A hierarchy of the functional organization for color, form and disparity in primate visual area V2

Daniel Y. Ts’o a,*, Anna Wang Roe b, Charles D. Gilbert c

a SUNY Health Science Center, 750 East Adams Street, Syracuse, NY 13210, USA
b Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06511, USA
c The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Abstract

By combining optical imaging, single unit electrophysiology and cytochrome oxidase (CO) histology, we sought to reveal in greater detail the functional organization within the CO stripes of visual area V2 of primates. To visualize the disparity selective regions of V2, the imaging of binocular interaction was employed. These imaging maps guided single unit penetrations that then revealed a columnar organization for disparity. Our studies also showed a pattern of intermixing between the color and disparity pathways of V2, including the existence of single cells tuned for both color and disparity. While previous studies have suggested that the CO stripes of V2 constitute the fundamental organizational unit within V2, our results show a further level of organization consisting of functionally distinct subcompartments, 0.7–1.5 mm in diameter, within individual stripes. These subcompartments, which are not clearly revealed by CO histochemistry, lie within each of the thin, pale, and thick CO dense stripes in V2 and are specific for aspects of color, orientation and retinal disparity, respectively. The present results favor an architectural view of V2, not unlike that of V1, as a collection of functionally distinct subcompartments or modules situated within each of the V2 stripes. These modules also support the notion that for each cortical area (e.g. V1, V2, V4), there exists a stereotyped cortical module with a geometry that is characteristic for each area. These modules exist as a middle tier in a hierarchy of functional organization within V2. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Functional architecture; Visual cortex; Optical imaging; Cytochrome oxidase; Columnar organization; Color vision; Stereopsis

1. Introduction

Within the visual modality, the attributes of form, color, depth and motion are thought to be analyzed by distinct populations of cells, and processed along separate, parallel pathways. In several visual cortical areas, the submodalities of vision are segregated into discrete compartments, which can be identified by histochemical staining for the mitochondrial enzyme cytochrome oxidase (CO) (Wong-Riley, 1979; Horton & Hubel, 1981; Horton, 1984). In the primary visual cortex of the primate (area V1), for example, cells found in the patches of CO staining known as ‘blobs’ are often selective for color, while cells selective for stimulus orientation (and therefore involved with the processing of form vision) are found in the interstices between the blobs (Livingstone & Hubel, 1984; Ts’o & Gilbert, 1988).

In the second visual area of the primate (area V2), the segregation and compartmentalization of cells processing form, color and depth information, is evident as a series of large (~1.3 mm wide) stripes seen CO histology. Two types of alternating CO-rich stripes have been described, the thick and thin stripes that are separated by a third type of lightly stained stripe, the pale stripe (Tootell & Hamilton, 1989). Electrophysiological and anatomical studies exploring the functional correlates of this striped pattern of staining have suggested a tripartite organization of visual processing in V2, wherein the thick stripes contain cells selective for disparity and motion, the thin stripes, cells selective for color, and the pale stripes, cells selective for orientation (DeYoe & Van Essen, 1985; Shipp & Zeki, 1985; Hubel & Livingstone, 1987). The cytochrome oxidase staining of an individual stripe, though, is not homogeneous but rather consists of a collection of lighter and darker patches that overall form the V2 stripe (Tootell &
Hamilton, 1989; Wong-Riley et al., 1993). A similar patchiness has also been observed in anatomical studies of V2 connectivity (Levitt, Yoshioka, & Lund, 1994; DeYoe, Felleman, Van Essen, & McClendon, 1994; Malach, Tootell, & Malonek, 1994). These observations suggested an underlying substructure or series of sub-compartments within the V2 stripes.

To explore the functional properties of these sub-compartments in the V2 stripes, we combined conventional single-unit electrophysiology with in vivo optical imaging (Ts'o, Frostig, Lieke, & Grinvald, 1990) of area V2 in the macaque. A key strength of using the optical imaging technique is that it allows one to visualize the functional compartments in the intact animal, allowing detailed study of the functional characteristics of the various compartments by subsequent electrode penetrations targeted to selected sites within or at the boundaries of compartments. This strategy eliminates the need to rely heavily on postsilent CO histology to locate the positions of the recording sites relative to the V2 stripes, as most previous electrophysiological studies have done. In addition, these previous studies primarily utilized tangential electrode penetrations traversing the stripes to maximize the opportunities for recording from several neighboring stripes (DeYoe & Van Essen, 1985; Hubel & Livingstone, 1987), and thus did not provide information on the fine structure of columnar functional organization of properties within individual stripes. The optical imaging results of area V2 reveals a heterogeneity and a compartmentalization on a smaller scale than the CO stripes, and subsequent electrophysiology shows the functional specificity of these sub-compartments. By optically imaging the cortical surface to localize the V2 stripes just prior to the single-unit recording sessions, we were able to place vertical electrode penetrations in known positions relative to the V2 stripes, and more closely study the functional heterogeneity within single stripes.

