Different sensations from cones with the same photopigment

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We used adaptive optics to study color fluctuation in the appearance of tiny flashes of light. For five subjects, near threshold, monochromatic stimuli with full widths at half maximum of 1/3 arcmin were delivered throughout a patch of retina near 1 deg in which we also determined the locations of L, M, and S cones. Subjects reported a wide variety of color sensations, even for long-wavelength stimuli, and all subjects reported blue or purple sensations at wavelengths for which S cones are insensitive. Subjects with more L cones reported more red sensations, and those with more M cones tended to report more green sensations. White responses increased linearly with the asymmetry in L to M cone ratio. The diversity in the color response could not be completely explained by combined L and M cone excitation, implying that photoreceptors within the same class can elicit more than one color sensation.

Keywords: cones, adaptive optics, color vision

Introduction

Color vision depends on three classes of cones that are interleaved spatially into a single layer of photosensitive cells. Therefore, the reconstruction of spectral variations across the scene requires the comparison of signals from cones with different pigments that are sampling somewhat different portions of the retinal image. This sampling strategy succeeds in normal scenes because it relies on the fact that the spectral reflectance varies slowly on the spatial scale of the cones. However, for extended stimuli of high spatial frequency, the grain of the trichromatic mosaic can sometimes intrude in visual experience. For example, high frequency black and white patterns appear to contain splotches of color (Brewster, 1832) caused by inability of the visual system to reconstruct color and brightness information from the undersampled or aliased retinal image (Williams, 1983; Williams, Sekiguchi, Haake, Brainard, & Packer, 1991; Sekiguchi, Williams, & Brainard, 1993).

A similar kind of chromatic artifact occurs with stimuli that are very small. Holmgren (1884) reported that tiny monochromatic flashes of light appear to fluctuate in color, presumably as involuntary eye movements cause each flash to stimulate different cones. Hartridge (1954) found more than three sensations under these conditions and concluded erroneously that there must be more than three kinds of receptors in the retina. Many investigators have subsequently studied the detection and appearance of tiny flashes (Bouman & Walraven, 1957; Krauskopf, 1964; Krauskopf & Srebro, 1965; Ingling, Scheibner, & Boynton, 1970; Williams, MacLeod, & Hayhoe, 1981; Cicerone & Nerger, 1989; Vimal, Pokorny, Smith, & Shevell, 1989; Wesner, Pokorny, Shevell, & Smith, 1991; Otake, Gowdy, & Cicerone, 2000). Both the chromatic aliasing with large stimuli and the fluctuation in color of small flashes of light could provide insight into the fine scale topography of the mechanisms responsible for color vision. While it has usually been assumed that these phenomena reveal the granularity of the cone mosaic, they may also reveal the discrete nature of the postreceptoral microcircuitry for color and spatial vision.

An understanding of the role of the cone mosaic in the fluctuations in color appearance of tiny flashes of light has been hampered for at least two reasons. First, it has not been possible to determine the topography of the three cone classes in the subject's eye. Second, blur by the eye's optics has prevented imaging a spot of light on the fovea with an area smaller than that of a dozen or more cones. We have overcome both these problems by using an adaptive optics system (Hofer et al., 2001) that removes blur caused by imperfections in the eye's optics.

Methods

Psychophysics

Adaptive optics combined with retinal densitometry (Rushton, 1972; Roorda & Williams, 1999; Roorda,

