The Journal of Neuroscience, March 1995, 15(3): 1808-1818

Concomitant Sensitivity to Orientation, Direction, and Color of Cells in Layers 2, 3, and 4 of Monkey Striate Cortex

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The receptive field properties of cells in layers 2, 3, and 4 of area 17 (V1) of the monkey were studied quantitatively using colored and broad-band gratings, bars, and spots. Many cells in all regions studied responded selectively to stimulus orientation, direction, and color. Nearly all cells (95%) in layers 2 and 3 exhibited statistically significant orientation preferences (biases), most exhibited at least some color sensitivity, and many were direction sensitive. The degree of selectivity of cells in layers 2 and 3 varied continuously among cells; we did not find discrete regions containing cells sensitive to orientation and direction but not color, and vice versa. There was no relationship between the degree of orientation sensitivity of the cells studied and their degree of color sensitivity. There was also no obvious relationship between the receptive field properties studied and the cells' location relative to cytochrome oxidase–rich regions. Our findings are difficult to reconcile with the hypothesis that there is a strict segregation of cells sensitive to orientation, direction, and color in layers 2 and 3. In fact, the present results suggest the opposite since most cells in these layers are selective for a number of stimulus attributes.

[Key words: visual cortex, monkey, orientation, direction, color sensitivity, blobs]

Orientation, direction, and color sensitivity are recognized as fundamental properties of neurons in the mammalian central visual pathways (Hubel and Wiesel, 1962, 1968). These specialized neurons are most likely involved in the perception of form, motion, and color. Historically, color sensitivity was thought to originate subcortically while both orientation and direction sensitivity were thought to originate in the cortex from nonorientation- and nondirection-sensitive inputs (Hubel and Wiesel, 1962, 1968).

In the first studies of monkey striate cortex (V1) the large majority of cells in layers 2 and 3 was reported to be orientation sensitive (Hubel and Wiesel, 1968; Schiller et al., 1976; DeValois et al., 1982). The orientation selectivity of these cells was originally hypothesized to arise from convergence of inputs from unoriented cells in layer 4 (Hubel and Wiesel, 1968). More recently it has been reported that layers 2 and 3 contain cytochrome oxidase–rich regions (blobs) of color-sensitive cells that are insensitive to stimulus orientation and direction separated by cytochrome oxidase–poor regions (interblobs) of broad-band cells that are sensitive to orientation and direction (Livingstone and Hubel, 1984). Blob cells and interblob cells are hypothesized to receive inputs from the color-sensitive parvicellular and broad-band magnocellular layers, respectively, of the dorsal lateral geniculate nucleus (Livingstone and Hubel, 1987, 1988; Casagrande and Norton, 1991; reviewed in LeVay and Nelson, 1991). Striate cortical cells exhibiting different properties are reported to project in parallel to separate regions (thick stripes, thin stripes, interstripes) of cortical area 18 (V2) that contain cells exhibiting corresponding properties. Regions of extrastriate cortex V4 (color area) and MT (motion area) are further hypothesized to receive inputs from the parvicellular and magnocellular pathways, respectively.

Taken together, the foregoing experiments and ideas led to the suggestion that cells signaling form, motion, and color are segregated in the visual pathways (Livingstone and Hubel, 1988; reviewed in LeVay and Nelson, 1991). In recent years this idea has gained popularity. However, despite its popularity, this suggestion has not received clear experimental support. Indeed, a number of studies have provided evidence that the segregation of cells exhibiting different selectivities into separate, parallel pathways may not be as strict as proposed originally (Lennie et al., 1990; Maunsell et al., 1990; Ferrera et al., 1992; Martin, 1992; DeBruyn et al., 1993; Kiper et al., 1994; Schiller, 1994; Zipser et al., 1994). This study was designed to test the hypothesis that different cells in V1 signal form, motion, and color.

Materials and Methods

Surgical preparation. Animals were prepared for electrophysiological recording as described previously (Leventhal and Schall, 1983; Schall et al., 1986). Subjects were sedated with ketamine (Ketalar, Parke-Davis) and then anesthetized with 3–5% halothane (Fluothane) in 75% NO_2:25% O_2. Intravenous and tracheal cannulae were inserted. Animals were placed in a stereotaxic apparatus and an acrylic chamber secured with dental cement was positioned over area 17 (V1). All pressure points and incisions were infiltrated with a long-acting anesthetic (1% Lidocaine HCl, Abbott Laboratories). A mixture of *d*-tubocurarine (0.4 mg/kg/hr) and gallamine triethiodide (7 mg/kg/hr) were continuously infused intravenously to induce and maintain paralysis. Animals were artificially ventilated continuously with a mixture of nitrous oxide (75%) and oxygen (25%) containing halothane (1%). Body temperature was maintained at 38°C. The electrocardiogram and electroencephalogram were monitored. Depth of anesthesia was assessed by monitoring EEG for absence of the 8–13 Hz α wave. Expired CO_2 was monitored and maintained at approximately 4%.

Received Mar. 22, 1994; revised Aug. 29, 1994; accepted Sept. 6, 1994.

This work was supported by EY04951 and EY08523 to A.G.L.

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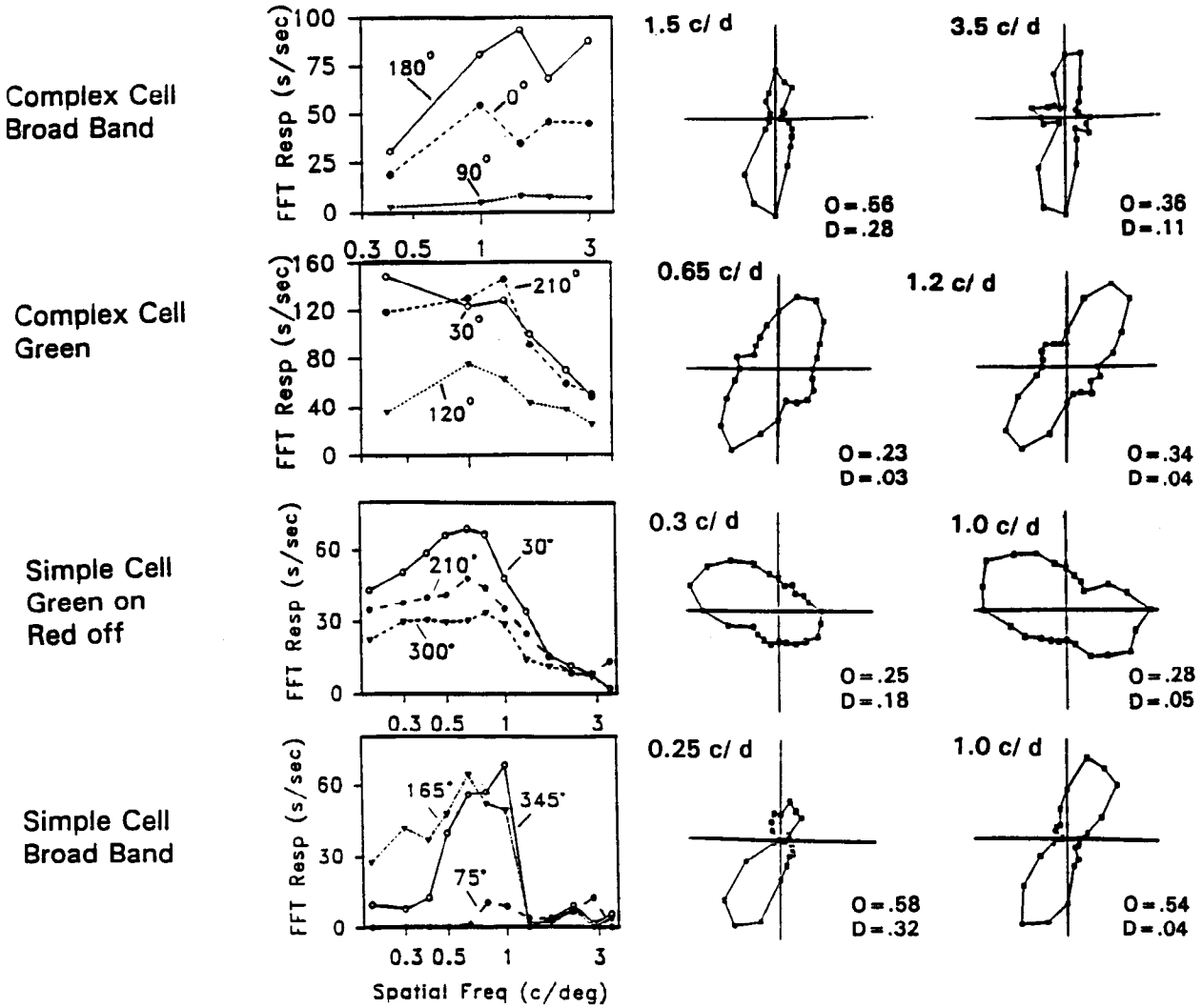

Figure 1. Typical spatial frequency, orientation, and direction tuning curves of color-sensitive and broad-band cells in layers 2 and 3 of monkey striate cortex. The polar plots were gathered using a variety of spatial frequencies (*c/d,* cycles per degree). The orientation (*O*) and direction (*D*) biases are indicated for each polar plot. The results in this figure were gathered using broad-band and colored sinusoidal gratings drifting across the receptive field. Cells were also tested using broad-band and colored alternating gratings, counterphased gratings, and moving spots and bars (see Materials and Methods). The orientation of each grating or bar was orthogonal to the direction. Fifteen presentations of each grating (temporal frequency of 2–4 Hz) at each of 24–36 orientations were used to compile tuning curves for the cells. The responses of the cells were tested at a variety of spatial frequencies. The various orientations and spatial frequencies were presented in random order to reduce sampling bias. The stimulus used was at least three times larger than the receptive field center of the cell. The temporal frequency employed was the one judged to be optimal for the unit. The responses of the cells to the visual stimuli presented were stored in the computer for analyses. Notice that the differences in the spatial frequency tuning of the cells at different orientations and directions were consistent with their orientation and direction tuning and that the color-sensitive simple and complex cells responded relatively better to lower spatial frequencies than did the broad-band simple and complex cells.