2. Methods

Ten young adult monkeys, Macaca fascicularis, were used in these studies, as well as in studies not reported here. All experimental procedures were conducted in accordance with protocols approved by the Rockefeller Animal Care and Use Committees and adhere to federal and NIH guidelines. The monkeys were initially anesthetized with ketamine HCL (10 mg/kg, I.M.) followed by sodium thiopental (20 mg/kg, I.V. supplemented by a constant infusion of 1–2 mg/kg/h). The animal was then either cannulated through a tracheotomy or intubated with an endotracheal tube, paralyzed with vecuronium bromide (0.1 mg/kg/h) and artificially resiredated. The EKG, EEG, temperature and expired CO2 were monitored throughout the entire experiment. A retinoscope was used to determine the appropriate contact lenses to focus the eyes on a color video monitor or tangent screen 1.5 m from the animal. The positions of the fovea of each eye were plotted on the screen with the aid of a fundus camera (Nikon). A hole above the visual cortex was made in the skull and a stainless steel chamber cemented over the hole. After the dura was opened, the chamber was sealed with a glass cover plate and filled with silicone oil. This sealed chamber minimized the movement of the cortex due to heartbeat and respiration. In some experiments, the cortex was covered with agar and a glass coverslip instead of the sealed chamber arrangement. The actual position of the visual field representation of the region of visual cortex under study was determined electrophysiologically and was in the eccentricity range of 2–5 deg parafoveal. The receptive field position and properties of single units were studied with slits of light from a hand-held projector. This recording arrangement was also used to perform electrophysiological confirmations of the functional maps obtained with optical imaging. The intrinsic signal imaging methods used have been described previously (Ts'o et al., 1990) and employed a slow scan 12-bit CCD camera (Photometrics) acquiring frames under 630 nm illumination. Imaging data were normalized with a ‘cocktail’ blank, computed from the sum of all the stimulated conditions. Normalized images from a given stimulus condition were then subtracted from an opponent condition (e.g. right from left eye stimulation for ocular dominance, luminance from red/green isoluminance for color preferring regions). In the case of imaging for general activation, the blank condition image data was subtracted from the summed images collected during the presentation of luminance gratings of a range of spatial frequencies and orientations.

Visual stimuli were presented on a Barco color monitor, either with the eyes converged using a Risley prism, or in the case of disparity stimuli, dichoptically with a split screen arrangement. During stimulus conditions involving monocular presentations, the visual stimuli was gated with the aid of electromechanical shutters (Uniblitz) in front of each eye. Most visual stimuli consisted of moving sine gratings of low (0.2–1 cpd) and medium (2–5 cpd) spatial frequency with temporal frequencies in the range of 1–5 Hz. Luminance gratings were near 100% contrast with a maximum luminance of 80 cd/m².

The isoluminant chromatic gratings were calibrated with the aid of a spectrophotometer (Photo Research) and based on the cone spectral data of Smith and Pokorny. Modulations in both the red/green and trianoptic confusion axes were employed. The mean luminance for these gratings was 30 cd/m². In sessions intended to compare responses to isoluminant chromatic gratings and luminance gratings, the spatial and
temporal frequencies of the gratings were the same for each set of grating conditions, and in the range of 0.2–0.5 cpd and 1–3 Hz.

Single unit recordings were performed with standard extracellular techniques using tungsten/glass electrodes. All the electrode penetrations for this study were made as close to perpendicular to the cortical surface as possible. Agar was placed over the cortex to minimize pulsations. Spike signals were fed through a window discriminator and collected by a PC computer for the construction of histograms and tuning curves. Cells were considered to be disparity sensitive if they exhibited a modulation in their firing rate of at least 2:1 as retinal disparity was varied. Disparity cells were classified according to type (far, near, tuned excitatory, tuned inhibitory) and peak (or trough) disparity (Poggio & Fischer, 1977). Special care was taken to ensure the stability of the eyes, particularly when studying disparity, by recording from a reference electrode placed close to the foveal representation of V1 and isolating a reference binocular V1 cell. In addition to the periodic referral to the fields of the V1 reference cell, contact lens were outfitted with a tiny mirror off which was reflected a laser pointer that provided a clear indication of any movement of the eyes.

During some recording sessions, stimuli were presented with a handheld projector capable of producing spots and bars of varying sizes, and filtered using a range of 30nm bandwidth interference filters spanning 450–630 nm, adjusted for rough equi-radiance. Recording positions were marked with electrolytic lesions. In some experiments, anatomical tracers, fluorescent latex beads (Lumafluor), were pressure-injected to study the connectivity at particular recording sites. At the end of data collection, animals were then deeply anesthetized with an intravenous dose of sodium thiopental (100 mg/kg) and perfused transcardially with 4% paraformaldehyde. Following extraction of the brain, the relevant cortical region was removed, flattened and immersed in 30% sucrose solution. The cortical tissue was then sectioned tangentially at 30μm and sections were reacted for cytochrome oxidase (CO) histochemistry (Wong-Riley, 1979). CO histology was used in conjunction with lesion, tracer and vasculature information to reconstruct and confirm the optical imaging and single-unit recording maps.