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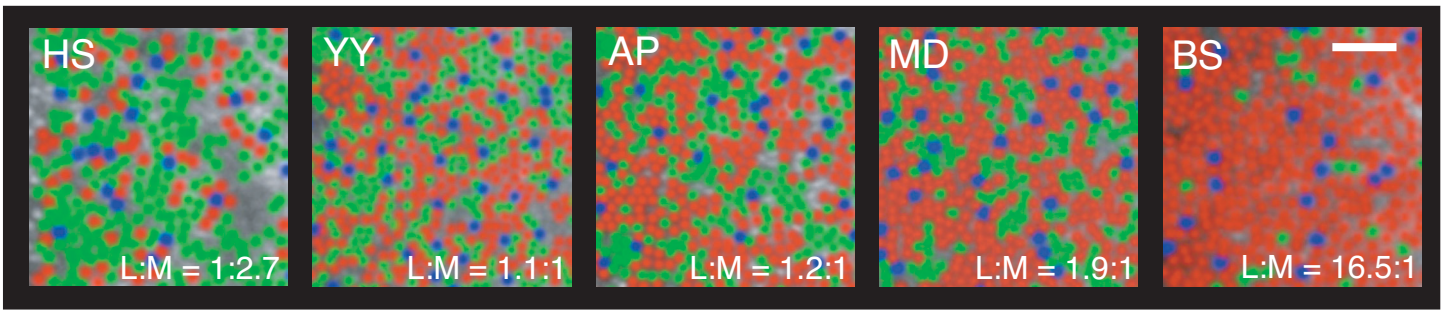

Figure 1. The retinal mosaics of the five subjects studied. Each figure shows the location of L (red), M (green), and S (blue) cones in patches of retina at approximately 1-deg retinal eccentricity. The ratio of L to M cones for these subjects is HS, 1:2.7; YY, 1.1:1; AP, 1.2:1; MD, 1.9:1; and BS, 16.5:1. The scale bar represents 5 arcmin. All images are shown to the same scale.

Metha, Lennie, & Williams, 2001) allowed us to determine the locations of L, M, and S cones in patches of retina near 1-deg retinal eccentricity in five subjects with normal color vision (Hofer, Carroll, Neitz, Neitz, & William, 2005) (see Figure 1).

Brief (<4 ms), monochromatic (500 nm, 550 nm, and 600 nm) test flashes were presented at ~1-deg retinal eccentricity. The subject viewed the stimulus through an adaptive optics system to minimize the diameter of the test flash. The stimulus consisted of a 25-micron pinhole backlit by a broad-band white light light-emitting diode (LED), which subtended just less than 0.3 arcmin at the retina. Based on convolutions of the pinhole with the point-spread functions calculated from wave aberration measurements for each observer, the test flash full width at half maximum was approximately one-third of an arcmin. This is less than half the diameter of an individual cone inner segment near 1 deg, which ranged from 0.8 to 1.0 arcmin for the subjects we used. Wavelength was controlled with narrow-band (10 or 25 nm) interference filters, and a suitable focus correction was made for the chromatic aberration of the eye at each wavelength. Stimuli were presented near threshold on an otherwise dark field except for an 820-nm point source, which served as the fixation target as well as the wave-front sensing beacon necessary to measure the eye's optical quality during adaptive correction. The intensity of the beacon required for accurate wave-front sensing was higher than that required for fixation alone. For this reason a control experiment was performed on two subjects (YY and AP) to ensure that the brightness of the beacon did not affect spot detection (see Figure 2). To suppress any contribution from rods, trials were performed in 7-min blocks from 4-11 min after a white light bleach of both rod and cone pigment.

We sought to distribute the test flashes fairly uniformly throughout the retinal area that had been characterized in each subject, so the results would not be biased by local variation in L and M cone density. Flashes were presented to one of five retinal locations, four of which lay at the corners of a square retinal region 14 arcmin on a side, and one of which lay at the center of the square. Fixational eye movements further dispersed the test flash location throughout the characterized region. The average standard deviation in fixation measured under similar experimental conditions in three of the subjects from the displacements between multiple retinal images was about 3.5 arcmin. The position of the stimulus was controlled manually with precision micrometers. The five locations were randomly permuted between each 7-min block of stimulus trials.

