V2 Thin Stripes Contain Spatially Organized Representations of Achromatic Luminance Change

A considerable amount of research over the last decades has focused on the apparent specialization of V2 thin stripes for the processing of color in diurnal primates. However, because V2 thin stripes are functionally heterogeneous in that they consist of largely separate color- and luminance-prefering domains and because the color-prefering domains contain a systematic representation of hue, we hypothesized that they contained functional maps that subserve luminance processing. Here we show, using optical imaging of intrinsic cortical signals and microelectrode recording, that the V2 thin stripe luminance-prefering domains contain spatially segregated modules that encode the direction of relative luminance change. Quantitative analysis of the cortical responses to luminance increments or decrements indicates that these luminance-sensitive modules also encode the magnitude of the luminance change. These results demonstrate an important role of V2 thin stripes in the processing of luminance and thus suggest that thin stripes are involved in the overall processing of the surface properties of objects rather than simply the processing of color.

Keywords: cytochrome oxidase stripes, functional imaging, luminance coding, macaque monkey, optical recording, visual cortex

Introduction

Luminance is an important visual attribute that describes a key surface property of objects. Luminance differences can be used to discriminate an object from its background, and luminance gradients can provide cues about the 3-dimensional structure of objects (Gibson 1950; Cutting and Millard 1984; Kleffner and Ramachandran 1992). Furthermore, luminance processing is critical to object vision at scotopic light levels, which eliminate the contributions of color processing (Kayama and others 1979). Despite the importance of luminance for object recognition, the cortical mechanisms underlying the processing of luminance are not well understood.

Functional imaging and single-unit studies demonstrate that V2 thin stripes contain regions that respond best to chromatic modulation (DeYoe and Van Essen 1985; Hubel and Livingstone 1987; Tootell and Hamilton 1989; Roe and Ts’o 1995, 1999; Xiao and others 2003), and these color-prefering domains contain spatially organized representations of hue such that perceptually similar hues are represented in adjacent cortical modules (Xiao and others 2003). However, despite the prevailing view that V2 thin stripes are specialized for processing color, they are functionally heterogeneous in that they contain segregated chromatic- and luminance-prefering modules (Roe and Ts’o 1995; Ts’o and others 2001; Wang and Felleman 2002; Xiao and others 2003). A subset of V2 neurons is tuned to a narrow range of luminance and thus appears to encode specific absolute luminances, and some of these cells also discriminate the direction of luminance change (Peng and Van Essen 2005). Furthermore, the importance of V2 in the processing of apparent brightness was demonstrated by Roe and others (2005), who showed that portions of V2 are activated by stimuli that induce changes in perceived luminance in the absence of changes in physical luminance in the receptive field. In the current study, we sought to determine whether V2 luminance-prefering neurons were organized into functional maps that represent absolute luminance or relative luminance change from background. The results demonstrate that V2 thin stripes contain segregated domains that encode luminance increases or decreases relative to the background luminance. Furthermore, these luminance-change domains encode the magnitude of the relative luminance change by the magnitude of the evoked neuronal or optical response.

Materials and Methods

The general animal preparation and experimental procedures have been described elsewhere (Xiao and others 1999, 2003). All procedures were in compliance with the guidelines of the Society for Neuroscience for the use of laboratory animals and approved by the Animal Welfare Committee of University of Texas-Houston Medical School. Recordings were performed on 11 thin stripes in 8 hemispheres of 7 juvenile monkeys (Macaca fascicularis) initially sedated with ketamine (25 mg/kg, intramuscularly), anesthetized with sufentanil citrate (6-12 μg/kg/h), and paralyzed with pancuronium bromide (0.05 mg/kg/h, intravenously). The electrocardiogram, expired CO2 level (4.0%), SpO2, and body temperature were continuously monitored during the experiment. The eyes were brought into focus and converged on the screen of a video display using contact lenses and a prism. The screen (19 x 14 degrees) covered the visual field of the recorded portions of V2.

Optical images of cortical intrinsic signals activated by visual stimuli were acquired with a slow-scan charge-coupled device array camera (Photometrics CH 250 EV-0206) that was focused 0-300 μm below the surface of V2 by a tandem lens system (50 mm x 50 mm or 50 mm x 35 mm). Each stimulus was presented for 3 s, with 13 s between stimuli. Each stimulus was repeated 50 times and was interlaced with other stimuli in a pseudorandom order. For each presentation, 9 frame images (each for 103 ms) were acquired at 2 frames per second from 0.7 s before to 3.3 s after stimulus onset.

To investigate the cortical activation evoked by achromatic luminance stimuli Commission Internationale de l’Eclairage (CIE)-xy chromaticity coordinates, (0.27, 0.29)), we acquired optical images in response to static square-wave gratings (0.25 cycles/degree [c/d] at 2 alternating spatial phases; and 4 orientations; 0°, 90°, 45°, and 135°) at a high resolution (8.1 μm/pixel). Response images to different orientations and phases of each stimulus were summed across 50 repetitions. We presented grating stimuli, consisting of bars either darker or brighter than the background luminance starting from 3 different background luminances: 1) from 0 cd/m² background to 5, 10, and 20 cd/m² average luminance gratings (all brighter); 2) from 10 cd/m² background to 5 (darker), 15, and 25 cd/m² (brighter); and 3) from 40 cd/m² background to 30, 25, and 20 cd/m² (all darker). These
backgrounds were used as the control stimuli as well. In some experiments, we set the background at 20 cd/m² and the luminance of one phase of a grating was changed to 0 or 10 cd/m² (darker) or to 30 or 40 cd/m² (brighter). The resulting stimuli had mean luminances of 10, 15, 25, and 30 cd/m², respectively. We also presented uniform luminance stimuli that covered the full screen. Their luminance was changed from the 10 cd/m² background to -0 (luminance decrement), 20, and 40 cd/m² (luminance increments) or from the 20 cd/m² background to -10, 10 (luminance decrements) and to 30, 40 cd/m² (luminance increments). These stimuli also allowed us to begin to evaluate the role of stimulus contrast on the location and magnitude of evoked optical responses.