3. Results

3.1. The alternation of color and disparity dark stripes in V2

We began each experiment by using a slow scan CCD (charge-coupled device) camera to acquire an image of the cortical surface and vasculature at 570 nm. The CCD camera was then used to image the intrinsic activity-dependent optical signals in primate V1 and V2 in vivo. As shown previously (Ts’o et al., 1990), the dark CO stripes of V2 (thick and thin) can be visualized by optical imaging in response to general activation. The greater activation of thick and thin stripes can be obtained with a broad range of stimuli and may be related to the higher metabolic/activity levels of the dark cytochrome stripes during visual activation. These patterns are not obtained without visual stimulation (i.e. blank condition). An example of such activation is shown in Fig. 1B, which was obtained by summing response to moving gratings of all orientations (0, 45, 90, 135 deg) and presented to both eyes. The images of the cortical surface vasculature and V2 stripe map then served as a guide for the positioning of single unit recording electrodes in V2. Since the optical imaging technique yields indirect information about the overall population response to the various stimulus conditions presented, it may not reveal the particulars of the diverse range of individual cell response properties. We therefore used single unit recordings not only to confirm the optical imaging data, but to also explore in greater detail the receptive field properties of individual cells in V2.

We made vertical electrode penetrations at various sites in and around the dark stripes seen in the optical imaging map (see Fig. 1). In the rightmost dark (thin) stripe in the field, we found cells that were unoriented and color-selective. These cells were binocular and could be classified as either type II (center-only, color opponent) or modified type II (type II with broadband surround antagonism) receptive fields, (Wiesel & Hubel, 1966; Livingstone & Hubel, 1984; Ts’o & Gilbert, 1988) or complex unoriented (spot) (Hubel & Livingstone, 1987; Baizer, Robinson, & Dow, 1977) or oriented and color-selective. Penetrations were continued down to an average depth of 1mm and all the cells in each penetration exhibited the same color selectivity. However, the six penetrations differed in their color selectivity. This pattern of clustering of color selectivity was a common finding in our V2 recording experiments and suggests an internal organization within a color stripe according to preferred wavelength or direction in color space (Ts’o & Burkitt, 1998). As a group, V2 color cells exhibited a greater range of color properties (Roe & Ts’o, 1995, 1999) than what we had previously found in V1 (Ts’o & Gilbert, 1988), where most color cells tended to cluster along the cardinal red/green and blue/yellow opponent axes (Ts’o & Burkitt, 1998, but see Lennie, Krauskopf, & Sclar, 1990).

In the middle (thick) dark stripe (Fig. 1C, marked with the letter D), we found cells selective not for color but for retinal disparity. These cells had complex, oriented receptive fields. Many of the cells in the disparity zones did not respond to monocular visual stimuli and
Fig. 1. Optical imaging and single-unit recording in V2, revealing color and disparity stripes. (A) Postmortem cytochrome oxidase (CO) histology, showing two dark thin stripes, a thick stripe (middle), and intervening pale stripes. (B) Optical imaging of activity in the same region of cortex (framed in white borders in (A)), also showing three dark stripes. The analysis performed was a multiple 'single-conditioned stimulus', taking the sum of four different binocularly presented orientations and subtracting the blank condition. (C) Single-unit recording map, showing surface vasculature and color properties of clusters of cells encountered in several vertical penetrations in the rightmost thin stripe. Each colored dot represents clusters of color cells recorded at each site. The wavelengths (nm) of monochromatic light that elicited the greatest response is shown beside each dot. The neighboring thick stripe contained disparity selective cells, as indicated by the penetration labeled with a D. Note nonuniformity in both the optical imaging map and in the CO histology, suggesting functional substructure in the stripes.
responded only to binocular stimuli over a narrow range of disparities (see Fig. 2, typically half-width at half-height of 1/8 of a degree). This class of disparity cell is termed ‘obligatory binocular’ (Hubel & Wiesel, 1970; Kennedy, Martin, & Whitteridge, 1983). Thus in this and other V2 recording experiments, in confirmation of previous studies (Zeki, 1980; DeYoe & Van Essen, 1985; Hubel & Livingstone, 1987) we found an alternating pattern of functional properties among the dark stripes, seen by optical imaging and subsequent histology. One type of dark stripe (thin) contained color cells, and alternated with the second type of dark stripe (thick), which contained cells selective for disparity. Given the frequent difficulty of discerning the thick versus the thin stripes in CO histology of macaque V2, the imprecise correlation between CO staining patterns and functional specificity and our overall greater emphasis on the functional subdivisions determined by physiological data rather than the histological differentiations, we prefer to refer to the V2 stripe compartments as ‘color’ stripes and ‘disparity’ stripes rather than the histologically determined ‘thin’ and ‘thick’ stripes (Roe & Ts’o, 1998).