Receptive field mapping procedures. The responses of single cells in areas of interest to drifting high and low spatial frequency sinusoidal gratings as well as to alternating counterphased gratings were used to determine whether the cell summated linearly or nonlinearly. Cells were classified as simple and complex on the basis of the segregation (simple cells) or overlap (complex cells) of on and off subfields, as well as the modulation of the cells' response to gratings of the optimal spatial frequency (Hubel and Wiesel, 1968; Schiller et al., 1976). Special complex cells were classified using the criteria of Gilbert (1977). Color sensitivity was studied in detail (see below). Spatial frequency tuning, receptive field size, response tonicity, response to moving and flashing bars and spots, orientation and direction sensitivity, and sluggishness of response were also studied. Layer 4 cells were identified as X- or Y-type (Dreher et al., 1976; Shapley et al., 1981) based upon attributes such as response linearity, transientness of response, receptive field size, and response to rapid stimulus motion. Visual stimuli were generated on a Tektronix 608 display driven by a Picasso (Innisfree Electronics) image synthesizer. The Picasso was controlled by computer. Our system is capable of generating a broad spectrum of visual stimuli under computer control, collecting the data from multiple channels, and performing on-line statistical analyses and correlations. In addition, an apparatus was employed which allowed the oscilloscope display to be moved to any point in the animal's visual field while at the same time maintaining a fixed distance between the display and the animal's retina. At each visual field position the center of the display screen was exactly 171 cm from the animal's retina. Thus, we are able to accurately study cells subserving different parts of the visual field without distortion. The eccentricity of each cell's receptive field was defined as the distance from the center of the receptive field (determined by presenting stimuli to the dominant eye) to the projection of the fovea for that eye.

Procedures for the determination of orientation and direction sensitivity. The physiological orientation and direction biases of cells were studied using sinusoidal gratings drifting across the receptive field as well as alternating counterphased gratings, and moving spots and bars. The orientation of each grating or bar was orthogonal to its direction of motion. Fifteen presentations of each grating (temporal frequency of

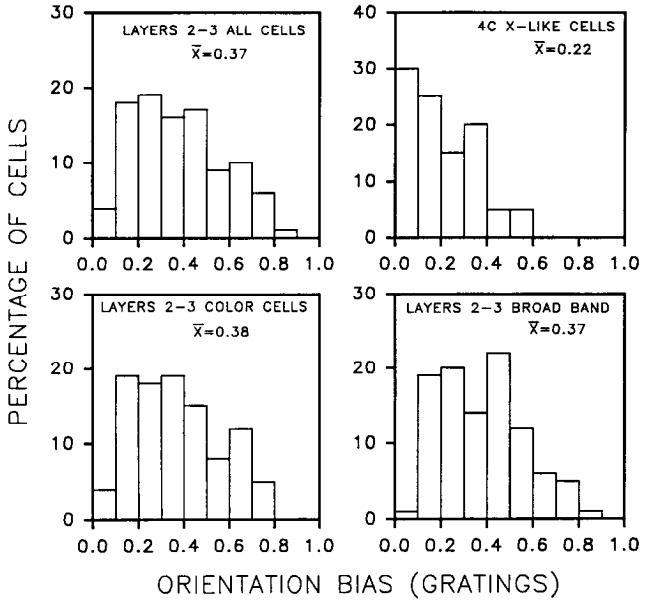

Figure 2. Histograms illustrating the distribution of the orientation biases of cells in monkey striate cortex. Results for cells in different layers, color-sensitive cells, and broad-band cells are shown separately. The mean orientation biases for each group are shown.

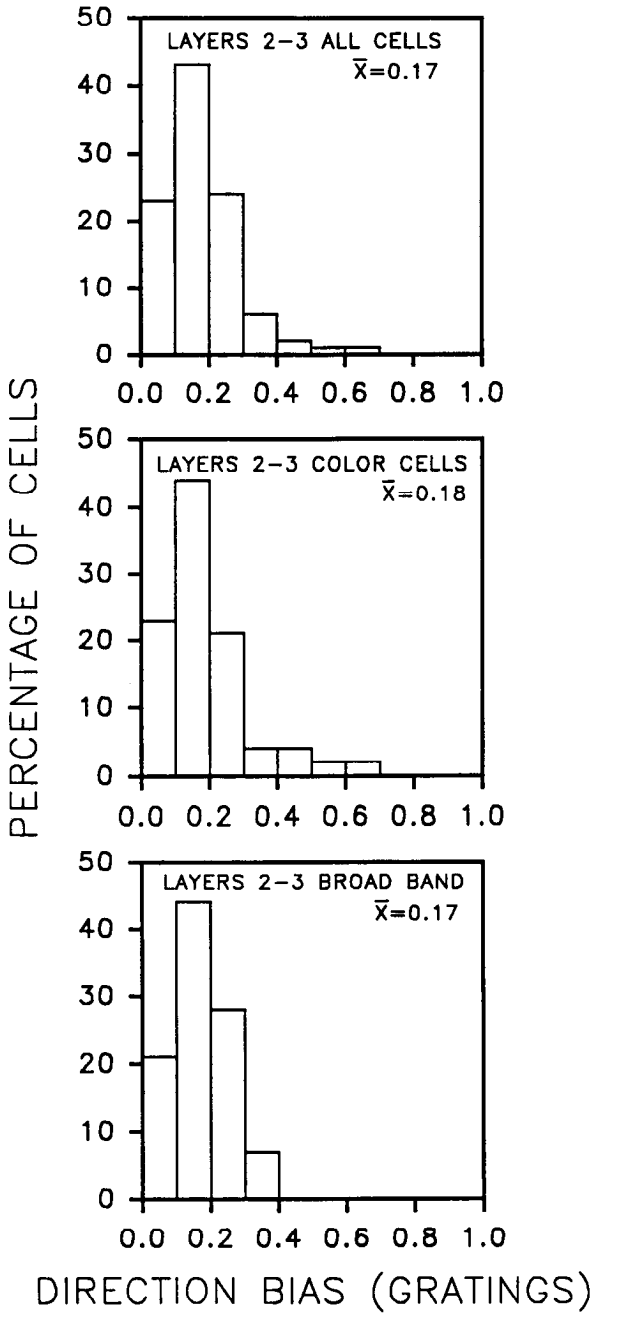

Figure 3. Histograms illustrating the distribution of the direction biases of cells in monkey striate cortex. Results for cells in different layers, color-sensitive cells, and broad-band cells are shown separately. The mean direction biases for each group are shown.

2–4 Hz) at each of 24–36 orientations were used to compile the turning curves for the cells. The responses of the cells were studied at a variety of spatial frequencies. The various orientations and spatial frequencies were presented in random order to reduce sampling bias. For cells not displaying end inhibition, the stimulus used was at least three times larger than the receptive field center of the cell. The temporal frequency employed was the one judged to be optimal for the unit by presenting moving gratings of the optimal spatial frequency at various temporal frequencies. For cells that displayed end-zone inhibition (end-stopped cells), stimuli of the appropriate length were employed.

Orientation and direction preferences and sensitivities were calculated for each cell. The statistical methods employed are described in detail in Batschelet (1981) and Zar (1974). These methods have been previously used in the calculation of the orientation sensitivities of retinal ganglion cells (Levick and Thibos, 1982; Thibos and Levick, 1985), LGNd relay cells (Shou and Leventhal, 1989), and visual cortical cells to moving stimuli (Wörgötter and Eysel, 1987; Wörgötter et al., 1990; Thompson et al., 1994).

Briefly, the responses of each cell to the different directions of the stimulus presented were stored in the computer as a series of vectors. The vectors were added and divided by the sum of the absolute values of the vectors. The angle of the resultant vector gives the preferred direction of the cell. The length of the resultant vector, termed the orientation or direction bias, provides a quantitative measure of the orientation or direction sensitivity of the cell. Because the periodicity of orientation is 180°, the angles of the direction of the stimulus grating, bar, or spot are multiplied by a factor of two when calculating orientation preferences. However, direction is cyclic over 360°, therefore the actual directions of the stimulus gratings, bars, or spots are used to calculate the direction preferences of the cell. Orientation and direction biases range from 0 to 1, with 0 being completely insensitive to orientation or direction and 1 responding to only one orientation or only one direction. Although in theory the range is from 0 to 1, the observed range of orientation biases for cortical cells is from 0 to about 0.75, and the range of direction biases observed is from 0 to about 0.5.