The stimulus duration was chosen to minimize the motion blur due to eye movements. The frequency of motion artifacts could be readily estimated from the individual retinal images, which were acquired with a 4-ms imaging flash, obtained in the same subjects while classifying cones. Roughly 5% of imaging trials were subject to motion blur. The color-naming stimuli were always briefer than 4 ms. On those few trials where motion blurred stimuli they probably went undetected by the subject. This is because

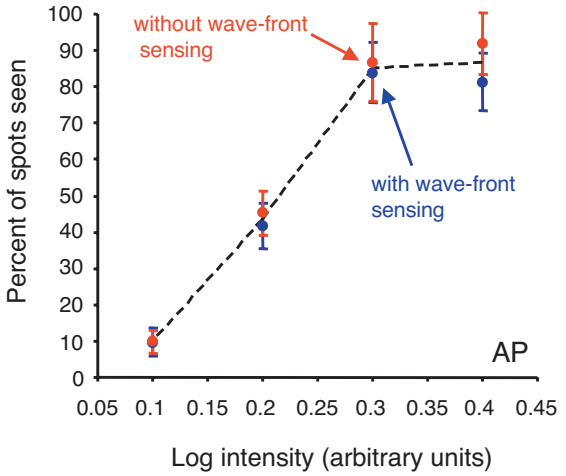

Figure 2. The detection curve for one subject (AP) for a 550-nm small spot of light when using a dim 820-nm fixation target (~0.25 μw incident on the eye's pupil) judged just bright enough for fixation (red dots, without wave-front sensing), and when using the relatively bright 820-nm wave-front sensor beacon (~1.25 μw incident on the eye's pupil) as the fixation target (blue dots, with wave-front sensing). The brightness of the wave-front sensing beacon did not affect the number of flashes seen. Results for another subject (YY) were similar. Flashes were presented without aberration correction to a single location at 1.25 deg retinal eccentricity through a 3-mm artificial pupil.

the motion would have caused the tiny threshold stimulus to be spread over a large number of cones, which makes it unlikely that enough quanta would be absorbed by those cones for detection to occur. Our experiments indicated the main result of the study was obtained even when using stimuli as brief as 100 microseconds, which is an order of magnitude too brief to be affected by eye movements.

Adaptive correction and stimulus presentation were self-initiated by subjects. On each trial subjects were asked to report whether or not the test flash was seen, and if so its appearance using one of eight hue categories (red, orange, yellow, yellow-green, green, blue, blue-green, blue, and purple) or white. Two subjects (AP and YY) required an additional "indescribable" category for when the flash was seen yet caused no definable perceptual response. When analyzing the data, trials were kept only if the adaptive correction had reached an acceptable level, chosen to be a residual root-mean-square wave-front error over a 6-mm pupil of 0.11 microns or less. For most subjects about 25% of trials were rejected because they did not meet these criteria. This was important to ensure a relatively constant retinal stimulus profile. Typically, stimuli were presented at 5-6 intensity levels spanning each subject's detection curve for each wavelength. Intensity was randomized from trial to trial. Approximately 10% of trials contained no stimulus. These trials were used to assess the subjects' error rates, which were always less than 1.5%. For two subjects, BS and AP, the experiment was repeated for one wavelength (BS, 600 nm; AP, 550 nm) on a different occasion separated by some months from the main experimental sessions. They did not show any significant difference in their responses.

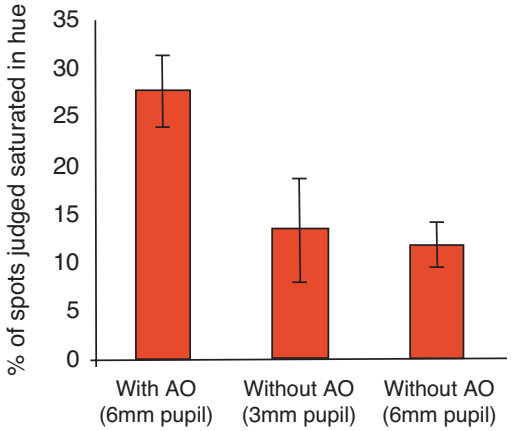

Figure 3. The percentage of 560-nm flashes seen that were judged saturated in color when aberrations were corrected with adaptive optics (with AO) and when aberrations were uncorrected (without AO). Twice as many flashes were judged saturated in color when adaptive optics was used to sharpen the small spot stimulus. All stimuli with adaptive optics were viewed through a 6-mm artificial pupil. Without adaptive optics, data are shown for stimuli viewed through both 3-mm and 6-mm artificial pupils. Data are averaged for three subjects and error bars represent ±1 *SD*.

