibody labeling of functional subdivisions in visual cortex: Cat-301 immunoreactivity in striate and extrastriate cortex of macaque monkey. Vis. Neurosci. *5:*67–81.

DeYoe, E.A., D.J. Felleman, D.C. Van Essen, and E. McClendon (1994) Multiple processing streams in occipitotemporal visual cortex. Nature *371:*151–154.

Felleman, D.J., and D.C. Van Essen (1991) Distributed hierarchical processing in the primate cerebral cortex. Cereb. Cortex *1:*1–47.

Felleman D.J., E.A. DeYoe, J. Knierim, J. Olavarria, and D.C. Van Essen (1988) Compartmental organization of projections from V2 to extrastriate visual areas V3, V3A, and V4t in macaque monkeys. Invest. Ophthal. Vis. Sci., ARVO Abstr. Suppl. *29:*115.

Ferrera, V.P., T.A. Nealey, and J.H.R. Maunsell (1994) Responses in macaque visual area V4 following inactivation of the parvocellular and magnocellular LGN pathways. J. Neurosci. *14:*2080–2088.

Fitzpatrick, D., W.M. Usrey, B.R. Schofield, and G. Einstein (1994) The sublaminar organization of corticogeniculate neurons in layer 6 of macaque striate cortex. Vis. Neurosci. *11*:307–315.

Fries, W. (1984) Cortical projections to the superior colliculus in the macaque monkey: A retrograde study using horseradish peroxidase. J. Comp. Neurol. *230*:55–76.

Fries, W. (1986) Distribution of Meynert cells in primate striate cortex: Spatial relationships with cytochrome oxidase blobs. Naturwissenschaften *73:*S557.

Fries, W., K. Keizer, and H.G.J.M. Kuypers (1985) Large layer VI cells in macaque striate cortex (Meynert cells) project to both the superior colliculus and prestriate visual area V5. Exp. Brain Res. *58:*613–616.

Gattass, R., C.G. Gross, and J.H. Sandell (1981) Visual topography of V2 in the macaque. J. Comp. Neurol. *201:*519–539.

Gegenfurtner, K.R., D.C. Kiper, and S.B. Fenstemaker (1996) Processing of color, form and motion in macaque area V2. Vis. Neurosci. *13:*161–172.

Girard, P., P.-A. Salin, and J. Bullier (1991) Visual activity in macaque area V4 depends upon area 17 input. NeuroReport *2*:81–84.

Gottman, J.M. (1981) Time Series Analysis. A Comprehensive Introduction for Social Scientists. Cambridge: Cambridge University Press.

Graham, J., C.-S. Lin, and J.H. Kaas (1979) Subcortical projections of six visual cortical areas in the owl monkey *Aotus trivirgatus*. J. Comp. Neurol. *187*:557–580.

Gross, C.G. (1991) Contribution of striate cortex and the superior colliculus to visual function in area MT, the superior temporal polysensory area and the inferior temporal cortex. Neuropsychologia *29:*497–515.

Harting, J.K., M.F. Huerta, A.J. Frankfurter, N.L. Strominger, and G.J. Royce (1980) Ascending pathways from monkey superior colliculus: An autoradiographic analysis. J. Comp. Neurol. *192*:853–882.

Hendry, S.H.C., E.G. Jones, S. Hockfield, and R.D.G. McKay (1988) Neuronal populations stained with the monoclonal antibody Cat-301 in the mammalian cerebral cortex and thalamus. J. Neurosci. *8:*518–542.

Horton, J.C. (1984) Cytochrome oxidase patches: A new cytoarchitectonic feature of monkey visual cortex. Phil. Trans. R. Soc. Lond. B *304:*199–253.

Horton, J.C., and D.H. Hubel (1981) Regular patchy distribution of cytochrome oxidase staining in primary visual cortex of macaque monkey. Nature *292:*762–764.

Hubel, D.H., and M.S. Livingstone (1987) Segregation of form, color and stereopsis in primate area 18. J. Neurosci. *7:*3378–3415.