In order to provide a second measure of orientation and direction sensitivity, the maximum to minimum response ratios were calculated for each cell. In the calculation of orientation sensitivity, the responses evoked by the preferred orientation were averaged and divided by the averaged responses evoked by the nonpreferred orientation. Similarly, in the calculation of direction ratios the responses in the preferred direction were averaged and divided by the averaged response in the nonpreferred direction. Theoretically, the range is from 1 to ∞. The observed range for orientation is from 1:1 to over 100:1 and direction is from 1:1 to over 20:1. Since the vector analysis described above takes into account the responses at all directions of stimulus motion it can account for lopsided (non-Gaussian) (Thompson et al., 1989) orientation and direction tuning. Thus, it is more accurate than some previously used methods (i.e., half-width-at-half-height, direction index, and maximum to minimum response ratio; see Orban, 1984) to predict the orientation and direction preferences of visual neurons (see also Wörgötter et al., 1990, for discussion).

Reliability of mapping procedures. In order to test whether or not the preferred orientations and directions of the cells studied could be determined accurately and consistently over time, many cells were studied for 1 hr or more, and multiple tuning curves were compiled at a variety of spatial frequencies. This analysis indicates that an orientation or direction bias of 0.08 or greater is significant and shows that the circular

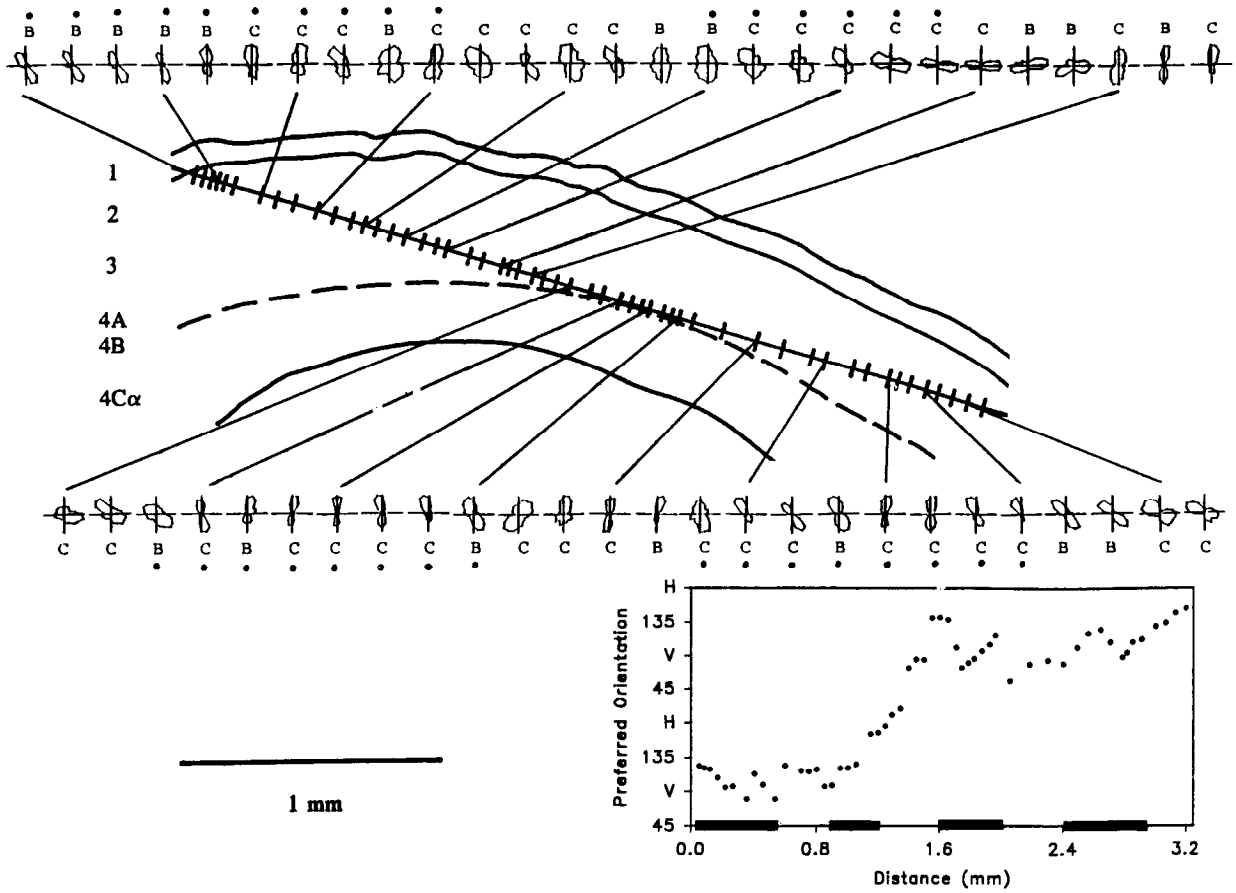

Figure 4. Reconstruction of an electrode penetration through layers 2 and 3 of area 17 of the rhesus monkey. The polar plots illustrate the orientation and direction tuning of the units encountered. *C* and *B* (*top penetration*) indicate whether the cells were color sensitive or broad band. The *spots* indicate cells that were in the regions that stained most darkly for cytochrome oxidase. The scatter plots illustrate the change in preferred orientation with distance along the penetration. The *thick bars* on the abscissa (*top*) indicate regions that stained most darkly for cytochrome oxidase. Notice that orientation preference changes more rapidly in some regions than in others (compare 0 to 0.8 mm vs 0.8 mm to 1.6 mm). Notice that, in general, preferred orientation changed gradually and systematically along the penetration.

distribution of the cells' responses to moving stimuli is nonrandom (Rayleigh test, $p < 0.05$; described below) (Zar, 1974). An orientation or a direction bias of 0.1 or greater indicates significance at the $p < 0.005$ level (Rayleigh test). Moreover, cells with biases of 0.1 or greater exhibited preferred orientations and directions within 5–10° with repeated testing; their degree of bias varied very little between trials. Thus, in our studies, a cell exhibiting a bias of 0.1 or greater will be considered to be orientation or direction sensitive; cells with biases less than 0.1 will be defined as being nonorientation or nondirection sensitive. Since orientation bias relates nonlinearly to response ratio, orientation biases of 0.1, 0.3 and 0.5 correspond to maximum to minimum response rates of 1.5:1, 3.7:1, and 10.8:1, respectively.

Color sensitivity. Receptive fields were studied with achromatic and chromatic stimuli in order to determine the spatial and temporal properties of the cells. A preliminary evaluation of the cells' chromatic properties was carried out using Kodak wratten and neutral density filters in conjunction with a hand-held projector (Pantoscope-Keeler Instruments). Next, color sensitivity was determined quantitatively by generating colored spots, bars, and gratings on a modified Tektronix 608 display that contains a special phosphor designed to emit a uniform mix of wavelengths. Intensity and wavelength were controlled by using our visual stimulation hardware and software in conjunction with the appropriate Kodak wratten filter. Colored stimuli were presented on dark backgrounds and/or backgrounds of the opponent color. Care was taken to present stimuli under equiluminant conditions in order to isolate inputs from different types of cones (Lennie et al., 1990). The luminance and chromaticity of the stimuli were measured using a chronometer (Tektronix J17 Luma Color photometer) that employs the Yxy color notation system. In this system, x and y express color tone while Y is a measure of luminance.

In addition to deciding subjectively which cells were color sensitive, a quantitative measure of color sensitivity was determined for each cell as follows. First, the width of the excitatory receptive field was determined by flashing spots and bars of different colors and sizes. The stimulus dimensions and color that elicited the maximum responses were determined from the poststimulus time histograms. The maximum response elicited by a stimulus of the preferred color on a dark background was then divided by the maximum response evoked by an identical stimulus of equal luminance of the opponent color. The response ratio obtained in this fashion is termed the color index. It ranges from 1 to ∞, with 1 being a cell that responded equally well to red and green or to blue and yellow. Progressively higher values indicate progressively stronger degrees of color sensitivity. Many cells responded to all colors tested. In these cases the larger of the two ratios (red vs green or blue vs yellow) was taken to be the color index of the cell.

Histology and histochemistry. At the conclusion of each experiment the animal was deeply anesthetized and perfused through the heart with 700 ml of lactated Ringer's solution containing 0.1% heparin, followed by 1000 ml of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4, followed by 600 ml of lactated Ringer's solution containing 5% dextrose. After the perfusion the head was placed back in the stereotaxic apparatus and the brain was blocked parallel to the electrode tracks. The blocks containing the electrode tracks were stored for 2–4 d in 30% sucrose solution. Coronal sections that were parallel to the electrode tracks were cut using a freezing microtome. The sections were mounted on gelatinized slides and sections were stained for cytochrome oxidase. Every second section was lightly counterstained with thionin.