The average number of stimulus trials per wavelength for each subject was HS, 90; BS, 525; AP, 823; YY, 826; and MD, 1495.

All research on human subjects adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board at the University of Rochester. Informed consent was obtained from all subjects after explanation of the nature and possible consequences of the study. None of the data reported here were obtained on the eyes of the authors; however, the first author verified the main conclusions of the experiment on her own eye.

Results and discussion

Figure 3 illustrates the benefit of using adaptive optics to observe the color fluctuations of tiny spots. In preliminary experiments, sharpening the flash of light imaged on the retina with adaptive optics increased two-fold the fraction of 560-nm flashes that appeared a saturated color.

A main result of this initial investigation of the color fluctuations of tiny flashes of light is that subjects required a large number of hue categories to describe their percepts, in disagreement with previous work that has suggested that only two hue categories are needed to describe tiny flashes in the long wavelength end of the spectrum (Cicerone & Nerger, 1989; Krauskopf, 1978). To facilitate a comparison of the color-naming results across subjects, data were interpolated at the 50% probability of seeing using a linear interpolation of the data between 20% to 85% probability of seeing, where the percentage of flashes seen in each hue category tended to be approximately constant or else vary in an approximately linear way, given the uncertainty of the data, for each subject. Subjects' responses for 550-nm flashes of light at 50% probability of seeing are shown in Figure 4. Subjects with L-rich retinas report a larger fraction

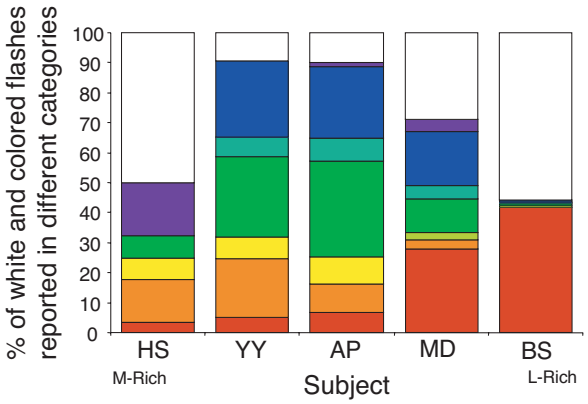

Figure 4. The color sensations reported by subjects when viewing a small spot of 550-nm light. At this wavelength only L and M cones participate in detection. Shown are the percentages of white and colored responses that were placed in each response category, interpolated at 50% frequency of seeing. Percentages for BS are white, 56%; red, 42%; yellow-green, 0.7%; green, 0.7%; blue-green, 0.3%; and blue, 0.8%. In addition to white, each subject used at least five different hue categories.

of flashes as red, and M-rich subjects tend to report a larger fraction of flashes as green. However, the most striking observation is that all subjects required five to seven of the eight hue categories and all required white.

Table 1 lists the percentage of spots each subject placed in each color category, interpolated at 50% probability of seeing, for each wavelength. The large variety of colors seen and the general trend of increasing reds and decreasing greens with higher L to M ratios are present at all three wavelengths tested. The difference we observed in color-naming behavior across subjects is different from the results of previous experiments performed without adaptive optics, where the statistics of the color names given to small, dim stimuli presented to the fovea were found to be constant across individuals (Bouman & Walraven, 1957; Ingling et al., 1970; Cicerone & Nerger, 1989; Krauskopf, 1978). The dependence of red and green responses on L to M cone ratio is strikingly different from what is known about the color appearance of macroscopic stimuli. In the latter case, color appearance as assessed, for example, by unique yellow is completely independent of L to M cone ratio (Brainard et al., 2000; Neitz, Carroll, Yamauchi, Neitz, & Williams, 2002).