Hübener, M., and J. Bolz (1992) Relationships between dendritic morphology and cytochrome oxidase compartments in monkey striate cortex. J. Comp. Neurol. *324:*67–80.

Kennedy, H., C. Dehay, and J. Bullier (1986) Organization of the callosal connections of visual areas V1 and V2 in the macaque monkey. J. Comp. Neurol. *247*:398–415.

Kuypers, H.G.J.M., and D.G. Lawrence (1967) Cortical projections to the red nucleus and the brain stem in the rhesus monkey. Brain Res. *4:*151–188.

Lachica, E.A., P. Beck, and V.A. Casagrande (1992) Parallel pathways in macaque monkey striate cortex: Anatomically defined columns in layer III. Proc. Natl. Acad. Sci. USA *89:*3566–3570.

Levitt, J.B., D.C. Kiper, and J.A. Movshon (1994a) Receptive fields and functional architecture of macaque V2. J. Neurophysiol. *71:*2517–2542.

Levitt, J.B., T. Yoshioka, and J.S. Lund (1994b) Intrinsic cortical connections in macaque visual area V2: evidence for interaction between different functional streams. J. Comp. Neurol. *342*:551–570.

Levitt, J.B., T. Yoshioka, and J.S. Lund (1995) Connections between the pulvinar complex and cytochrome oxidase-defined compartments in visual area V2 of macaque monkey. Exp. Brain Res. *104:*419–430.

Lia, B., and J.F. Olavarria (1996) The distribution of corticotectal projection neurons correlates with the interblob compartment in macaque striate cortex. Vis. Neurosci. *13:*461–466.

Livingstone, M.S., and D.H. Hubel (1982) Thalamic inputs to cytochrome oxidase-rich regions in monkey visual cortex. Proc. Natl. Acad. Sci. USA *79:*6098–6101.

Livingstone, M.S., and D.H. Hubel (1983) Specificity of cortico-cortical connections in monkey visual system. Nature *304*:531–534.

Livingstone, M.S., and D.H. Hubel (1984) Anatomy and physiology of a color system in the primate visual cortex. J. Neurosci. *4:*309–356.

Livingstone, M.S., and D.H. Hubel (1987a) Connections between layer 4B of area 17 and the thick cytochrome oxidase stripes of area 18 in the squirrel monkey. J. Neurosci. *7:*3371–3377.

Livingstone, M.S., and D.H. Hubel (1987b) Psychophysical evidence for separate channels for the perception of form, color, movement, and depth. J. Neurosci. *7:*3416–3468.

Livingstone, M.S., and D.H. Hubel (1988) Segregation of form, color, movement, and depth: Anatomy, physiology, and perception. Science *240:*740–749.

Lui, F., K.M. Gregory, R.H.I. Blanks, and R.A. Giolli (1995) Projections from visual areas of the cerebral cortex to pretectal nuclear complex, terminal accessory optic nuclei, and superior colliculus in macaque monkey. J. Comp. Neurol. *363:*439–460.

Lund, J.S., A.E. Hendrickson, M.P. Ogren, and E.A. Tobin (1981) Anatomical organization of primate visual cortex area VII. J. Comp. Neurol. *202:*19–45.

Lund, R.D. (1972) Synaptic patterns in the superficial layers of the superior colliculus of the monkey, Macaca mulatta. Exp. Brain Res. *15:*194–211.

Lynch, J.C., A.M. Graybiel, and L.J. Lobeck (1985) The differential projection of two cytoarchitectonic subregions of the inferior parietal lobule of macaque upon the deep layers of the superior colliculus. J. Comp. Neurol. *235:*241–254.

Malach, R. (1992) Dendritic sampling across processing streams in monkey striate cortex. J. Comp. Neurol. *315:*303–312.

Malach, R., R.B.H. Tootell, and D. Malonek (1994) Relationship between orientation domains, cytochrome oxidase stripes, and intrinsic horizontal connections in squirrel monkey area V2. Cereb. Cortex *4:*151–165.