Cytochrome oxidase staining. Sections of striate cortex containing

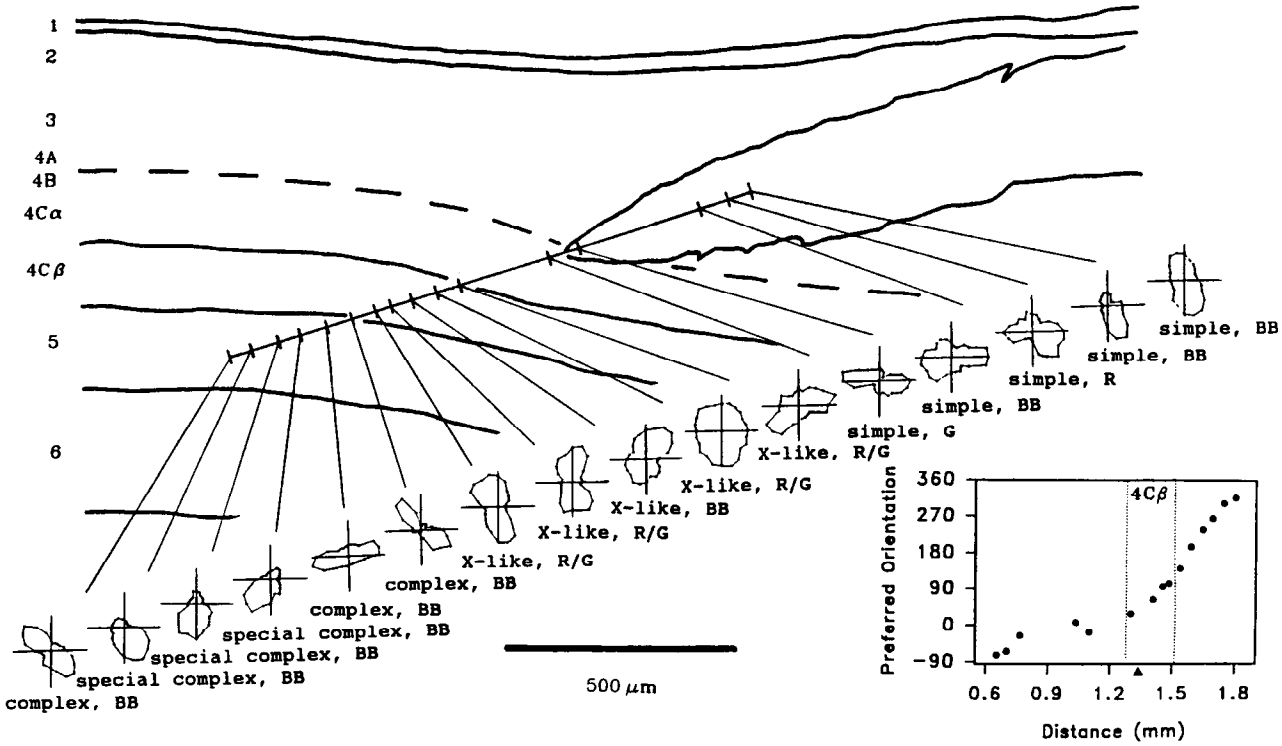

Figure 5. Reconstruction of an electrode penetrates through layers 2 and 3 of area 17 of the rhesus monkey. Conventions are as in Figure 4. In this figure, 0°, 180°, and 360° are horizontal, and 90° and 270° are vertical. The cell types and the color sensitivities of the cells encountered are shown. The *triangle below the abscissa in the lower scatter plot* indicates the only cell encountered in the penetrations that did not exhibit a statistically significant orientation bias. The rate of change in the preferred orientations of the cells in layer 4Cβ was consistent with the rate of change in the preferred orientations of the cells above and below them.

the electrode tracks were stained as outlined below and in Wong-Riley (1979).

(1) The animals were perfused with a mixture of 4% paraformaldehyde, 0.5% glutaraldehyde, and 4% sucrose in 0.1 M phosphate buffer (pH 7.4). The brain was removed, blocked, and left in the fixative for 1 hr or longer at 4°C. (2) The blocks were washed with several changes of the original buffer. The blocks were stored in 30% sucrose in 0.1 M phosphate buffer for 1–2 d until they sank. (3) Sections were cut with a freezing microtome at thicknesses between 75–100 μm. Sections were incubated at 37°C in the dark for 1–2 hr. The incubation medium was prepared freshly before use and consisted of 50 mg DAB, 90 ml 0.1 phosphate buffer, pH 7.4, 15–30 mg cytochrome C, type III (Sigma), and 4 gm sucrose. (4) The tissues were checked after 0.5–1 hr for the presence of light to dark brown reaction products. The incubation was ceased when there was a clear differentiation between highly reactive and nonreactive regions. At that point, the sections were rinsed in three changes of 0.1 M phosphate buffer.

Electrode track reconstruction. The position of the electrode track was identified by locating gliosis resulting from lesions made with the recording electrode. Since most of our penetrations lasted for 2 d, there was often also a thin line of gliosis along the penetration that was visible in the two or three sections containing the electrode tracks. In some experiments, the electrode tip was broken off at the end of the final penetration. The animal was then perfused with the electrode tip in place. In these cases the sections containing the electrode tracks were obvious during sectioning. In cases where the assignment of cells to CO-rich and -poor regions could be made, cells in layers 2 and 3 were divided into two groups. These were CO-rich regions (blobs) and CO-poor regions (interblobs) (Livingstone and Hubel, 1984).

Data analysis. Paired and unpaired *t* tests as well as the Mann–Whitney *U* test were used to compare distributions of biases. Also, several statistical techniques designed specifically to analyze distributions of angles (circular statistics) were used to help us interpret our data. A short description of each test is given below. A complete account of circular statistics can be found in Batschelet (1981).

The Rayleigh test determines if a distribution of angles differs significantly from a random distribution; that is, whether the angles are clustered about some value. If a certain angle is expected, then the V test is a more powerful test of whether a distribution of angles is peaked about the expected value. To determine if the mean of the sample of angles differs significantly from an expected angle, the confidence intervals given by Batschelet (1981) are used. Watson's U^2 test compares two distributions of angles (unimodal or multimodal) in order to determine whether the two samples differ significantly. High U^2 values result if the two distributions are different.

Results

The visual responses of 355 cells in layers 2, 3, and 4 of nine monkeys were studied. Cells subserved regions of retina from 1 to 15° from the fovea. Ninety percent of the cells subserved regions of retina from 2 to 10° from the fovea.

Orientation and direction biases of cells in layers 2 and 3

Figure 1 illustrates the orientation, direction, and spatial frequency tuning of typical cells recorded from layers 2 and 3. For each cell, the long axis of the polar plot illustrates the cell's orientation preference. The spatial frequency of the sinusoidal grating employed to generate each plot is shown, as is the degree of orientation and direction sensitivity. The spatial frequency tuning of each cell is also shown, as is the orientation of the sinusoidal grating used to generate each spatial frequency tuning curve.

Figure 2 shows the distribution of the orientation biases of all cells recorded from layers 2 and 3. It is evident from Figures 1 and 2 that the large majority of cells sampled (over 95%) from layers 2 and 3 exhibited a significant degree of orientation bias (≥0.1). Similarly, many cells exhibited statistically significant preferences for the direction of stimulus motion (Figs. 1, 3). In general, orientation sensitivity was clearest when the test stimuli

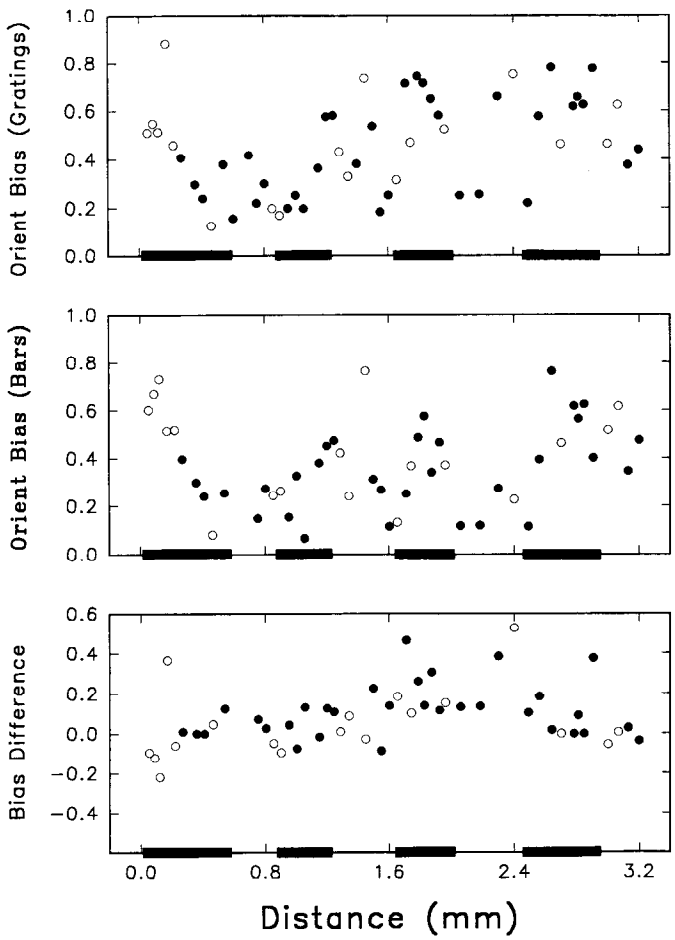

Figure 6. The orientation biases of color-sensitive (●) and broad-band cells (○) encountered along the penetration through layers 2 and 3 shown in Figure 4 (top). The biases elicited by drifting sinusoidal gratings (*top*) and moving bars (*middle*) are shown separately. The *thick bars* on the abscissa indicate regions that stained the most darkly for cytochrome oxidase. Although virtually all cells exhibited orientation biases that were statistically significant (bias ≥ 0.1), many cells exhibited lower biases when bars rather than gratings were the test stimulus. This was especially true for color-sensitive cells. The *bottom* scatter plot shows the difference in the orientation biases elicited by moving bars and gratings. Positive values indicate that the cells exhibited a stronger bias when sinusoidal gratings were the test stimuli; negative values indicate a stronger bias was exhibited when bars were the test stimulus.

were gratings of relatively high spatial frequency and direction sensitivity was clearest when gratings of relatively low spatial frequency were employed (see also Blasdel and Fitzpatrick, 1984; Hammond and Pomfret, 1990; Thompson et al., 1994). However, this is not always the case (Fig. 1; see also Hammond and Pomfret, 1990; Thompson et al., 1994). When the test stimuli were optimized to best reveal the cell's selectivity, the degree of orientation and direction sensitivity was observed to vary continuously among cortical cells. The distributions of the orientation and direction biases of cells in layers 2 and 3 were unimodal and consisted of cells exhibiting a wide range of selectivities (Figs. 2, 3).