3.2. The optical imaging of disparity stripes by imaging binocular interaction

In the experiment illustrated in Fig. 3, we employed a variant of the imaging of general activity to differentiate between the stripe regions in V2 that prefer color from those that prefer disparity stimuli. The fact that the disparity sensitive stripes of V2 contain a preponderance of obligatory binocular cells (Hubel & Wiesel, 1970; Hubel & Livingstone, 1987; Ts’o, Gilbert, Frostig, Grinvald, & Wiesel, 1989) suggested that we might be able to image the disparity regions by identifying the areas that, unlike the color stripes, require binocular stimulation to be activated (Fig. 2). That is, a

![Fig. 2](image-url)
monocular stimulus should be ineffective in stimulating the obligatory binocular cells in the disparity stripes, but would continue to excite cells in the color selective and the pale stripes. Thus, with monocular stimulation, the color regions should continue to image as dark patches, while the unactivated disparity regions should become white. The pale stripe regions should remain gray since pale stripe cells are binocular. Fig. 3B shows an example of this type of imaging experiment, in which stimulation at all orientations was presented to the left eye only and those images were blank-subtracted. The resultant images reveals three dark regions in the field (far left, middle, far right) that responded well to monocular stimulation and presumably are color stripes. In addition to the dark regions and gray regions (pale stripes), optical imaging with monocular stimulation also revealed white regions, indicating zones of cells that were unresponsive to the monocular stimuli, presumably corresponding to the disparity stripes. A closer inspection of the optical imaging map

Fig. 3. Cytochrome oxidase (CO) histology, monocular optical imaging and single-unit recording map from a portion of V2 near the V1/V2 border, spanning ~7 mm. (A) CO histology. Note the pattern of the V2 stripes, which at times suggests the close apposition of neighboring ‘thin’ (color) and ‘thick’ (disparity) stripes, sometimes to the point of merging into a single region of CO-rich staining. Note also the CO blobs of V1 near the V1/V2 border. (B) Optical imaging of binocular interaction, using a monocular (left eye) stimulation paradigm, resulting in imaged regions of V2 with high activity to monocular stimulation (black), moderate activity (gray) and low activity (white). These regions correspond to subcompartments within the color, disparity and pale stripes, respectively. Note the black patch (color subcompartment) in the center of the field, in direct apposition to a white patch (disparity subcompartment). Note also some V1 blobs in the left ocular dominance column. From the same session we also obtained a right eye imaging map (not shown) which exhibited the identical labeling pattern in V2, but reveals the pattern of right eye blobs in V1. (Ts’o et al., 1990) (C). Recording map showing the surface vasculature and the results of single-unit recording penetrations guided by the optical imaging map in (B). In each recording site, at least three different cells were isolated and characterized at penetration depths between 0.1 and 1 mm. As in Fig. 1, colored dots indicate sites in which unoriented color cells were found, oriented bar symbols indicate sites in which oriented, non-color selective cells were found and the D symbol indicates sites in which tuned excitatory disparity cells were found. The Dn symbol indicates a column of near disparity cells. The results from the single-unit recordings match the expectations from the optical imaging maps as to the functional specificity of different V2 subcompartments within the stripes.
in this case shows that at times (see the middle disparity region indicated by middle blue arrow and adjacent color region of Fig. 3B), there is little if any intervening pale (gray) stripe between the alternation of the color (black) and disparity (white) regions, but instead disparity regions appeared side by side with color regions (Ts’o, Gilbert, & Wiesel, 1990). Note that these regions of strong monocular and binocular activation are not stripe-like in appearance, but rather appear as patches of activation within an appropriate stripe. Such images suggest the existence of subcompartments within the V2 stripes.

We made vertical electrode penetrations at various sites in and around the dark and white patches seen in the V2 optical imaging map (Fig. 3C). In the leftmost dark stripe in the field, we found cells that were unoriented and color-selective. Adjacent to this region of color cells was a site in which oriented, non-color selective cells were found, presumably a pale stripe (seen as a gray region in Fig. 3B). In the neighboring region that was white in the optical imaging map (left blue arrow), we found complex oriented cells that were selective for retinal disparity (sites marked with D). In the middle of the field we found an imaged dark patch that contained red/green color opponent cells (marked with a red dot) and a neighboring imaged white patch that contained disparity cells (D, middle blue arrow). Finally, in the rightmost edge of the field, we recorded in an imaged white zone and found disparity cells and a neighboring dark patch and again found color selective cells. We also found disparity columns populated by near cells (Dn, Fig. 3B), as well as far cell columns (Df, Fig. 5C) and tuned inhibitory columns (not shown), although all less frequently than the tuned excitatory and obligatory binocular columns. Our single unit electrical recordings guided by the optical imaging maps found in every case examined that the darkened (monocularly activated) zones in these images contained color selective cells, while whiter (monocularly inhibited) zones contained disparity selective cells that were usually obligatory binocular.

3.3. Color and disparity pathways intermix in V2

The experiment in Fig. 3, then, supports the general rule that there is an alternation of color stripes and disparity stripes. However, it also suggests that color and disparity regions in V2 are sometimes found next to each other, without any apparent intervening pale stripe. Indeed even in CO histology in the macaque monkey, the V2 stripes are not perfectly delineated and often merge or are bridged by bands of dense staining running across the stripes. Six additional experimental cases (e.g. Figs. 4 and 5) confirm this configuration as a common occurrence. The postmortem CO histology from these experiments shows that often adjoining regions of color and disparity cells were contained in a single CO-rich region (see Figs. 4A and 5A). In Fig. 4B, the optical imaging indicates a clear distinction between a particular color stripe (left most dark patch in Fig. 4B) and its neighboring disparity stripe (white patches in the center of Fig. 4B). The optical imaging further shows this disparity stripe extending more posteriorly (towards the V1/V2 border) in a region that the CO histology shows lighter staining. None of these significant functional features are discernable in the CO staining pattern. Thus the CO histology gave no hint of the greatly differing functional specialization within a V2 ‘stripe’, as was obvious from the optical imaging maps.