Malpeli, J.G., and P.H. Schiller (1981) Response properties of single cells in monkey striate cortex during reversible inactivation of individual lateral geniculate laminae. J. Neurophysiol. *46:*1102–1119.

Martin, K.A.C. (1988) From enzymes to visual perception: A bridge gone too far? Trends Neurosci. *11:*380–387.

Maunsell, J.H.R. (1992) Functional visual streams. Curr. Opin. Neurobiol. *2*:506–510.

Maunsell, J.H.R., and W.T. Newsome (1987) Visual processing in monkey extrastriate cortex. Annu. Rev. Neurosci. *10*:363–341.

Maunsell, J.H.R., and D.C. Van Essen (1983) The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey. J. Neurosci. *12:*2563–2586.

Merigan, W.H., and J.H.R. Maunsell (1990) Macaque vision after magnocellular lateral geniculate lesions. Vis. Neurosci. *5*:347–352.

Merigan, W.H., and J.H.R. Maunsell (1993) How parallel are the primate visual pathways? Annu. Rev. Neurosci. *16*:369–402.

Mesulam, M.M. (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. *26*:106–117.

Mishkin, M., L.G. Ungerleider, and K.A. Macko (1983) Object vision and spatial vision: Two cortical pathways. Trends Neurosci. *6*:414–417.

Nakamura, H., R. Gattass, R. Desimone, and L.G. Ungerleider (1993) The modular organization of projections from areas V1 and V2 to areas V4 and TEO in macaques. J. Neurosci. *13:*3681–3691.

Nealey, T.A., and J.H.R. Maunsell (1994) Magnocellular and parvocellular contributions to the responses of neurons in macaque striate cortex. J. Neurosci. *14:*2069–2079.

Ogren, M.P., and A.E. Hendrickson (1977) The distribution of pulvinar terminals in visual areas 17 and 18 of the monkey. Brain Res. *137:* 343–350.

Olavarria, J., and P.L. Abel (1996) The distribution of callosal connections correlates with the pattern of cytochrome oxidase stripes in visual area V2 of macaque monkeys. Cereb. Cortex *6*:631–639.

Olavarria, J., and R.C. Van Sluyters (1985) Unfolding and flattening the cortex of gyrencephalic brains. J. Neurosci. Methods *15:*191–202.

Olavarria, J., E.A. DeYoe, and D.C. Van Essen (1989) Pattern of cytochrome oxidase staining in the unfolded and flattened V2 of macaque monkey. Invest. Ophthal. Vis. Sci., ARVO Abstr. Suppl. *30:*298.

Partlow, G.D., M. Colonnier, and J. Szabo (1977) Thalamic projections of the superior colliculus in the rhesus monkey, *Macaca mulatta*. A light and electron microscopic study. J. Comp. Neurol. *171:*285–318.

Payne, B.R., and A. Peters (1989) Cytochrome oxidase patches and Meynert cells in monkey visual cortex. Neuroscience *28:*353–363.

Peterhans, E., and R. von der Heydt (1993) Functional organization of area V2 in the alert macaque. Eur. J. Neurosci. *5*:509–524.

Rockland, K.S. (1985) A reticular pattern of intrinsic connections in primate area V2 (area 18). J. Comp. Neurol. *235:*467–478.

Roe, A., and D.Y. Tso (1995) Visual topography in primate V2: Multiple representation across functional stripes. J. Neurosci. *15:*3689–3715.

Sawatari, A., and E.M. Callaway (1996) Convergence of magno- and parvocellular pathways in layer 4B of macaque primary visual cortex. Nature *380:*442–446.

Schiller, P.H. (1981) Input and output specificity of striate cortex. In O. Pompeiano, and C.A. Marsan (eds): Brain Mechanisms and Perceptual Awareness. New York: Raven Press, pp. 21–36.