To compare the cortical region activated by these luminance stimuli with the hue map also found in thin stripes, we presented isoluminant chromatic/grey grating (mean luminance 10 cd/m²; 0.25 c/d, 2 alternating spatial phases: 0°, 90°, 45°, 135°) of red (0.55, 0.33), yellow (0.45, 0.47), green (0.27, 0.49), and blue (0.16, 0.08) stimuli and in some cases, orange (0.54, 0.40), aqua (0.25, 0.36), and purple (0.23, 0.11) stimuli.

In these experiments, the response images in each condition were averaged over 50 repetitions of each stimulus. Differential images were used to identify the color-prefering and luminance-prefering domains coincident with thin stripes and the orientation-selective domains coincident with interstripes and thick stripes. Subsequently, the organizations of luminance and hue domains were evaluated using single-condition images. For differential images, we averaged the fifth through eighth response frames (occurring from 1.3 to 2.8 s after stimulus onset) from one stimulus condition and divided it by the first (prestimulus) background frame and then subtracted this image from that similarly calculated from the other condition. For single-condition images (Fig. IA), we first calculated the mean response during the fifth through eighth response frames and then divided this mean response by the first of 2 background frames acquired prior to stimulus onset. For each stimulation condition a control image was calculated by dividing the second prestimulus frame by the first prestimulus frame. A difference of Gaussian (DOG) spatial filter (with standard deviations [SDs] of 49 and 326 µm) was used to remove the high and low spatial frequency noise of each frame (Fig. 1B). All image processing was performed using standard and custom functions within ImageJ.

Each single-condition image was analyzed to determine both the total domain of the statistically significant response and the domain of the peak significant response. To evaluate the significance of the responses to a given stimulus, we first performed a t-test by comparing the single-condition response (average activity across frames 5–8) with the activity induced by the background alone (initial prestimulus frame across 4 stimulus conditions) on a pixel-by-pixel basis. Figure 1C illustrates the spatial extents of statistically significant activations of the single-condition image from Figure 1B evaluated at P < 0.05 (green contour), P < 0.005 (red contour), and P < 0.0005 (yellow). Each statistical criterion provides important information about the configuration and extent of significant cortical activations due to each stimulus. In this and our previous study (Xiao and others 2003), the statistical significance of single-condition responses was evaluated at P < 0.005. Once the domain of significant pixel activations was determined (at P < 0.005), the relative response magnitude was then evaluated in order to determine
the location and extent of the peak statistically significant response. Three criterion levels (Fig. 1D) were evaluated to identify all pixels having a value $-\Delta R/R \times 10^\circ$ (where $R$ indicates reflectance) $> 50\%$, 75\%, and 90\% of the maximal response in the single-condition image. In this and our previous work (Xiao and others 2003) the criterion of 75\% of the maximum response was used to identify the location and extent of peak statistically significant responses.

In order to reduce the artifacts produced by major blood vessels and the lunate sulcus, a blood vessel mask was added to the statistical response maps described above. Because the typical surface view of cortex acquired with green light (Fig. 1E) was generally unevenly illuminated, simple threshold masking would fail to capture most of the blood vessel details. In order to correct for this illumination artifact, a Gaussian filtered image (SD = 326 \mu m) of the surface (Fig. 1F) was subtracted from the original surface image to produce a “flat-field” surface image (Fig. 1G) that was then subjected to a threshold and inversion to produce a blood vessel mask of fine detail (Fig. 1H). This blood vessel mask was then added to the single-condition statistical images ($P < 0.005$; e.g., Fig. 1I) to produce the masked statistical image (Fig. 1J). This masked significant response domain was then evaluated using the 75\% criteria to identify the extent of the significant peak response (Fig. 1K). These significant peak response domains were then projected onto the image of the cortical surface (Fig. 1L) or onto the filtered functional image using the CONTOUR function of Interactive Data Language (V6.0, Research Systems Inc, Boulder, CO).

After the optical imaging experiments in 2 monkeys, electrode penetrations, vertical to the cortical surface, were made under direct visual guidance of the cortical surface through the clear lid of the microelectrode microdrive recording chamber. Electrode penetrations were directed at specific functional loci determined from the prior functional imaging that were marked on high-resolution images of the cortical surface using the detailed pattern of the vasculature as reference. This method of penetration visualization allows for an accuracy of electrode guidance of 100 \mu m or less. All units were recorded within a depth of 1 mm from the cortical surface. Neurons were isolated using manually controlled uniform luminance stimuli or drifting luminance gratings of various orientations. The neural activity was evaluated first using the amplified audio signal of the raw response, and then single-or multunit activity was selected using a time-amplitude window discriminator (BAK Electronics, Mount Airy, MD). The receptive field was mapped with a bright or dark bar and flashed or moved through the receptive field as controlled by a computer mouse. The receptive field borders were then established by identifying that region of space in which no evoked visual response could be obtained through flashed or moving stimuli. After the receptive field of single- or multunis was plotted, flashed uniform luminance stimuli, either completely restricted to or slightly larger than the plotted receptive field (Kinoshita and Komatsu 2001), were presented for 1.28 s every 3.28 s if a neuron did not respond well to the uniform stimuli, drifting luminance or isoluminant color/gray gratings (1.5 c/d, 1 cycle/s/cycle) were presented. Net neuronal responses were obtained by subtracting the spontaneous firing rate during 1 s before the presentation of each stimulus from the firing rate during stimulus presentation. Ten to twenty repetitions of each stimulus were interleaved in a pseudorandom order with other stimuli, and their net neuronal responses were averaged to obtain the mean response to the stimulus. The CIE-xy chromaticity coordinates, luminance levels, and background configurations of these stimuli were the same as those used in the optical imaging experiments. The intensity of optical signals at the electrode penetration sites was measured as the average response within a 10 x 10 pixel (81 x 81 \mu m) region of interest (ROI) to determine the degree of correlation between tunings of the neuronal and optical responses to a set of stimuli. The position of this ROI was determined from the electrode penetration site recorded at the time of electrode penetration, which was then transferred to the corresponding functional images using the direct image coordinates.