Closely spaced electrode penetrations (Figs. 4C and 5C) in and around these color/disparity interface zones revealed a transition from unoriented, color cells insensitive to disparity, to cells exhibiting both color and disparity selectivity (see Fig. 6), to cells that are not color selective, but are sharply tuned to disparity. This interaction between color and disparity in V2 is reminiscent of interactions between color and orientation in V1 (Ts’o & Gilbert, 1988) and V2 (Roe & Ts’o, 1995), in which color oriented cells tended to be located at the borders between oriented (form) and color unoriented regions. In Fig. 6 is shown a recording from a V2 cell near the border between color and disparity region. This cell exhibited both color selectivity, with a preference for short wavelengths, and a tuning for far disparities. These observations suggest that there are interactions and intermixing of functional properties between the various functional subcompartments in the stripes of V2, and indicate a less than strict segregation of these visual submodalities (Ts’o, Gilbert, & Wiesel, 1991; Lund, Yoshioka, & Levitt, 1993; Levitt, Kiper, & Movshon, 1994).

3.4. Anatomical evidence of disparity and color cross-connectivity

Injections of red and green fluorescent tracers were used in the experiment illustrated in Fig. 5 to reveal the connectivity of color and disparity compartments in V2. The locations of a disparity stripe and a color stripe were identified with optical imaging (Fig. 5B) and verified with single unit recordings (Fig. 5C). These two sites were separated by over 6mm. Green beads were injected into the disparity stripe and red beads were injected into the color stripe (see Fig. 5A). The resulting pattern of retrograde transport of tracer indicates the presence of intermixing and cross-connectivity between neighboring color stripes and disparity stripes (cf. Levitt et al., 1994; Malach et al., 1994). Both the green bead (disparity) injection and red bead (color) injection resulted in transported label to neighboring
Fig. 4. Optical imaging and single-unit recording maps in V2 revealing zones of color and disparity interaction. (A) Postmortem cytochrome oxidase (CO) histology, showing several V2 stripes. The site marked L indicates an electrolytic lesion used to aid in the histological reconstruction. (B) Optical imaging of activity in response to monocular stimuli, in vivo, in the same region of cortex, showing three distinct regions in V2, two dark (color) and one elongated white (disparity) region. (C) Single-unit recording map, showing surface vasculature and properties of cells encountered in vertical penetrations among several stripes in V2, indicated by the arrows at the top. In the leftmost color stripe, clusters of unoriented color cells were found (labeling conventions as in Fig. 1). The neighboring (white) thick stripe contained disparity selective cells. The rightmost dark stripe also contained color cells. In the regions between the color (dark) and disparity (white) regions, we found single cells selective for both color and disparity, as indicated by penetrations marked both with a D and a colored bar. The CO histology gives little hint of the functional distinctness of the color and disparity regions. Also apparent in the CO histology and the optical image are the blobs and ocular dominance columns of V1.

color and disparity stripes. Moreover, intermixing of red and green bead-labeled cells and some double-labeled cells were found in the center region of the field, where the optical imaging results indicated a border between a color and a disparity stripe, and where single unit recordings found cells tuned to both color and disparity (overlapping color bar and D symbols in Fig. 3C). These findings imply, specifically, that color disparity cells project to neighboring disparity-only and color-only stripes, and more gener-
ally, that there is considerable cross-connectivity and interaction between disparity and color stripes in V2. We have observed this pattern of anatomical labeling and intermixing between the disparity and color pathways of V2 in two additional experimental cases (not shown).

Fig. 5.
These anatomical studies suggest a mixing of color and disparity information within V2, one which is widespread, and, at the same time, clearly patchy in distribution (note periodicity of label in V2). Such specificity can also be observed in the pattern of tracer transport from the green (disparity) bead injection to V1 (Fig. 5A). The green bead labeling pattern systematically avoids the CO blobs of V1 and instead localizes in the CO-sparse interblob regions. As evident from the CO histology, it is clear that the interblob regions from both left and right ocular dominance columns are labeled, indicating the convergence of visual information from both eyes in the disparity stripes of V2. An analogous labeling pattern indicating the convergence of monocular information from both the left eye CO blobs and the right eye CO blobs in V1 resulting from tracer injections in a V2 color stripe was observed in other experimental cases (not shown). These findings underscore the fact that these distributed patterns of connectivity, both between V1 and V2 (cf. Roe & Ts’o, 1999) and within V2, are not diffuse but rather quite specific and are likely to play a role in the generation and organization of higher order properties (e.g. V2 color and non-color disparity cells).