Schiller, P.H., M. Stryker, M. Cynader, and N. Berman (1974) Response characteristics of single cells in the monkey superior colliculus following ablation or cooling of the visual cortex. J. Neurophysiol. *37:*181–194.

Schiller, P.H., J.G. Malpeli, and S.J. Schein (1979) Composition of the geniculostriate input to superior colliculus of the rhesus monkey. J. Neurophysiol. *42:*1124–1133.

Schiller, P.H., N.K. Logothetis, and E.R. Charles (1990) Role of the color-opponent and broad band channels in vision. Vis. Neurosci. *5:*321–346.

Shipp, S., and S. Zeki (1988) The functional logic of cortical connections. Nature *335*:311–317.

Shipp, S., and S. Zeki (1985) Segregation of pathways leading from area V2 to areas V4 and V5 of macaque monkey visual cortex. Nature *315:*322–325.

Shipp, S., and S. Zeki (1989a) The organization of connections between areas V5 and V1 in macaque monkey visual cortex. Eur. J. Neurosci. *1:*309–332.

Shipp, S., and S. Zeki (1989b) The organization of connections between areas V5 and V2 in macaque monkey visual cortex. Eur. J. Neurosci. *1:*333–354.

Tigges, J., and M. Tigges (1981) Distribution of retinofugal and corticofugal axon terminals in the superior colliculus of the squirrel monkey. Invest. Ophthal. Vis. Sci. *20:*149–158.

Tootell, R.B.H., and S.L. Hamilton (1989) Functional anatomy of the second visual area (V2) in the macaque. J. Neurosci. *9:*2620–2644.

Tootell, R.B.H., M.S. Silverman, R.L. DeValois, and G.H. Jacobs (1983) Functional organization of the second visual area in primates. Science *220:*737–739.

Ungerleider, L.G., and M. Mishkin (1982) Two cortical systems. In D.J. Ingle, M.A. Goodale, and R.J.W. Mansfield (eds): Analysis of Visual Behavior Cambridge: MIT, pp. 549–585.

Valverde, F. (1978) The organization of area 18 in the monkey: A Golgi study. Anat. Embryol. *154:*305–334.

Van Essen, D.C., D.J. Felleman, E.A. DeYoe, J. Olavarria, and J. Knierim (1990) Modular and hierarchical organization of extrastriate visual cortex in the macaque monkey. Cold Spring Harbor Symp. Quant. Biol. *55:*679–696.

Van Essen, D.C., W.T. Newsome, and J.L. Bixby (1982) The pattern of interhemispheric connections and its relationship to extrastriate visual areas in the macaque monkey. J. Neurosci. *2:*265–283.

Van Essen, D.C., C.H. Andersen, and D.J. Felleman (1992) Information processing in the primate visual system: An integrated systems perspective. Science *255:*419–423.

Williams, E.A., and J.M. Gottman (1982) A User's Guide to the Gottman–Williams Time-Series Analysis Computer Programs for Social Scientists. Cambridge: Cambridge University Press.

Wilson, M.E., and M.J. Toyne (1970) Retino-tectal and cortico-tectal projections in *Macaca mulatta*. Brain Res. *24:*395–406.

Wong-Riley, M. (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res. *171:*11–28.

Wong-Riley, M.T.T., and E.W. Carroll (1984) Quantitative light and electron microscopic analysis of cytochrome oxidase-rich zones in VII prestriate cortex of squirrel monkey. J. Comp. Neurol. *222:*18–37.

Yoshioka, T., J.B. Levitt, and J.S. Lund (1994) Independence and merger of thalamocortical channels within macaque monkey visual cortex: Anatomy of interlaminar projections. Vis. Neurosci. *11:*467–489.

Zeki, S., and S. Shipp (1988) The functional logic of cortical connections. Nature *335:*311–317.

Zeki, S., and S. Shipp (1989) Modular connections between areas V2 and V4 of macaque monkey visual cortex. Eur. J. Neurosci. *1:*494–506.