Orientation and direction biases of cells in layer 4

As reported previously, layer 4 cells outside of layer 4Cβ exhibited clear orientation biases (Figs. 4–8; Blasdel and Fitzpatrick, 1984). Overall, the distribution of the orientation biases of layer 4 cells outside of layer 4Cβ did not differ from those of cells in layers 2 and 3. However, in contrast to previous reports, the present findings indicate that most cells in layer 4Cβ also exhibit a significant degree of orientation bias and some are direction biased (Figs. 1–3). As a group, layer 4Cβ cells exhibited much lower biases than did cells in the other layers (Figs. 1–3). Within layer 4Cβ the degree of selectivity varied among cells and the distributions of both the orientation and direction biases of layer 4Cβ cells were unimodal. As a result of the absence of very selective cells in layer 4Cβ, the range of orientation biases encountered was narrower than in the other layers.

Organized arrangement of orientation- and direction-sensitive cells

Figures 4–8 illustrate the results obtained from long penetrations through striate cortex made approximately parallel or at a shallow angle to the pial surface. In Figures 4, 5, and 7 the borders of the different layers and the types of cells encountered are indicated. The orientation and direction tuning of all cells studied are shown; the scatter plots below the penetrations show the preferred orientations of the successively encountered cells. The scatter plots shown in Figures 6 and 8 illustrate the orientation biases of each of the cells encountered along the penetrations shown in Figures 4 and 7.

These figures indicate that the large majority of cells studied exhibited significant orientation biases and the preferred orientations of the cells varied systematically along the penetrations (Figs. 4, 7). There was a weak tendency for adjacent cells to exhibit a similar degree of selectivity (Figs. 6, 8). However, areas of selective cells separated by regions containing unselective cells were not observed. In fact, the degree of selectivity of successively encountered cells within layers 2 and 3 could differ markedly. This was true whether bars or gratings were the test stimuli, although gratings elicited somewhat stronger orientation biases than bars (Figs. 6, 8). This difference was especially marked for color-sensitive cells (Fig. 6). The penetrations shown in Figures 4–8 also show the orientation and direction sensitivity of successively encountered cells in layer 4Cβ. These cells were much less selective than cells sampled from other layers in the same penetration. Nevertheless, the preferred orientations of layer 4Cβ cells changed gradually and systematically in a fashion consistent with the changes observed in the layers above and below them (Fig. 5).

Orientation and direction sensitivity of color and broad-band cells

Figure 1 shows the orientation and direction tuning of typical color-sensitive and broad-band cells in layers 2 and 3. Figures 2 and 3 show separately the distributions of the orientation and direction biases of color-sensitive and broad-band cells in these layers. The locations of the color-sensitive and broad-band cells encountered in layers 2, 3, and 4 are shown in the penetrations illustrated in Figures 4, 5, and 7. The locations of cytochrome oxidase–rich and cytochrome oxidase–poor regions are also illustrated in Figures 4 and 6.

Overall, color-sensitive and broad-band cells in layers 2 and 3 exhibited the same degree of orientation sensitivity when tested with drifting sinusoidal gratings of the appropriate spatial frequency (Fig. 2). As was the case for orientation and direction sensitivity, color sensitivity varied continuously among cortical

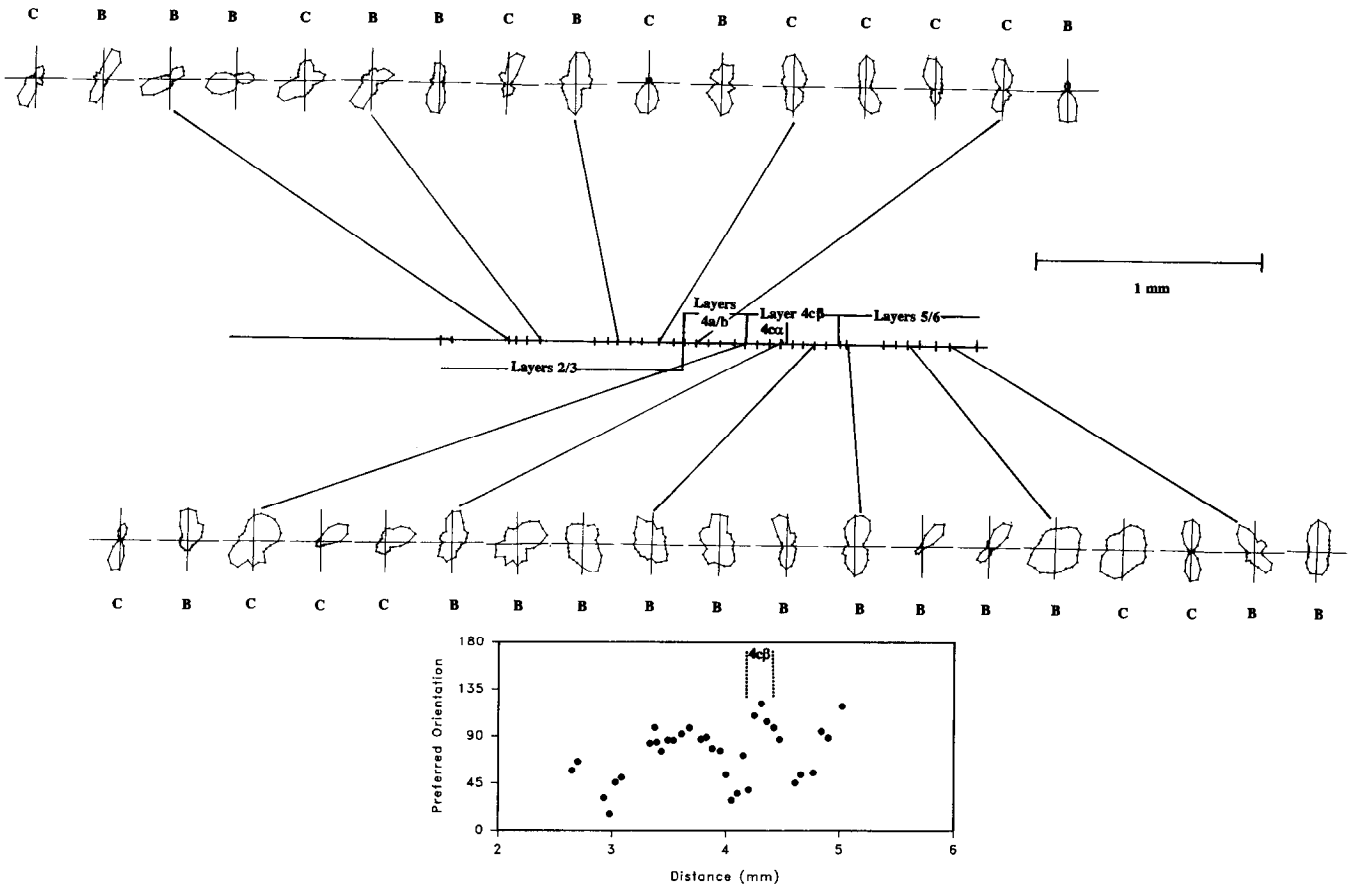

Figure 7. Reconstruction of an electrode penetration through layers 2–6 of area 17 of the rhesus monkey. The polar plots illustrate the orientation and direction tuning of the units encountered. Conventions are as in Figure 4. Notice that, in general, preferred orientation changed gradually and systematically along both penetrations. The rate of change in the preferred orientations of the cells in layer 4Cβ was consistent with the rate of change in the preferred orientations of the cells above and below them. Cells in layer 4Cβ exhibited lower biases than cells in other layers.

cells (Fig. 9; see also Lennie et al., 1990). There was no evidence for distinct populations of cells sensitive to orientation but not color and vice versa. Moreover, reconstructions of long penetrations approximately parallel or at a shallow angle to the pial surface (Figs. 4, 5, 7) provided no evidence for clusters of cells selective for color but not orientation nor for regions selective for orientation but not color. Studies employing cytochrome oxidase staining revealed light and dark regions that did not correlate well with the properties studied (Figs. 4–6). These findings are consistent with the observation that there was no clear relationship between a cell's degree of orientation sensitivity when tested with drifting sinusoidal gratings and its responses to colored stimuli. Figure 9 shows the orientation biases of cells exhibiting different degrees of color sensitivity. Notice that there is no correlation (R^2 = 0.004).