3.5. Columnar organization of disparity in V2

Guided by the optical imaging maps indicating the location of the disparity regions in V2, we made vertical penetrations into the disparity stripes, extending 1–2 mm. In such recordings, we observed: (1) a preponderance of oriented cells with vertical or near-vertical preferred orientation; (2) a remarkable constancy in preferred orientation, from cell to cell, more so than in other oriented regions in V2 or in V1 — and when oriented changed, it did so abruptly, jumping to a new orientation, not gradually as in V1; (3) a columnar-like clustering of disparity properties from cell to cell along the penetration, including disparity type (far, near, tuned excitatory, tuned inhibitory) and preferred disparity; (4) particularly in a tuned excitatory, obligatory-binocular column, an orderly and gradual shifting of preferred disparity, generally around zero disparity, for example, from slightly near, through zero, to slightly far, then reversing in progression. Fig. 7 illustrates such a penetration in a V2 disparity stripe, and is representative of the 22 similar penetrations we made. We cannot rule out the possibility that the curious gradual shifting of preferred disparity is a result of the curvature of the cortex with respect to our electrode, thus leading to a sampling that wandered into adjacent disparity columns. By backtracking penetrations and confirming

Fig. 5. Cross-talk between color and disparity pathways shown physiologically and anatomically. (A) CO histology of a portion of V2, showing several color and disparity stripes and the blobs of V1. Also shown are the results of the anatomical reconstruction using fluorescence microscopy of the pattern of transport of a red bead and a green bead injection in V2. The injection sites are marked with the large red and green spot, with the size of the spot indicating the extent of the injection site. The smaller red and green symbols indicate the location of clusters of labeled cells. Note the pattern of tracer transport which was not restricted to like typed V2 stripe. In particular, note the region in the center of the field where both tracers were transported and where color disparity cells were found. Note also the pattern of green label (disparity-injected) in V1, where it was found only in the interblob regions, systematically skipping the V1 blobs. (B) Optical imaging of binocular interaction from this case, again showing regions of high activity (color subcompartments), and low activity (disparity subcompartments). Note that the green bead injection was placed in a disparity subcompartment and the red bead injection was placed in a color subcompartment. The position of the arrows marking the various V2 stripes differs between (A) and (B) since the image in (B) is taken starting from the middle of the field in A and the course of the V2 stripes across the field is slanted and irregular. (C) Recording map showing surface vasculature and the property of sites in various V2 subcompartments. Note the color subcompartments in the center of the field, and the transition from unoriented color cells, to oriented color cells and color disparity cells, to disparity, non-color selective cells. The $D_s$ symbol indicates a column of far disparity cells.
Fig. 7. Shifting peak disparity tuning along a vertical penetration down a V2 disparity column. Starting at 50 μm down from the pial surface, cells were isolated at ~50 μm intervals, disparity tuning curves and preferred orientation were measured. There is a shifting and meandering of the peak disparity tuning of the progression of recorded cells along the penetration.

recording sites earlier in the penetration we were convinced that this behavior was not the result of any wandering of the eyes. It remains for future studies to determine whether this systematic shifting of peak disparity is truly a property of these disparity columns that perhaps plays a role in the sharpening or shaping of the tuning curves of V2 disparity cells.

3.6. Remarkably constant orientation preference within disparity columns, compared with other regions of V2

A comparison of the progression of preferred orientation within a vertical penetration is shown in Fig. 8 for penetrations in disparity stripes, pale stripes and color stripes. Preferred orientation tends to remain remarkably constant within a disparity penetration, compared to penetrations in pale and color stripes. If the preferred orientation does change within a vertical penetration, it jumps to a new orientation, rather than gradually shifting to another orientation. An analogous pattern of preferred orientation can be observed in the tangential domain, across V2, as well, in the optical imaging maps of orientation preference in V2 (see Fig. 10B). Relatively large regions (0.5–1.5 mm) of single orientation preferences are seen followed by a jump to another orientation in a neighboring orientation module. One can speculate that for sharply tuned disparity processing or for the grouping of like-oriented objects (Merigan, Nealey, & Maunsell, 1993) or texture processing, this organization of orientation preference in which a relatively large region of V2 represents a single unvarying orientation preference (but probably varying in visual field position and other properties) may hold some special significance.

3.7. Functionally distinct subcompartments within single V2 stripes

The existence of subcompartments within individual V2 stripes has been hinted at by anatomical evidence from CO histology and anatomical tracing (e.g. DeYoe et al., 1994; Malach et al., 1994). In addition to the evidence for V2 stripe subcompartments using the optical imaging for binocular interaction, described above, we have seen further evidence in imaging studies using isoluminant chromatic grating stimuli. In the experiment shown in Fig. 9, three high magnification images are shown. The ocular dominance map shown in Fig. 9C reveals the V1/V2 border location. The optical imaging of general activity in V2 revealed a single dark stripe (Fig. 9B). When we used optical imaging to compare regions activated by isoluminant chromatic gratings to those activated by luminance gratings, we visualized interdigitating functional patches within that single dark stripe (Fig. 9A). This image data indicated that there were several subcompartments within the stripe, some containing cells preferring color stimuli (darker patches) and other subcompartments containing cells preferring stimuli defined by luminance (lighter patches). We confirmed the stimulus preference of these subcompartments by making electrode penetrations in each subcompartment and recording from single units. As suggested by the optical imaging map, we found that the darker patches contained color cells whose firing was elevated and modulated in response to a drifting isoluminant grating in the red/green direction of color space (Fig. 9E), while in the lighter patch, we found cells with elevated and modulated responses to a drifting luminance grating (Fig. 9D). Thus we have found that single V2 color stripes are actually composed of multiple regions or subcompartments of different functional specificity, indicating an additional level of functional organization not clearly discernable in CO histology.
4. Discussion