**Results**

Optical imaging of intrinsic cortical signals and microelectrode recording of single- and multunit activity were used to characterize the functional organization of V2 thin stripes (Roe and Ts’o 1995; Xiao and others 1999, 2003; Ts’o and others 2001). Recording sites in V2 thin stripes were determined through a stepwise procedure that first identified V2 and then identified the color- and luminance-prefering modules of thin stripes that were finally distinguished from the V2 thick stripes and interstripes by the latter’s orientation-selective modules. V2 was identified as a narrow strip of binocularly activated cortex immediately anterior to V1, which contains the characteristic ocular dominance stripes (differential image of left- vs. right-eye stimulation viewing white-black square gratings, 2 c/d, 4 c/s; Roe and Ts’o 1995, 1999; Xiao and others 1999, 2003; here in Fig. 2A) or by the different patterns of orientation modules in V1 and V2 (e.g., Ts’o and others 2001, fig. 10b; here in Figs. 2C, 5ADGJ, and 8B). Within this binocular zone, V2 thin stripes were identified first at a low spatial resolution (22 \mu m/pixel) by the presence of color-prefering and luminance-prefering modules in the differential image of responses to isoluminant red/green square-wave gratings (0.25 or 2.0 c/d, 1 or 2 c/s; 10 cd/m$^2$) and achromatic luminance-contrast gratings (2 c/d, 2 c/s; mean luminance = 10 cd/m$^2$; Fig. 2B; also in Figs. 2B, 8E, H, K and 8C). Although isoluminant red/green and luminance-contrast gratings were often presented with different spatial frequencies (0.25 vs. 2.0 c/d) to maximize the differential response, multiple control experiments (not illustrated) demonstrated that the same spatial configurations of color-prefering and luminance-prefering modules were achieved when the spatial and temporal frequencies of the 2 stimuli was equal. In this differential image (Fig. 2B), the color-prefering module is dark (black arrow) and the luminance-prefering module is bright (white arrow). The region bounded by the black box represents the cortical territory that was subsequently imaged at higher magnification. The black and white dashed contours encircling the luminance- and color-prefering modules are subsequently reproduced in Figure 2I, J to facilitate the comparison between the low-power differential image and the higher magnification images of single-condition activations of luminance increments, luminance decrements, and isoluminant hues. The identification of the thin stripe was confirmed by the lack of an organized map of orientation and by the presence of a robust map of orientation in neighboring interstripes revealed in the differential image of responses to 45\° versus 135\° (or 0\° vs. 90\°) white-black square-wave gratings (1 c/d, 2.5 c/s; 100% contrast; Fig. 2C). Once a thin stripe was identified using these criteria, the cortical region containing the luminance- and color-prefering modules were then imaged at high magnification (8.1 \mu m/pixel) to determine the spatial organization of single-condition activations to luminance increment, luminance decrement, and isoluminant hue stimulation.

**Achromatic Luminance Responses**

The locations of cortical activations to luminance stimuli were determined using single-condition images in response to luminance increments or decrements from a uniform background. In an effort to minimize the contributions of the cortical vasculature and sulcal movement to the statistical analysis of the optical responses, single-condition statistical response maps (e.g., Fig. 1F) were filtered by flat-field maps (e.g., Fig. 1G) of the cortical vasculature captured under green illumination. This filtering process resulted in the removal of many artifactual signals originating from the large cortical vessels including those overlying the lunate sulcus.
In this exemplar thin stripe, cortical foci activated by achromatic luminance stimuli were determined by presenting static grating stimuli whose mean luminance were either 5 or 10 cd/m² less (Fig. 2D,E) or 5 or 10 cd/m² greater (Fig. 2G,H) than the initial background luminance level (Fig. 2F; 20 cd/m²). In each single-condition image, the pixels demonstrating a statistically significant response (t-test, P < 0.005; see Materials and Methods), whose absolute response was within the top 25% of the peak pixel response, were outlined by a solid red contour that was superimposed on the gray-scale single-condition response image.

In this thin stripe, the significant peak cortical responses to luminance-decrement stimuli were restricted to a small patch near the edge of the lunate sulcus (Fig. 2D,E). In contrast, luminance-increment stimuli produced a single small significant peak response focus, whereas the peak response to the +10 cd/m² stimulus was more extensive. Finally, the constant background luminance condition (Fig. 2F) failed to produce any statistically significant response.

In order to determine the relationship between the luminance-preferring and color-preferring modules (Fig. 2B) and the luminance increment- and decrement-preferring modules, the bright (luminance-preferring) region of the isoluminance red/green-luminance (R/G-Luminance) response was outlined in a black dashed line and the dark (color-preferring) region was outlined in a white dashed line, and these contours were superimposed. Case w02L10.
comparison of these single-condition luminance responses with the contours derived from the luminance-preferring (black dashed) and color-prefering (white dashed) modules observed in the R/G-Luminance stimulation revealed that nearly all these significant peak luminance responses were contained within the luminance-preferring module. The black square in Figure 2f corresponds to the field of view illustrated in Figure 2D,H.

Finally, in order to determine the relationship between the luminance increment- and decrement-preferring modules and the previously described hue map found in thin stripes (Xiao and others 2003), the single-condition peak response domains to both hue and achromatic luminance stimuli are illustrated in Figure 2B. As expected, the majority of the peak hue responses were found up and to the left of the achromatic luminance response peak. This pattern of single-condition hue and luminance activations is entirely consistent with the segregation of dark (color-preferring) and bright (luminance-preferring) regions in the R/G-Luminance map illustrated in Figure 2B. Specifically, the single-condition peak hue responses to red, orange, yellow, and green, but not blue or purple, fall entirely within the color-prefering module (thin dashed white contour in Fig. 2f). This result is not entirely unexpected because the color-prefering module was defined using a red/green isoluminant stimulus.

**Time Course of Achromatic Luminance Responses**

The validity of the observed optical responses to luminance-change stimuli is supported further through the analysis of their temporal dynamics. Figure 3A illustrates single-condition response images as a function of time for the 5 stimulus conditions in the thin stripe illustrated in Figure 2. Images were acquired at a rate of 2 frames per second beginning 0.2 s prior to stimulus onset. As expected, no discernable response was observed at any time point in the background stimulus condition (luminance change = 0 cd/m² relative to the background). In each of the remaining stimulus conditions, observable pixel darkening was first observed beginning at the second frame (+0.3 s) and the response continued to increase (darken) over the remaining 3 s. The locations of the statistically significant peak (75%) response contours are illustrated on the column of functional images that represent the mean response during the period from 1.3 to 2.8 s. In the right-hand portion of Figure 3A, in order to make each cortical response prominent, the image contrast is held constant within each stimulus time series but differs between stimulus conditions. However, in order to represent the relative efficacy of each stimulus, the image contrast was held constant across all stimulus conditions in the right-most column.