Properties of cells within cytochrome oxidase–rich and cytochrome oxidase–poor regions

Histograms illustrating the orientation, direction, and color sensitivities of cells within CO-rich and CO-poor regions are shown in Figure 10. In both regions cells exhibited varying degrees of orientation, direction, and color specificity. No significant relationships between cytochrome oxidase staining and the properties of the cells were observed (Fig. 10).

Differential responses of color-sensitive and broad-band cells to grating and bar stimuli

The foregoing findings appear to be different from those of other workers who report that cells in layer 4Cβ are not orientation sensitive (Hubel and Wiesel, 1968; Blasdel and Fitzpatrick, 1984; Livingstone and Hubel, 1984) and that layers 2 and 3 contain regions of color-sensitive, unoriented cells separated by regions of broad-band, orientation-sensitive cells (Livingstone and Hubel, 1984). A possible reason for the apparent disagreement is that other workers used mainly moving bars, not drifting sinusoidal gratings, as the test stimuli. To see if this was the case, we reanalyzed our results when moving bars, not drifting sinusoidal gratings, were used as the test stimuli. In this analysis the size and velocity of the moving bars were optimized to best reveal the selectivity of the cells studied.

The results of this analysis confirmed that the large majority of both color-sensitive and broad-band cells in layers 2, 3, and 4Cβ exhibited statistically significant orientation preferences. However, color-sensitive cells in layers 2 and 3 were less orientation sensitive (mean bias = 0.29) than broad-band cells (mean bias = 0.38) when bars were the test stimuli (Fig. 11). This difference is significant ($p < 0.01$, Mann–Whitney U test). It is noteworthy that orientation bias relates nonlinearly with response ratio (see Materials and Methods). As a result, in layers 2 and 3 the average color-sensitive cell and broad-band cell re-

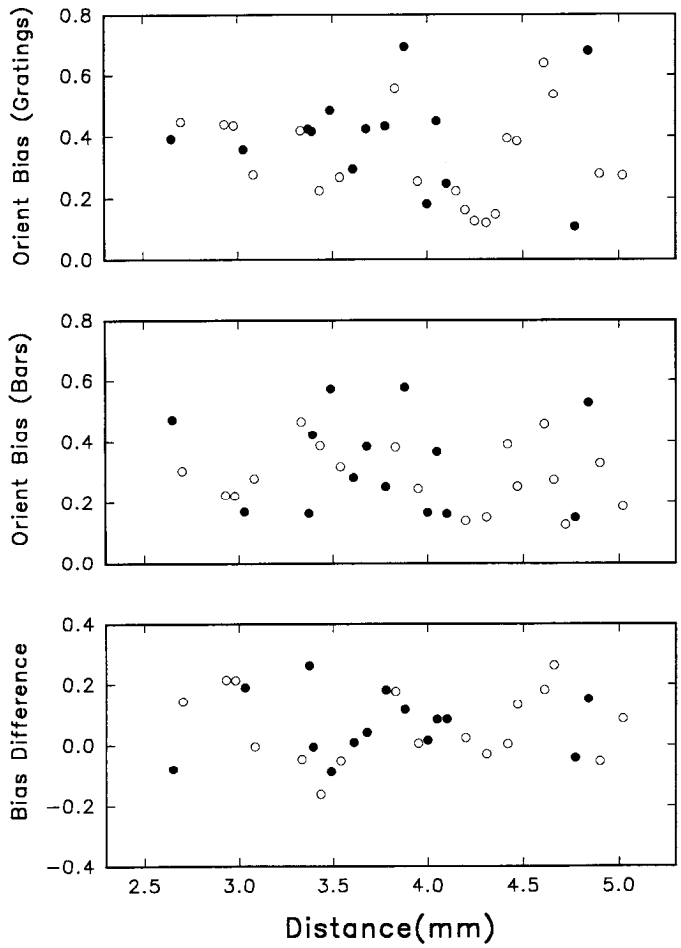

Figure 8. The orientation biases of color-sensitive and broad-band cells encountered along the penetration shown in Figure 7. The biases elicited by drifting sinusoidal gratings (*top*) and moving bars (*middle*) are shown separately. The differences in the orientation biases elicited by moving bars and drifting sinusoidal gratings are also shown (*bottom*). Positive values indicate that gratings elicited stronger orientation biases than did bars. Notice that regions of orientation-sensitive cells separated by regions of unoriented cells were not observed regardless of the test stimuli.

sponded about 3.5 and 5.6 times better, respectively, to the preferred than to the nonpreferred orientation when bars were the test stimuli. The relatively weak orientation sensitivity of cells in layer 4Cβ also appeared much weaker ($p < 0.001$, Mann–Whitney U test) when bars were the test stimuli.

Spatial frequency tuning of color-sensitive and broad-band cells

Striate cortical cells exhibit a range of preferred spatial frequencies. A number of studies have provided evidence that there is a weak relationship between spatial frequency tuning and cytochrome oxidase staining (Tootell et al., 1988; Silverman et al., 1989; Born and Tootell, 1991; DeBruyn et al., 1993). In this study, the spatial frequency tuning of cells within and outside of cytochrome oxidase–rich regions was also investigated. No clear differences in the cutoff spatial frequency of cells in the two regions were observed when the cells were studied using optimally oriented and directed sinusoidal gratings.

Cortical cells in layers 2, 3, and 4 exhibit the clearest orientation biases when tested with gratings of a relatively high spatial frequency. In view of this, the distributions of optimal spatial frequencies of color-sensitive and broad-band cells in layers 2 and 3 were compared. The results of this analysis showed that, as a group, color-sensitive cells exhibited significantly lower optimal spatial frequencies than did broad-band cells ($p < 0.001$, Mann–Whitney U test).

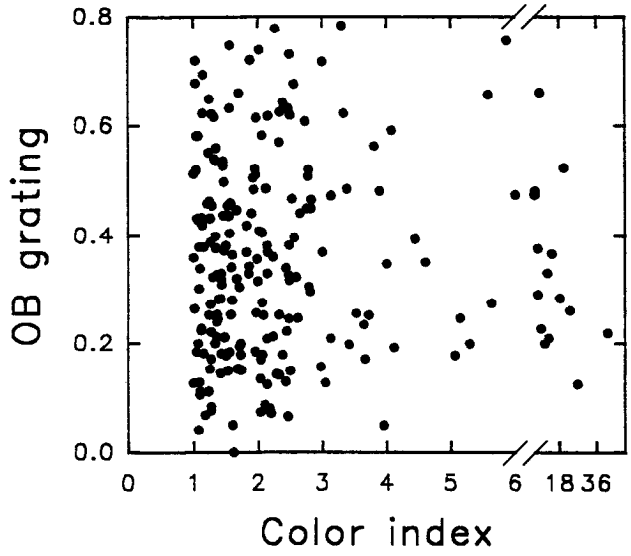

Figure 9. The orientation biases of cells exhibiting different degrees of color sensitivity. The color index (see Materials and Methods) provides a means of illustrating which cells were insensitive to wavelengths (index = 1.0) and which exhibited different degrees of color sensitivity (indexes > 1.0).

The foregoing findings combined with the observation that most cortical cells appear to be less orientation sensitive when tested with sinusoidal gratings of relatively low spatial frequency (Fig. 1; Hammond and Pomfret, 1990; Thompson et al., 1994) can explain why color-sensitive cells appear less orientation sensitive than broad-band cells in response to moving bars. In particular, a bar or edge stimulates a cell over its full range of spatial frequencies but a sinusoidal grating does not. As a result, cells that respond better to low spatial frequencies such as LGNd cells, layer 4Cβ cells, and color-sensitive cells in layers 2 and 3 tend to appear less orientation sensitive in response to bar stimuli than to sinusoidal gratings of the appropriate spatial frequency (Thompson et al., 1994). The reason for this is that the responses of these cells are especially dependent upon the relatively unoriented, low spatial frequency component of bar stimuli. Thus bars are not the stimuli of choice if one wishes to reveal the cell's orientation preference. In contrast, broad-band cells in layers 2 and 3 do not respond as well to low spatial frequencies, and thus appear more orientation sensitive to bar stimuli. These cells are more dependent upon the relatively orientation-sensitive, high spatial frequency component of bar stimuli. The orientation and spatial frequency tuning curves of the color-sensitive and broad-band simple and complex cells in Figure 1, for example, show that color-sensitive cells responded better than broad-band cells to the lowest spatial frequencies tested.