The results from the single-unit recordings in general matched the expectations from the optical imaging maps (e.g. Figs. 3–5) in confirming the functional specificity of the various subcompartments within the V2 stripes. In cases where examination of the post-mortem CO histology would have proved inconclusive, the optical imaging maps indicated the layout and functional properties of V2 subcompartments with much higher degree of certainty. Indeed given the uncertainties associated with CO histology, it seems that caution is warranted against overreliance on CO histology to define the locations, borders and functional specialization of the V2 stripes. We have found a similarly imperfect relationship between the V1 CO blobs defined histologically and the actual location, extent and borders of functionally defined color patches in V1 of the macaque (Landisman & Ts’o, 1992).

Levitt et al. (1994) have questioned the extent of functional segregation in V2, when examining the distribution of spatial and temporal frequency, and orientation tuning within specific CO stripe types defined histologically. The present results emphasize the importance of choosing the appropriate stimulus dimensions when determining the extent of functional segregation.

![Orientation vs Penetration depth](image)

Fig. 8. Constancy of preferred orientation along vertical penetrations in disparity, pale and color stripes. There is a greater constancy of orientation preference within disparity stripes (A), compared with pale (B) and color (C) stripes. In addition, when preferred orientation does change within a disparity column, it often does so abruptly, jumping to a new orientation, rather than gradually transitioning to a new preferred orientation. Note also in the pale and color stripes where vertical penetrations sometimes found no clear columnar organization to orientation, even though many oriented cells were encountered in that column. The (R) symbol indicates a site where red/green color opponent cells were found.
Fig. 9. Optical imaging and single-unit electrophysiology in subcompartments of a single V2 color stripe. On the left, three images taken of the same region of V2, under three different imaging paradigms, designed to reveal the ocular dominance columns of V1 (C), the stripes of V2 (B), and regions that prefer color over luminance stimuli (A). When imaging for color vs. luminance (A), the single V2 thin stripe can be seen to be actually composed of several distinct modules or subcompartments. On the right are single-unit recordings into two different subcompartments, one that preferred luminance stimuli (D), as seen by the cell’s modulated response to a 2Hz low spatial frequency (1 cpd) luminance grating, and the other site in a color-preferring subcompartment (E), as seen by the cell’s modulated response to an isoluminant color grating in the red/green (R/G) direction in color space. This particular cell was qualitatively described as red-ON green-OFF Type II and did not respond to modulations in the blue/yellow (B/Y) direction in color space.

In a given cortical area. Our findings indicate that the stimulus dimensions of color, disparity and orientation tuning constitute a clear basis for segregation within V2.

In Fig. 10, the images of subcompartments within V2 underscores the remarkable similarity in the size and shape of the color, orientation and disparity (Burkitt & Ts’o, 1998) subcompartments (Fig. 10A–C), regardless of the functional submodality represented. Each subcompartment is ~ 0.7–1.5 mm in diameter and sometimes elongated in appearance, spanning the width of the stripe. The pattern of these V2 subcompartments suggests a relationship with the clusters of connections demonstrated by anatomical studies (Levitt et al., 1994; DeYoe et al., 1994; Malach et al., 1994). These findings indicate that V2 is comprised of modules of a characteristic size and shape, analogous to the modules of V1 (the blobs and orientation columns) but of a larger scale. The existence of these modular subcompartments in V2 suggests that the overall architecture of V2 may be more similar to that of V1 than suggested by the CO staining in V2. The apparent similarity in the geometry of the different subcompartments within V2, regardless of the particular functional specificity, supports the notion of a cortical module characteristic in size for each cortical area, with V2 modules approximately six times the size of V1 modules, a figure also corroborated by the analysis of cortical magnification in V1 and V2 (Roe & Ts’o, 1995). This notion is further supported by analogous results in V4, suggesting a characteristic V4 module size that is larger than that of either V2 or V1 (Amir, Harel, & Malach, 1993; Lund et al., 1993; Ghose & Ts’o, 1995; Ghose & Ts’o, 1997). Our findings of the modular subcompartments of V2 describe, in
functional terms, an additional level within the V2 organizational hierarchy, and at a finer grain than the view of V2 as a collection of CO stripes.

The subcompartments for color and luminance seen in color stripes seen in optical imaging undoubtedly intermesh with the also observed representation of

![Subcompartments for color, orientation and disparity within stripes of V2. The three optical images were obtained from different animals.](image)