The time course of the evoked optical response is illustrated in Figure 3B. In this analysis, the absolute change in cortical reflectance was calculated within a 10 × 10 pixel (81 × 81 μm) ROI centered on the pixel exhibiting the peak response in the final response frame (see ROI plotted in the 3.3-s column in Fig. 3A). In each stimulus condition, the optical response increases slowly with a latency to half-maximum response at approximately 1.0–1.3 s. Whereas each stimulus condition exhibited a similar temporal response function, the peak change in reflection varied approximately 2-fold between the 2 decrement and 2 increment stimuli. That is, the 2 luminance-decrement stimuli evoked larger responses than the 2 luminance-increment stimuli. Nevertheless, when replotted as a function of the normalized peak response, each stimulus condition resulted in a highly similar temporal response function (Fig. 3C).

**Spatial Coding of Absolute or Relative Luminance**

The spatial segregation of responses to dark and bright luminance stimuli raises the possibility that the whole range of absolute luminance values might be represented systematically in the region between these dark and bright luminance domains. Alternatively, these luminance domains might simply represent the direction of luminance change relative to the initial background luminance. In order to determine whether these segregated luminance domains code the absolute or relative luminance of visual objects, the spatial positions of responses to specific stimuli were evaluated in blocks of trials that varied across different initial background luminance conditions. In these experiments, we determined the spatial positions of the response to the same physical stimulus (average luminance 5 or 20 cd/m²) when preceded by 2 of 3 different initial background luminance levels (~0, 10, or 40 cd/m²). If V2 thin stripe luminance domains encode absolute luminance, the cortical location of the peak cortical activations produced by each physically identical stimulus should be nearly identical across the different background conditions. However, if the location of the cortical activations produced by each physically identical stimulus shifts position as a function of the preceding background, then these luminance domains must encode the direction of luminance change relative to background. Figure 4 illustrates luminance-change responses from 9 different V2 thin stripes in which the positions of statistically significant peak cortical activations, following physically identical stimuli, shift across the cortex as a function of the initial background condition. In Figure 4A,B, cortical responses to a 5 cd/m² average luminance grating were evaluated under background conditions of 0 and 10 cd/m². In the first case (w11L01), one major cortical response was observed in the 0 cd/m² background condition (luminance increment; Fig. 4A1), whereas a spatially offset response was observed in the 10 cd/m² background condition (luminance decrement; Fig. 4A2). The significant peak responses are projected onto the cortical surface in Figure 4A3 to illustrate that the luminance-increment responses (white contour) were spatially segregated from the luminance-decrement responses (black contours). A similar pattern of segregated activations in response to physically identical stimulation beginning from different luminance backgrounds is illustrated from another thin stripe in Figure 4B1–B3.

The same type of spatial shifting of cortical responses to physically identical stimuli under different background conditions is illustrated for larger luminance changes in Figure 4C. In this case (w11r07), a 20 cd/m² average luminance increase resulted in a robust cortical activation on the right (Fig. 4C1), whereas a 20 cd/m² luminance decrease produced a robust cortical activation on the left (Fig. 4C2). This pattern of luminance-increment and luminance-decrement segregation, demonstrating luminance coding relative to the background, is summarized in Figure 4C3. In each of these 3 cases, all stimuli were of 100% contrast, yet the resultant activation shifted as a function of the preceding background. This result demonstrates that these cortical foci represent the direction of stimulus luminance change rather than the stimulus contrast. In order to determine if this result is
preserved for lower contrast stimuli, we illustrate a case using stimuli of 33% contrast both preceded by the same uniform background luminance (20 cd/m²). In this case (w02L10), 2 stimuli, both of 33% contrast, elicited different patterns of cortical activations depending solely on the direction of luminance change from the background. The +10 cd/m² stimulus elicited a significant peak activation up and to the left of the recording field (Fig. 4D1), whereas the –5 cd/m² stimulus elicited a peak activation down and to the left (Fig. 4D2). These differential activation patterns elicited by low-contrast stimuli in this hemisphere are summarized in Figure 4D3. Thus, the direction of luminance change from a common background, rather than the contrast of the presented stimulus, was responsible for the spatial segregation of the luminance-increment and luminance-decrement domains.

Organization of Luminance-Change Domains in V2 Thin Stripes
Luminance-change domains were imaged in 11 thin stripes from 7 monkeys, and the results from 4 additional cases are summarized in Figure 5. In these cases, a surface view of the cortical vasculature and/or the pattern of ocular dominance in V1 was used to identify the position of V1 prior to the identification of the thin and interstripes of V2 (not shown). In each case, a differential image of orientation selectivity was used to identify the orientation-specific domains of the adjacent interstripes that are lacking in thin stripes (Figs. 5A,D,G, and 8B), and the differential pattern of orientation selectivity between V1 and V2 helps identify the V1/V2 border. Next, a low-magnification differential image of the activation to red/green isoluminant stimulation minus the activation to luminance-contrast stimulation was used to identify the luminance-preferring and color-preferring modules of each thin stripe (Figs. 5B,E,H,K, and 8C). These contours were then overlaid on the cortical maps illustrating the single-condition luminance-change responses in that thin stripe. This allows for the clear specification of each thin stripe and for the determination of the relationship between the luminance-preferring and color-preferring modules (in the differential R/G-L image) and the...
luminance-decrement and luminance-increment foci. In Figure 5A a parafoveal portion of V2 was imaged just posterior to the lunate sulcus. The outlined box indicates the cortical region later imaged at high magnification for the determination of luminance-change and hue domains. The differential image of orientation selectivity (45°/C176 - 135°/C176) demonstrates clear orientation-selective V2 interstripes (and thick stripes) and a homogeneous gray region (nonorientation selective) that corresponds to the thin stripe (Fig. 5A; compare with Fig. 5B). The different patterns of orientation activation in V1 and V2 identify the V1/V2 border in this case. The differential image of the R/G-Luminance stimulation conditions (Fig. 5B) clearly demonstrates a dark, color-preferring module (white arrow and white dashed contour) and a bright, luminance-preferring module (black arrow and black dashed contour). A high-magnification (8.1 μm/pixel) image of the cortical surface upon which the single-condition luminance-change responses are projected is illustrated in Figure 5C. In this thin stripe, spatially segregated luminance-increment (L+; solid and fine dashed white contours) and luminance-decrement (L-; solid black contours) domains are clearly visible and are largely contained within the R/G-L-derived luminance-preferring module (dashed black