Discussion

This study provides evidence that cells selective concomitantly for orientation, direction, and color are common in layers 2, 3, and 4Cβ of visual cortex. Discrete regions in layers 2 and 3

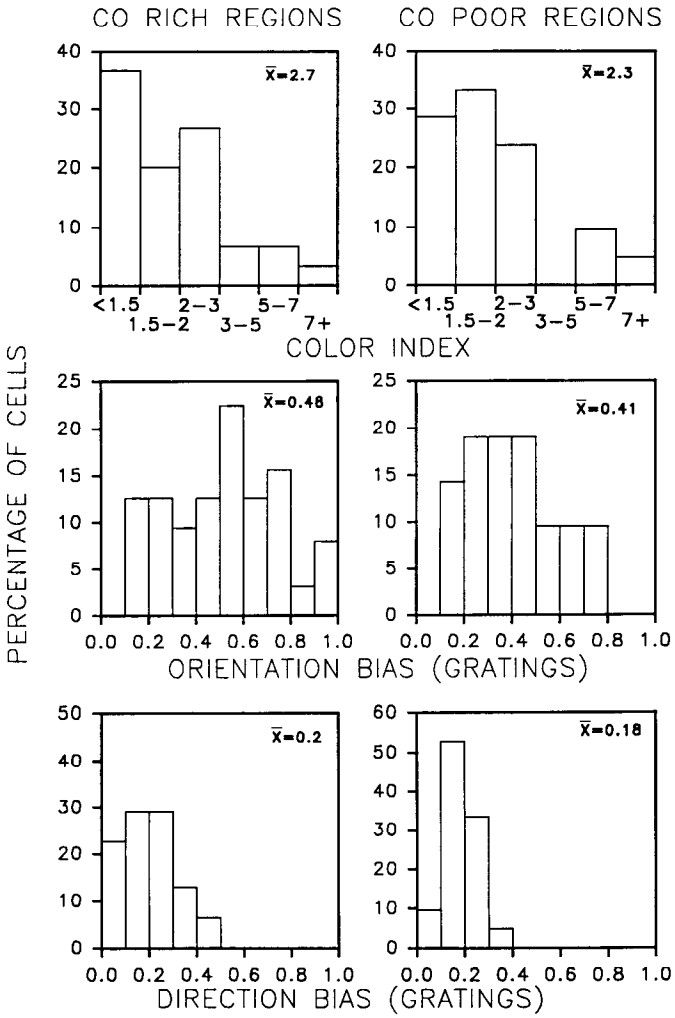

Figure 10. Histograms illustrating the distributions of the orientation, direction, and color sensitivities of cells in layers 2 and 3 that could be assigned to locations within CO-rich and CO-poor regions. The mean for each distribution is illustrated. Orientation and direction bias and color index are defined in Materials and Methods.

containing cells that are color sensitive but not orientation sensitive separated by regions containing cells that are orientation sensitive but not color sensitive were not encountered. The distributions of cells sensitive to orientation, direction, and color were not obviously related to the distributions of CO-rich and CO-poor regions.

Technical considerations

Our finding that cells sensitive to orientation, direction, and color are not segregated in layers 2 and 3 underscores the need to carefully quantify and statistically analyze these properties. Deciding subjectively when a cell is selective enough to be considered oriented, directed, or color sensitive is inappropriate since these properties vary continuously among LGNd and cortical cells (see also Derrington et al., 1984; Lennie et al., 1990).

The differences in the responses of cortical cells to bars and sinusoidal gratings must also be taken into account in any attempt to understand cortical function since the orientation and direction sensitivities of cells throughout the retinogeniculocortical pathways are spatial frequency dependent (Hammond 1973, 1974; Levick and Thibos, 1980, 1982; Shou and Leventhal,

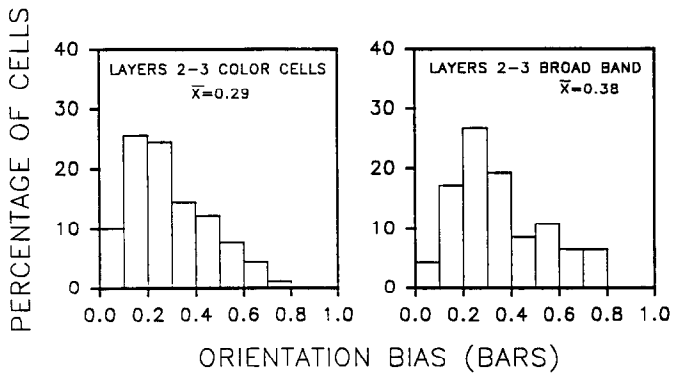

Figure 11. Histogram illustrating distribution of orientation biases of color-sensitive and broad-band cells in layers 2 and 3 studied with moving bars. The mean biases of color-sensitive cells (0.29) and broad-band cells (0.38) correspond to preferred orientation to nonpreferred orientation response ratios of about 3.5:1 and 5.6:1, respectively.

1989; Smith et al., 1990; Soodak et al., 1987; Hawken et al., 1988; Hammond and Pomfret, 1990). Deciding subjectively which stimulus is the one of choice in order to test selectivity and infer function seems inappropriate since a cell's selectivity can appear quite different depending upon which stimulus is employed. Indeed, some of the differences between this and previous reports (discussed below) are likely to reflect differences among experimenters in their choices of stimuli and their subjective impressions of what degree of stimulus selectivity is meaningful.

Relation to previous work

The receptive field properties of cells in layers 2, 3, and 4 have been the subject of a number of studies. Hubel and Wiesel (1968) found that most cells in layers 2 and 3 were orientation sensitive and many were direction sensitive. Most cells in layer 4C were found not to be orientation or direction sensitive. The orientation and direction sensitivities of striate cortical cells were described quantitatively by Schiller et al. (1976) and DeValois et al. (1982). These authors also found that the large majority of cells outside of layer 4 were orientation sensitive and many were direction sensitive. Blasdel and Fitzpatrick (1984) studied the specificity of layer 4 cells in more detail and confirmed a lack of orientation and direction sensitivity among layer 4Cβ cells but reported the presence of orientation sensitivity of cells in other parts of layer 4.

The chromatic properties of cells in layers 2, 3, and 4 were also reported by Hubel and Wiesel (1968). Many subsequent workers have confirmed the presence of cells in these layers that are sensitive to wavelength. Lennie et al. (1990) studied the chromatic properties of cells in monkey striate cortex in detail. They concluded that many cells in the upper layers responded selectively to chromatic stimuli and that the degree of chromatic sensitivity varied continuously among cortical cells. Thus, cells could not easily be classified as purely color sensitive or purely broad band.

Livingstone and Hubel (1984, 1987, 1988) also studied the chromatic and spatial properties of cells in layers 2, 3, and 4 of monkey striate cortex. They reported that layers 2 and 3 contain regions of color-sensitive, unoriented cells separated by regions of broad-band, orientation-sensitive cells. Many cells in layer 4Cβ were reported to be color opponent but not orientation sensitive. Ts'o and Gilbert (1988) reported similar results. The func-

tionally distinct regions in layers 2 and 3 were reported to correlate with the presence (color-sensitive, unoriented cells) or absence in layers 2 and 3 (broad-band, oriented cells) of cytochrome oxidase–rich regions (Livingstone and Hubel, 1984, 1988). Functionally distinct regions of area 17 were reported to project to functionally distinct regions of area 18 exhibiting corresponding properties (Livingstone and Hubel, 1988).

The present findings are consistent with the work of Hubel and Wiesel (1968) and the quantitative studies of Schiller et al. (1976) and DeValois et al (1982). In this study, distinct regions of unoriented cells were not encountered. The present findings are also consistent with the work of Lennie et al. (1990), since our results support the idea that stimulus selectivity varies continuously among cortical cells.

A number of studies have provided evidence that there is at least some segregation of cells preferring different spatial frequencies in visual cortex (Tootell et al., 1988; Born and Tootell, 1991; DeBruyn et al., 1993). The segregation does not appear to be strict (see Born and Tootell, 1991). Moreover, cells in cytochrome-rich regions in the macaque are reported to prefer relatively low spatial frequencies while those in cytochrome-rich regions in bush babies (*Galago crassicaudatus*) are reported to prefer relatively high spatial frequencies (DeBruyn et al., 1993). The present results did not reveal a clear relationship between spatial frequency tuning and cytochrome oxidase staining, although color-sensitive cells in layers 2 and 3 did tend to respond relatively better to low spatial frequencies than did broad-band cells.

One aspect of our findings can, however, shed some light on the differences among studies of the orientation, direction, and color sensitivity of cells in striate cortex. In particular, color-sensitive cells appear less sensitive to orientation than do broad-band cells when bars are the test stimulus. This difference is not evident when gratings are the test stimulus. Since most previous investigations of cells in layers 2 and 3 employed moving bars, not sinusoidal gratings, it is understandable that color-sensitive cells appeared less sensitive to orientation than did broad-band cells. Previous studies of cells in layers 4Cβ also used mainly bars as the test stimuli. This can explain why the orientation sensitivity of these cells has been overlooked and/or viewed as too weak to be meaningful.

Are form, color, and motion segregated in visual cortex?

The present findings do not support the idea, in its simplest form, that cells subserving form, motion, and color are segregated in layers 2 and 3 (Livingstone and Hubel, 1988). Such a model requires that most V1 cells exhibit pronounced selectivity for one stimulus attribute such as color but not another such as orientation. In fact, it appears that most cells in layers 2 and 3 are selective concomitantly for aspects of form, motion, and color. Moreover, the stimulus specificities of V1 cells vary continuously amongst cells. Thus, cells exhibiting the sort of stimulus selectivity required by the Livingstone and Hubel model do not exist in significant numbers in monkey striate cortex (see also Lennie et al., 1990).