(A) Color-prefering and luminance-prefering subcompartments within a single thin stripe. (B) Pseudo-color coded image of orientation selectivity in V2, showing domains of orientation (blue arrows indicate zones containing pale and thick stripes, large patches of saturated colors), separated by regions of little apparent organization for orientation (thin stripes, lacking patches of saturated color). Color code: blue = horizontal, red = 45°, yellow = vertical, green = 135°. (C) Patches of tuned excitatory disparity cells (white patches, left blue arrow) within thick stripes. Also patches of color cells (the dark patches, right blue arrow) can be seen within thin stripes of V2. Note the similarity of the geometry of the subcompartments, 0.7–1.5 mm in size, regardless of functional type, whereas subcompartments for color (blobs) or (iso)orientation in V1 are smaller than those in V2, at ~0.2 mm in size.
color within a single color stripe (e.g. Fig. 1) (Ts’o & Burkitt, 1998). Similarly the optical imaging studies also revealed the subcompartments of obligatory binocular cells within single disparity stripes, which must intermesh with the full representation of disparity as well as a range of orientation columns (Burkitt & Ts’o, 1998). Our electrophysiological studies have shown further organization within disparity stripes, with separate clusters or columns of near, far, tuned excitatory and tuned inhibitory cells contained within a single disparity stripe. Given our results (see also Burkitt & Ts’o, 1998), it seems likely that one key functional role of area V2 lies in the establishment of a definitive functional organization and cortical map for retinal disparity. The disparity selective compartments in V2 have also been shown to play a role in the three-dimensional representation of surfaces in space, sensitive to global disparity cues involved in model and amodal completion and disparity capture (Bakin et al., 2000). Beyond the properties of orientation, color and disparity are others that have been reported in primate V2, such as illusory contour analysis (Peterhans & von der Heydt, 1989) and elemental grouping (Merigan et al., 1993), at least some of which are likely to be organized into subcompartments and/or cortical maps that must also co-exist with those we have described in these studies.

As can be seen in our CO histology and optical imaging of the V2 stripes and V1 ocular dominance (OD) columns, it is well known that both of these structures run approximately perpendicular to the V1/V2 border, which is also the representation of the vertical meridian. Whatever the primary reason for this topographic relationship, perhaps the result of the general strategy of the cortex to map two distinct functional dimensions orthogonally, such as OD versus orientation in V1, or retinotopy (vertical meridian) versus other properties, this arrangement may produce added benefit for the disparity system in that it permits spatial computations along the horizontal axis without potentially troublesome breaks in the retinotopic map, in both V1 and V2 (Roe & Ts’o, 1995).

Another interesting feature apparent in Fig. 8 is the indication that there are regions in V2 that contain oriented cells but may not have a columnar organization for orientation. In these regions, one may speculate that the imperative to produce an orderly map of orientation has taken a lower priority in V2 to permit the mapping of other functional properties. The fact that under our imaging conditions monocular stimuli can elicit such a striking suppression of activity within some disparity patches (seen as white patches) might be taken as optical imaging evidence for a strongly inhibitory/suppressive component to the receptive field properties of V2 disparity cells. These large white regions of suppressed activity also would seem to supplement our findings from single unit recording (and those original findings of Hubel & Wiesel, 1970) of a large number of obligatory binocular disparity cells in V2 disparity stripes. These cells are not all tuned at zero disparity, but rather are apparently organized into columns with preferred disparities representing a range tightly clustered around zero disparity. Although such a well-developed representation might subserve stereoscopic depth perception, it seems at least as likely to play a vital role in vergence mechanisms, projecting into the eye movement pathways of MT (DeYoe & Van Essen, 1985; Shipp & Zeki, 1985). Indeed it also seems likely that the well-organized representation of disparity seen in MT (DeAngelis & Newsome, 1999) is inherited from these disparity stripes of V2.

We observed an intermixing of the color and disparity pathways (in addition to color and orientation), both at the anatomical and single-cell level. Often at the boundaries between a color stripe and a disparity stripe (without an intervening pale stripe), we found a gradual transition from color, non-disparity selective, to color and disparity selective, to disparity but not color selective. Such color disparity or color oriented cells (Roe & Ts’o, 1995), located at the borders of otherwise segregated visual pathways, may contribute to the creation of a unified percept from the separate and parallel processing of form, color and depth information and help solve the ‘binding problem’. Alternatively, psychophysical studies (e.g. Nakayama, He, & Shimojo, 1995) have shown the powerful role that stereoscopic depth information can play in scene segmentation and similar visual tasks (for a physiological correlate, see Bakin, Nakayama, & Gilbert, 2000). In these situations, stereoscopic depth can provide a strong cue in the process of sorting out form and surface (color) information to their appropriate object ‘owners’ (cf. Zhou, Friedman, & von der Heydt, 2000). These perceptual phenomena may have their neural basis in the substantial cross-connectivity and interactions of the disparity pathways observed in our present experiments. Such intermixing submodalities involving disparity in V2 may also underlie reports of disparity tuning among V4 cells (Hinkle & Connor, 2000), an important target of V2 projections.

In sum, our results, using the combination of optical imaging and single unit recordings, clearly show a high degree of functional segregation within V2, at the level...
of the V2 stripe and the various subcompartments within single stripes. At the same time, our physiological and anatomical results also indicate substantial interaction and crosstalk between the pathways of V2, the perceptual and neural computational implications of which we can only speculate. Finally, we suggest that constructing a definitive organization for retinal disparity is one of the key contributions of area V2 to visual processing.

Acknowledgements

We thank T.N. Wiesel for participation in some of the experiments, Carmela Lorusso, Jennifer Carroll and Shari Zagorski for excellent technical assistance, and support by grants from the National Eye Institute, the Office of Naval Research, and the Whitaker and McKnight foundations.

References