Figure 4. Luminance domains encode the direction of luminance change relative to background. (A–C) The cortical activation in response to physically identical stimuli shifts position depending only on whether the stimulus represents a luminance increment (A1, B1, C1) or decrement (A2, B2, C2) from the initial background. The 2 stimuli in each pair (e.g., A1, A2) are physically identical and differ only by the luminance of the preceding background. Outlines of significant peak responses across 2 stimulus conditions were superimposed on the surface view of the cortex in each case (A3, B3, C3). In each case, the stimuli are 100% contrast, so the pattern of activation reflects the direction of luminance change and not the stimulus contrast. A, case w11L01; B, case w05R07; C, case w11R07; D, low-contrast stimuli (33%) also produce differential activations (D1 vs. D2) depending on direction of luminance change but not stimulus contrast. Case w02L10.
contour) and do not encroach upon the color-prefering modules (dashed white contour).

A similar pattern of spatially segregated luminance-change domains is illustrated in Figure 5D–F. First, area V2 was distinguished from V1 by its binocular activation in the differential image of ocular dominance (not shown). Next, the orientation-selective activity in V2 interstripes was identified in the differential image of orientation selectivity (45°–135°) identifying V2 thick stripes and interstripes flanking a homogeneous, nonorientation-selective zone corresponding to the thin stripe (see panel B). Differential image of red/green isoluminance minus luminance grating stimulation (R/G-L) reveals the 2 luminance-prefering (black arrows and black dashed contours) modules and 1 color-prefering module (white arrow and dashed contour) within the V2 thin stripe. Black square in panels A and B indicate region of higher magnification imaging shown in panel C. High-magnification (8.1 μm/pixel) image of the cortical surface illustrating the locations of luminance-prefering (black dashed contours) and color-prefering (white dashed contour) modules and their relationship with single-condition luminance-increment (L+; solid white contour) and luminance-decrement (L–; solid black contour). Case w04L02. Differential image of orientation selectivity (45°–135°) identifies V2 interstripes (black arrows) as well as thick stripes and the homogeneous gray, nonorientation-selective region characteristic of the intervening thin stripe. Differential image of red/green isoluminant minus luminance grating stimulation (R/G-L) reveals the luminance-prefering (black arrow and black dashed contour) and color-prefering (white arrow and white dashed contour) within the V2 thin stripe. Higher magnification view of the cortical surface illustrating the significant peak activation foci to luminance increments (L+; solid white contours) and luminance decrements (L–; solid black contours). The white and black dashed contours represent the positions of the color- and luminance-prefering modules from the R/G-L differential image (panel E). Case w02L08. Low-magnification differential image of orientation selectivity (45°–135°) illustrating a band of orientation-selective activity (black arrow) medial to the thin stripe recording site (black bounding box). Low-magnification differential image of red/green isoluminant minus luminance grating stimulation (R/G-L) reveals the luminance-prefering (black arrow and black dashed contour) and color-prefering (white arrow and white dashed contour) modules within the V2 thick stripe. Higher magnification view of the cortical surface upon which are projected the significant peak activation foci to luminance increments (L+; solid white contour) and luminance decrements (L–; solid black contour). The white and black dashed contours represent the positions of the color- and luminance-prefering modules from the R/G-L differential image (panel F). Case w05R07. Low-magnification differential image of orientation selectivity (45°–135°) illustrating distinctive pattern of orientation selectivity in V1 and bands of orientation selectivity in V2 interstripes (black arrows) and a homogeneous gray region that corresponds to the V2 thin stripe that lacks organized orientation selectivity (see panel K). Low-magnification differential image in response to R/G-Luminance stimulation identifies luminance-prefering (dashed black contour) and color-prefering (dashed white contour) modules. High-magnification view of the cortical surface illustrating the projections of single-condition luminance-decrement (L–; black contour) and luminance-increment (L+; solid white contour) domains with respect to the R/G-Luminance-derived luminance-prefering (dashed black contour) and color-prefering (dashed white contour) modules. Case w11L04.
dashed contour) were then identified in the R/G-luminance differential image (Fig. 5E). Next, single-condition activations to luminance decrement (L−; solid black contours) and luminance increment (L+; solid white contour) are projected onto the cortical surface to demonstrate their spatial segregation from each other. Finally, the overlay of the R/G-L-derived luminance-prefering and color-prefering modules onto this surface map demonstrates that the majority of the luminance-change responses are contained within the luminance-prefering module (black dashed contour; Fig. 5F).

The spatial organization of luminance responses in a third thin stripe is illustrated in Figure 5G–I. In this case, the differential image of orientation tuning could clearly identify only the lateral interstripe (Fig. 5G), and the differential image in response to the R/G-Luminance stimulation revealed one clear color-prefering module (white arrow and dashed white contour) and one distinct luminance-prefering module (black arrow and dashed black contour; Fig. 5H). The single-condition luminance-change activations from this case are projected onto the cortical surface in Figure 5I. Here, the luminance-increment domain (L+; solid white contour) is clearly segregated from the luminance-decrement domain (L−; solid black contour), and the luminance-decrement domain, but not the luminance-increment domain, is entirely contained within the R/G-L-derived luminance-prefering module. The absence of the luminance-increment domain from the luminance-prefering module may be due to its close proximity to the large vasculature of the lunate sulcus in this low-magnification image.

A fourth example of segregated luminance-change domains and their relationship with V2 thin stripe luminance-prefering and color-prefering modules is illustrated in Figure 5J–L. The imaging site in V2 was first identified as a binocular zone in the differential image of ocular dominance (not shown) that was flanked by orientation-selective modules in the differential image of 45°–135° stimulation (Fig. 5J). The differential image in response to the R/G-Luminance stimulation (Fig. 5K) revealed one clear luminance-prefering module (dashed black contour) and one clear color-prefering module (dashed white contour). When projected onto the cortical surface (Fig. 5L), the single-condition luminance-decrement activation (L--; solid black contour) was spatially segregated from the luminance-increment activation (L+; solid white contour). Furthermore, the luminance-decrement domain was totally confined to the R/G-L-derived luminance-prefering module (dashed black contour), and the luminance-increment domain was shifted to the left occupying a region that did not appear to contain a clear luminance-prefering module. However, this large luminance-increment domain did partially overlap the R/G-L-derived color-prefering module (for one additional case, see Fig. 8A–F).