A number of other lines of evidence also do not support the strict, parallel processing of form, motion, and color. For example, Zipser et al. (1994) reported that many V1 cells can signal aspects of depth, orientation, and color. A carefully done study by DeBruyn et al. (1993) in bush babies (*Galago crassicaudatus*) employed sinusoidal gratings to study the orientation sensitivity of cells in different layers as well as in CO-rich and CO-poor regions. These authors report that nearly all cells in CO-rich regions, CO-poor regions, and throughout layer IV are orientation sensitive. DeBruyn et al. (1993) did provide evidence that there were some differences in the properties of cells within and outside of CO-rich regions in bush babies. However, these properties did not relate in any simple way to the parallel LGN pathways hypothesized to give rise to the parallel processing of form, motion, and color in striate cortex (reviewed in LeVay and Nelson, 1991).

Recent studies of extrastriate cortex also do not support the idea that there is a strict segregation of cells signaling form, motion, and color. For example, cells in area V2 of macaque cortex exhibit properties similar to those of the cells we observed in layers 2 and 3, and cytochrome oxidase staining does not correlate well with the receptive field properties of V2 cells (Kiper et al., 1994). The strict parallel processing of color and motion by areas V4 and MT, respectively, is also in doubt (Maunsell et al., 1990; reviewed in Martin, 1992; Schiller, 1994).

It should be noted that the present results are not completely inconsistent with the idea that different cells signal form and color in monkey striate cortex. Color-sensitive cells do appear less orientation sensitive than broad-band ones when bars are the test stimuli. Thus, while the present results do not support a straightforward segregation of cortical cells according to orientation, direction, and color sensitivity, it remains possible that a parcellation of function by different cells in V1 will prove to be correct once additional information is obtained.

Conclusion

Our results do not support the idea that different cells in V1 subserve form, motion, and color. Nevertheless, cytochrome oxidase–rich and -poor regions are present in V1 of a variety of primate species, including nocturnal ones without significant color vision, such as *Galago* (DeBruyn et al., 1993). It is tempting to speculate that the heterogeneous distribution of cytochrome oxidase in visual cortex is functionally meaningful. However, it now appears additional work is needed to determine its significance.

References

Batschelet E (1981) Circular statistics in biology. New York: Academic.

Blasdel GG, Fitzpatrick D (1984) Physiological organization of Layer 4 in macaque striate cortex. J Neurosci 4:880–895.

Born RT, Tootell RBH (1991) Spatial frequency tuning of single units in macaque supragranular striate cortex. Proc Natl Acad Sci USA 88: 7066–7070.

Casagrande VA, Norton TT (1991) Lateral geniculate nucleus: a review of its physiology and function. In: The neural basis of visual function (Leventhal AG, ed), pp 41–84. London: Macmillan.

DeBruyn EJ, Casagrande VA, Beck PD, Bonds AB (1993) Visual resolution and sensitivity of single cells in the primary visual cortex (V1) of a nocturnal primate (bush baby): correlations with cortical layers and cytochrome oxidase patterns. J Neurophysiol 69:3–18.

Derrington AM, Krauskopf J, Lennie P (1984) Chromatic mechanisms in lateral geniculate nucleus of macaque. J Physiol (Lond) 357:241–265.

DeValois RL, Yund EW, Hepler N (1982) The orientation and direction selectivity of cells in macaque visual cortex. Vision Res 22:531–544.

Dreher B, Fukuda Y, Rodieck RW (1976) Identification, classification and anatomical segregation of cells with X-like and Y-like properties in the lateral geniculate nucleus of old-world monkeys. J Physiol (Lond) 258:433–452.

Ferrera VP, Nealy TA, Maunsell JHR (1992) Mixed parvocellular and magnocellular geniculate signals in visual area V4. Nature 358:756–758.

Gilbert GD (1977) Laminar differences in receptive field properties of cells in cat primary visual cortex. J Physiol (Lond) 268:391–421.
Hammond PW (1973) Is retinal receptive field shape systematically related to retinal location? J Physiol (Lond) 234:64P–66P.
Hammond PW (1974) Cat retinal ganglion cells: size and shape of receptive field centres. J Physiol (Lond) 242:99–118.
Hammond P, Pomfret CJD (1990) Influence of spatial frequency on tuning and bias for orientation and direction in the cat's striate cortex. Vision Res 30:359–369.
Hawken MJ, Parker AJ, Lund JS (1988) Laminar organization and contrast sensitivity of direction-selective cells in the striate cortex of the old world monkey. J Neurosci 8:3541–3548.
Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol (Lond) 160:106–154.
Hubel DH, Wiesel TN (1968) Receptive fields and functional architecture of monkey striate cortex. J Physiol (Lond) 195:215–243.
Kiper DC, Gegenfurtner KR, Fenstemaker SB (1994) Representation of color in macaque V2. ARVO Abstr 35:1972.
Lennie P, Krauskopf J, Sclar G (1990) Chromatic mechanisms in striate cortex of macaque. J Neurosci 10:649–669.
LeVay S, Nelson SB (1991) Columnar organization of the visual cortex. In: Vision and visual dysfunction. The neural basis of visual function, Vol 4 (Leventhal AG, ed), pp 266–315. London: Macmillan.
Leventhal AG, Schall JD (1983) Structural basis of orientation sensitivity of cat retinal ganglion cells. J Comp Neurol 220:465–475.
Levick WR, Thibos LN (1980) Orientation bias of cat retinal ganglion cells. Nature 286:389–390.
Levick WR, Thibos LN (1982) Analysis of orientation bias in cat retina. J Physiol (Lond) 329:243–261.
Livingstone MS, Hubel DH (1984) Anatomy and physiology of a color system in the primate visual cortex. J Neurosci 4:309–356.
Livingstone MS, Hubel DH (1987) 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 MS, Hubel DH (1988) Segregation of form, color, movement, and depth: anatomy, physiology, and perception. Science 240: 740–749.
Martin KAC (1992) Parallel pathways converge. Curr Biol 2:555–557.
Maunsell JHR, Nealy TA, DePriest DD (1990) Magnocellular and parvocellular contributions to responses in the middle temporal visual area (MT) of the macaque monkey. J Neurosci 10:3323–3334.
Orban GA (1984) Neuronal operations in the visual cortex. In: Studies of brain function, Vol 8 (Barlow HB, Bullock TH, Florey E, Grusser OJ, Peters A, eds). New York: Springer.
Schall JD, Perry VH, Leventhal AG (1986) Retinal ganglion cell dendritic fields in old-world monkeys are oriented radially. Brain Res 386:18–23.
Schiller PH (1994) Area V4 of the primate visual cortex. Curr Dir Psychol Sci 3:89–92.
Schiller PH, Finlay BH, Volman SF (1976) Quantitative studies of single-cell properties in monkey striate cortex. I. Spatiotemporal organization of receptive fields. J Neurophysiol 39:1288–1319.
Shapley R, Kaplan E, Soodak R (1981) Spatial summation and contrast sensitivity of X and Y cells in the lateral geniculate nucleus of the macaque. Nature 292:534–545.
Shou T, Leventhal AG (1989) Organized arrangement of orientation sensitive relay cells in the cat's dorsal lateral geniculate nucleus. J Neurosci 9:4287–4302.
Silverman MS, Grosof DH, DeValois RL, Elfar SD (1989) Spatial-frequency organization in primate striate cortex. Proc Natl Acad Sci USA 86:711–715.
Smith EL III, Chino YM, Ridder WR III, Kitigawa K, Langston A (1990) Orientation bias of neurons in the lateral geniculate nucleus of macaque monkeys. Vis Neurosci 5:525–545.
Soodak RE, Shapley RM, Kaplan E (1987) Linear mechanism of orientation tuning in the retina and lateral geniculate nucleus of the cat. J Neurophysiol 58:267–275.
Thibos LN, Levick WR (1985) Orientation bias of brisk transient y-cells of the cat retina for drifting and alternating gratings. Exp Brain Res 58:1–10.
Thompson KG, Shou T, Zhou Y, Leventhal AG (1989) Orientation sensitivity of relay cells in the cat lateral geniculate nucleus. Soc Neurosci Abstr 15:175.
Thompson KG, Leventhal AG, Zhou Y, Liu D (1994) Stimulus dependence of orientation and direction sensitivity of cat LGNd relay cells without cortical inputs: a comparison with area 17 cells. Vis Neurosci 11:939–951.
Tootell RBH, Silverman MS, Hamilton SL, Switkes E, DeValois RL (1988) Functional anatomy of macaque striate cortex. V. Spatial frequency. J Neurosci 8:1610–1624.
Ts'o DY, Gilbert CD (1988) The organization of chromatic and spatial interactions in the primate striate complex. J Neurosci 8:1712–1727.
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.
Wörgötter F, Eysel U (1987) Quantitative determination of orientation and directional components in the response of visual cortical cells to moving stimuli. Biol Cybern 57:349–355.
Wörgötter F, Grundel O, Eysel UT (1990) Quantification and comparison of cell properties in cat's striate cortex determined by different types of stimuli. Eur J Neurosci 2:928–941.
Zar JH (1974) Circular distributions. In: Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall.
Zipser K, Lee TS, Lamme VAF, Schiller PH (1994) Invariance of figure-ground segregation mechanisms in V1 for depth, orientation, luminance, and chrominance cues. ARVO Abstr 35:1973.