Spatial Separation of Luminance-Change Domains

In this section, the magnitude of the cortical separation between luminance change–activated foci is quantified for both luminance increments and decrements of varying magnitudes for stimulus pairs in the same and opposite directions from background. For this analysis, the location of the peak pixel response was determined for each luminance-change stimulus, and the shift in this peak position was evaluated as a function of luminance-change magnitude and direction relative to background. This distance in pixels (measured using coordinate differences in ImageJ) was then transformed to cortical distance using the known magnification factor of 8.1 μm/pixel in these high-magnification functional images.

On average, cortical activity peaks shifted only 127 μm (±19.58 standard error of the mean [SEM], n = 29) between pairs of foci elicited by luminance changes in the same direction (e.g., bright to brighter or dark to darker). In contrast, cortical activity peaks shifted on average 719 μm (±126 SEM, n = 14) between pairs of foci elicited by luminance changes in the opposite direction conditions. Overall, these 2 populations had very similar average changes in mean luminance (12.5 vs. 10.0 cd/m²), and the difference in spatial separations is highly statistically significant (Student’s t, degrees of freedom = 42, P < 0.0001). In order to explore further whether the magnitude of the cortical shift was correlated with stimulus change magnitude, the analysis was repeated separately for luminance differences of 5, 10, 15, and 20 cd/m² (Fig. 6). First, for either luminance changes in the same (P > 0.22) and opposite directions (P > 0.70), the separation of evoked cortical foci was not significantly correlated with the magnitude of the luminance change. Second, when the separation of evoked foci was compared between same and opposite luminance-change directions, statistically significant differences were observed for 10-cd/m² (P < 0.01) and 15-cd/m² (P < 0.05) changes in average luminance. Statistical tests could not be performed at 5 or 20 cd/m² due to the stimulus set used, which precluded examples of 5 cd/m² for luminance changes in the opposite direction and of 20 cd/m² for luminance changes in the same direction. From this analysis we conclude that the observed shift in peak activity locus is due primarily to differences in the direction of luminance change and that peak cortical activity shifts very slightly with increases or decreases in luminance in the same direction relative to background. These results also indicate that the magnitude of luminance change (either increases or decreases relative to background) is not encoded by a spatial shift in the evoked cortical response locus.

Coding of Luminance-Change Magnitude

In an effort to determine whether and how these luminance-increment and luminance-decrement domains encode the magnitude of luminance change, we determined the magnitude of the peak optical response and the spatial extent of the significant response domain for a series of luminance increments from a background of 0 cd/m² in 6 thin stripes and for a series of luminance decrements from a background of 40 cd/m² in 2 of the same and from one additional thin stripe. We measured the area and magnitude within the total significant response domain rather than the peak significant response domain elicited by each stimulus in an effort to be sensitive to changes in the weaker responses near the edges of the response domain. The spatial extents of these luminance-change domains are illustrated in Figure 7A–E. For case w11L01 (Fig. 7A–C), 2 segregated luminance-increment domains were observed (Fig. 7A). Within one of these two foci, individual significant response areas remained largely constant across luminance increases, whereas in the other focus the significant response areas to the 2 smallest luminance increases were nearly identical and the significant response area to the largest luminance stimulus was smaller (0 cd/m² background). Luminance decrements from the 40 cd/m² background elicited a single significant response focus that was largely similar in size across stimulus magnitude. The relationship between these luminance-change domains is illustrated in Figure 7C.
The optical response as a function of stimulus luminance change magnitude tuning curves and the resulting average of normalized to the peak response and were replotted in Figure 7. In order to better visualize the change in optical response as a function of absolute signal change varied between cases. In this analysis, the magnitude or position with increases in stimulus magnitude.

Two additional examples of magnitude coding in a luminance-decrement domain are illustrated in Figure 7D,E. In case w11R01 (Fig. 7D), all 3 luminance increments elicited a single cortical focus whose position and extent was nearly constant across stimulus magnitude. In the third case (w03R14; Fig. 7E), each luminance-increment stimulus elicted a single significant response domain that was nearly equal in area for the 2 smaller increments but increased in size with the largest luminance increase (20 cd/m²). However, the location of the peak response for each condition did not vary much across stimulus conditions (see Fig. 6). For illustration purposes, a single luminance-decrement domain in each case is illustrated by the dashed black contour.

Because the majority of luminance-change foci did not vary in extent or position with increases in stimulus magnitude change, we next determined the magnitude of the optical response as a function of stimulus magnitude. In this analysis, the magnitude of the optical signal was determined within a 10 × 10 pixel (81 × 81 μm) ROI centered on the pixel eliciting the maximum response to the largest stimulus magnitude. The absolute magnitude of the optical signal elicited by luminance increments from a background of 0 cd/m² in 6 thin stripes is illustrated in Figure 7F. In each case, the absolute magnitude of the optical signal increased with stimulus luminance, but the absolute signal change varied between cases. In order to better visualize the change in optical response as a function of luminance increment, the data from each case were then normalized to the peak response and were replotted in Figure 7G. This figure illustrates individual-response versus luminance-change magnitude tuning curves and the resulting average of the optical response as a function of stimulus luminance increase. Overall, these data are highly statistically significant ($r = 0.77, P < 0.00001$), thus indicating a systematic increase in peak optical response with increases in stimulus luminance.

A similar analysis was performed in 3 cases of luminance decrements from a background of 40 cd/m². The absolute magnitudes of the evoked optical signals are illustrated in Figure 7H, and the normalized, individual-response tuning curves and the resultant average tuning are illustrated in Figure 7I. In case w11L01, luminance decrements produced large, nearly linear increases in absolute optical signal. In the other 2 cases, luminance decrements produced smaller optical signals (approximately $-0.00015AR/R$) that did not vary linearly with stimulus magnitude. The normalized optical response functions are illustrated in Figure 7J, which demonstrates an overall linear trend for optical response magnitude with increases in stimulus luminance change ($r = 0.75, P < 0.005$). These results demonstrate that whereas the locus of the peak response to either a luminance increment or decrement does not change with luminance-change magnitude, the peak cortical response increases monotonically with luminance-change magnitude.

**Electrophysiological Underpinnings of Luminance-Change Domains**

The neurophysiological underpinnings of these functional imaging results were determined through microelectrode recordings directed at previously determined luminance-change loci in 2 hemispheres of 2 monkeys. In these experiments, microelectrode penetrations were directed under visual guidance at the functionally characterized loci using the fine details of the cortical surface vasculature as reference. In each penetration, 1–4 recording sites were examined for their luminance tuning with stimuli identical to, or in some cases of slightly higher spatial frequency, those used in the optical recording experiments. If a neuron did not respond to flashing stimuli, drifting gratings were presented. In this section, electrophysiological results from 4 penetrations directed at luminance-increment and luminance-decrement domains of one V2 thin stripe are illustrated.

First, a thin stripe in V2 was first identified by its position on the cortical surface (Fig. 8A) and by its lack of orientation selectivity as observed in the differential image of 45°–135° orientations (Fig. 8B) and the presence of such selectivity in the adjacent interstripes and in area V1. The homogeneous gray region in the orientation map bounded by the white box contains a V2 thin stripe as defined by the region preferentially activated by the R/G-Luminance stimulus that also lacked an organized pattern of orientation selectivity (Fig. 8C). This region was then imaged again at higher magnification to reveal the luminance-decrement (Fig. 8E) and luminance-increment (Fig. 8F) domains. The statistically significant peak luminance response domains were then projected on the cortical surface (Fig. 8D). The luminance-decrement domain is indicated by a solid black contour and the luminance-increment domain is indicated by a solid white contour. The dashed contours indicate the color-preferring (dashed white) and luminance-preferring (dashed black) modules derived from the R/G-Luminance differential image in Figure 8C. The fine structure of the cortical vasculature was then used to provide direct visual guidance of 3 electrode penetrations into the luminance-decrement domain and 1 penetration into the luminance-increment domain (Fig. 8D).

For each penetration, a normalized luminance tuning curve was calculated for each of 1–3 recording sites. Each tuning curve captured the relative responses to 3 achromatic luminance

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**Figure 6. Cortical separation of luminance-change foci.** Graph of the cortical distances separating the peak response pixels of pairs of activation foci in response to different magnitudes of luminance change. The gray bars represent average (±SEM) separation of foci in response to luminance changes in the same direction from background (e.g., from 40 cd/m² background to 30 and to 25 cd/m²), whereas the black bars represent average separation of foci to luminance changes in the opposite directions (e.g., from 40 cd/m² background to 20 cd/m² and from 0 cd/m² background to 20 cd/m²). Within each luminance-change class (the same change direction), the magnitude of foci separation is nonsignificant across luminance-change magnitude. However, the separation of cortical foci is significantly different between the same and opposite directions for luminance-change magnitudes of 10 and 15 cd/m². The stimuli used did not allow for evaluations of cortical foci separations for the luminance change of 5 cd/m² in opposite directions or for the luminance change of 20 cd/m² in the same direction.

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**Figure 7. Cortical separation of luminance-change foci.** Figure 7 illustrates the cortical separation of luminance-change foci. Graph of the cortical distances separating the peak response pixels of pairs of activation foci in response to different magnitudes of luminance change. The gray bars represent average (±SEM) separation of foci in response to luminance changes in the same direction from background (e.g., from 40 cd/m² background to 30 and to 25 cd/m²), whereas the black bars represent average separation of foci to luminance changes in the opposite directions (e.g., from 40 cd/m² background to 20 cd/m² and from 0 cd/m² background to 20 cd/m²). Within each luminance-change class (the same change direction), the magnitude of foci separation is nonsignificant across luminance-change magnitude. However, the separation of cortical foci is significantly different between the same and opposite directions for luminance-change magnitudes of 10 and 15 cd/m². The stimuli used did not allow for evaluations of cortical foci separations for the luminance change of 5 cd/m² in opposite directions or for the luminance change of 20 cd/m² in the same direction.
contrasts –5, +5, and +15 cd/m² relative to the blank background (0 change; 10 cd/m²). For each recording site, the magnitude of the optical signal was calculated as the average signal within a 10 × 10 pixel (81 × 81 µm) ROI centered on the location of the electrode penetration. These data allowed for a direct comparison of the electrophysiological single- and multiunit recording tuning with the optical population response tuning.

The average neuronal and optical luminance tuning curves from 2 recording sites in penetration D1 directed at the luminance-decrement–preferring module in this hemisphere were illustrated in Figure 8G. For both the neuronal (solid line) and optical (dashed line) responses, the maximum response was observed to the dark achromatic stimulus (–5 cd/m² relative to background). Whereas the optical response to bright stimuli was approximately 25% of the peak, the neuronal response to the brightest stimulus was nearly equal to the peak response to dark stimulus. The average neuronal and optical tuning curves from 2 recording sites in luminance-decrement penetration D2 are illustrated in Figure 8H. Like penetration D1, the peak neuronal and optical responses were observed to the –5 cd/m² luminance-decrement stimulus. Furthermore, the optical responses to luminance increments were near zero, whereas the neuronal responses to bright stimuli range from 30–42% of the maximum. The neuronal and optical tuning curves from a single recording site in luminance-decrement penetration D3 are illustrated in Figure 8I. In this penetration the optical response reached a maximum for the luminance-decrement stimulus (–5 cd/m²), and the responses to luminance increments ranged from 20–40% of the maximum. However, the maximal neuronal response was to the largest luminance increment (+15 cd/m²)
and that to the luminance decrement (–5 cd/m²) was approximately 65% of the maximum response.

Three recording sites were evaluated for luminance tuning in the 1 penetration directed at the luminance-increment focus of this thin stripe. The average neuronal and optical luminance tuning curves from the 3 recording sites in this penetration into the luminance-increment--preferring module is illustrated in Figure 8. Overall, the neuronal tuning curve showed a maximal response to the brightest achromatic stimulus tested (+15 cd/m²), the dimmer bright stimulus elicited only 55% of the peak response, and the dark achromatic stimulus elicited 47% of the peak bright response. The normalized luminance tuning curve derived from the optical responses at penetration B1 into the luminance-increment--preferring domain is illustrated by the dashed curve in Figure 8J. Consistent with the neuronal tuning, the optical response was maximal to the largest luminance increment, and the luminance-decrement stimulus resulted in a response of 70% of maximum. Thus, the average neuronal and optical luminance tuning curves for penetration B1 are highly consistent with each other. That is, both the neuronal and optical tuning curves peak at the brightest achromatic luminance (+15 cd/m²).

The correlation between the neuronal and optical responses in these 4 penetrations into luminance-decrement and luminance-increment domains is illustrated in Figure 8K and L. Overall, these data are highly correlated (r = 0.94 and p < 0.05). Case w13R02.
luminance-increment domains is illustrated in Figure 8K. Overall, there is a highly significant statistical relationship between these 2 cortical activity measures (r = 0.74, P < 0.0001). Thus, these results demonstrate both the qualitative validation of the optical imaging of luminance-change domains in V2 thin stripes and the quantitative relationship between optical imaging and microelectrode unit recording. Finally, in an effort to quantify the relative responses to equal magnitude luminance increments and decrements, a bright/dark ratio was calculated for both the neuronal and optical responses to stimuli of +5 and –5 cd/m² relative to the background. Figure 8L illustrates the correlation between the neuronal and optical bright/dark ratios from penetrations D1–D3 and B1 (Fig. 8G–J). Overall, the neuronal and optical responses are highly correlated (r = 0.94), and this relationship is statistically significant (P < 0.05).

Discussion
Several previous studies have shown that some cells of cat area 17 (Rossi and others 1996; Rossi and Paradiso 1999), cat area 18 (Hung and others 2001), and monkey areas V1 and V2 (Kayama and others 1979; Komatsu and others 1996; Kinoshita and Komatsu 2001; Peng and Van Essen 2005) respond to homogeneous surfaces defined by luminance. Furthermore, optical recording has shown that these cells are organized into modules in cat area 18 (Tani and others 2003) and monkey area V2 thin stripes (Roe and Ts’o 1995; Ts’o and others 2001; Roe and others 2005). The current study demonstrates that V2 thin stripes contain spatially segregated luminance-increment and luminance-decrement domains that represent the magnitude and the direction of luminance change relative to a background. This relative coding mechanism permits the brain to interpret object brightness in an economical way rather than inefficiently encoding many absolute gray scales in the face of varied lighting conditions. Moreover, these V2 luminance-increment and luminance-decrement domains are consistent with the observation that some neurons in alert macaque V2 are selective for the direction of luminance change of a homogeneous field modulated sinusoidally in time (Peng and Van Essen 2005). It is reasonable to expect that these neurons are located in the luminance-increment or luminance-decrement domains of V2 thin stripes. Thus, the sign of object luminance change is represented by the patch-like neuronal activity in the V2 thin stripe luminance-increment or luminance-decrement domains. This functional mapping, however, does not preclude the possibility that a subset of individual neurons in these luminance-change domains is selective for a particular luminance value (Peng and Van Essen 2005). However, as a group, they appear to contribute to luminance processing by signaling relative luminance change. Since a recent psychophysical study has shown that the perception of brightness in macaques and humans is quite similar (Huang and others 2002), the systematic cortical representation of luminance revealed in the current study may underlie human brightness perception as well.

The results of the current experiments provide a unique insight into the representation of achromatic stimuli in the primate brain. The spatial segregation of luminance increment-preferring and luminance decrement-preferring foci in V2 thin stripes indicates that luminance is not spatially represented along a single dimensional axis. Rather, these results are suggestive of V2 regions dominated by retinal ON- and OFF-pathways. Thus, luminance change, relative to the current background luminance, appears to be represented efficiently along 2 distinct channels within area V2 thin stripes. Furthermore, in area V2, the magnitude of luminance change appears to be represented in the peak optical response. This organizational scheme allows for the preservation of the spatial encoding of luminance change without requiring the representation of absolute luminance.

The current results on the representation of achromatic stimuli can now be integrated with our previous work on the spatially organized representation of isoluminant hue (Xiao and others 2003) to produce a model of surface representation in area V2 thin stripes (Fig. 9). According to this view, V2 thin stripes contain segregated luminance-preferring and color-preferring modules that are flanked by the orientation-selective modules of V2 interstripes. Luminance-preferring modules consist of spatially segregated luminance-increment and luminance-decrement domains that may abut each other or may be separated by some distance, often spanning a color-preferring domain. In contrast, the color-preferring module consists of a spatially organized map of hue such that perceptually similar hues activated nearby localized foci, whereas perceptually dissimilar hues tend to be located farther apart. Thus, in V2 thin stripes, the hue map and luminance-change domains are either adjacent to one another or the hue map is flanked on either side by segregated luminance-increment and luminance-decrement domains. Finally, the representations of hue and luminance in V2 thin stripes differ in a fundamental way. Variations in hue are encoded spatially by a systematic shift across the cortical surface of the peak activity locus. In contrast,

Figure 9. Schematic representation of luminance-change and hue mapping in V2 thin stripes. V2 thin stripes contain luminance-change and hue-selective modules and do not contain organized representations of orientation like those observed in V2 interstripes and thick stripes. Within the thin stripes, luminance-preferring modules contain spatially segregated luminance-change domains that are preferentially activated by luminance increments (white circles) or luminance decrements (black circles). The luminance-increment and luminance-decrement domains are located either adjacent to one another or they are separated by an intervening hue map. In the color-preferring module, different hues are spatially organized into a regular hue map in which the foci (colored circles) representing perceptually similar hues overlap partially, whereas the foci representing perceptually dissimilar hues tend to be located farther apart (Xiao and others 2003).
luminance appears not to be encoded as a continuous variable with associated spatial shifts in the peak activity locus. Rather, direction of luminance change, not absolute luminance, is encoded by V2 thin stripes. According to this functional organization, the magnitude of luminance change is encoded by the magnitude of the population optical signal (or neuronal signal) rather than by a spatial shift in the peak activity locus or by an increase in the evoked activity area. Thus, whereas V2 thin stripes encode both hue and luminance change, they do so in 2 fundamentally different ways.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

Notes
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References