Data in Figure 4 and Table 1 also reveal that subjects reported blue or purple sensations for both 550-nm and 600-nm flashes of light. These stimuli presented at threshold for the L and M cones are unlikely to stimulate S cones because S cones are over a 100 times less sensitive than L or M cones at these wavelengths. Moreover, S cones represent only about 5% of the cones at this retinal location. This implies that only L or M cones can contribute to detection for 550-nm and 600-nm flashes presented at threshold. That subjects report blue and purple sensations at these wavelengths indicates that light absorption in S cones is not essential for the sensation of these hues. If L and M cones contribute to sensations of red and green, respectively, as predicted by the standard model of color opponency, then blue and purple sensations would be prohibited. Our data support previous suggestions that M cones may contribute to sensations of blueness (Drum, 1989; DeValois & DeValois, 1993; Schirillo & Reeves, 2001). A possible explanation for the bluish sensations is that they occur when the test flash excites M cones much more strongly than L cones, which mimics the ratio of excitation that would occur when actually viewing a bluish light. Another possibility is that blue or purple sensations are the result of electrical coupling between L and M cones and S cones. However, this seems unlikely because blue responses decreased with wavelength in a manner suggestive of the relative excitation of M to L cones, and recent work has also suggested that S cones are not electrically coupled to L and M cones (Hornstein, Verweij, & Schnapf, 2004).

Jameson and Hurvich (1967) reported that the chromaticity of a fixation target can significantly bias color-naming behavior. While control experiments showed that dimming the 820-nm fixation point by a factor of 5 (see Figure 2) did not affect the detection of test flashes or color-naming (color-naming results not shown), it is still possible that the hue of the fixation target biased subjects' color responses. However, we do not believe this accounts for the blue sen-

500 nm, 50% probability of seeing

Subject	L:M	white	red	orange	yellow	yellowgreen	green	bluegreen	blue	purple	indescribable
HS	1:2.7	23.0	23.0	6.0	14.0	10.0	6.0	2.0	12.0	5.0	0.0
YY	1.1:1	4.5	0.6	1.5	2.0	0.0	21.0	6.5	12.0	0.0	52.0
AP	1.2:1	8.0	3.5	1.5	7.0	0.0	22.0	7.0	16.0	0.0	35.0
MD	1.9:1	22.0	21.0	1.0	0.0	2.0	16.0	3.9	31.0	4.0	0.0
BS	16.5:1	54.0	39.0	0.0	0.0	3.4	2.8	0.0	0.0	0.0	0.0

550 nm, 50% probability of seeing

Subject	L:M	white	red	orange	yellow	yellowgreen	green	bluegreen	blue	purple	indescribable
HS	1:2.7	50.0	3.6	14.3	7.1	0.0	7.1	0.0	0.0	17.9	0.0
YY	1.1:1	4.3	2.4	9.0	3.5	0.0	12.6	3.0	12.0	0.0	53.0
AP	1.2:1	7.3	5.0	7.3	6.8	0.0	24.0	6.0	18.0	1.0	24.5
MD	1.9:1	29.0	28.0	3.0	0.0	2.5	11.0	4.5	18.0	4.0	0.0
BS	16.5:1	56.0	42.0	0.0	0.0	0.7	0.7	0.3	0.8	0.0	0.0

600 nm, 50% probability of seeing

Subject	L:M	white	red	orange	yellow	yellowgreen	green	bluegreen	blue	purple	indescribable
HS	1:2.7	21.4	35.7	28.6	0.0	0.0	0.0	0.0	0.0	14.3	0.0
YY	1.1:1	3.5	8.0	28.0	5.0	0.0	2.7	0.3	0.3	0.0	52.0
AP	1.2:1	4.0	44.0	22.0	13.0	0.0	1.2	0.0	0.4	2.0	13.5
MD	1.9:1	18.0	55.0	5.0	0.0	2.8	8.0	1.5	5.5	3.5	0.0
BS	16.5:1	44.0	55.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0

Table 1. The percentage of spots seen at 500, 550, and 600 nm that subjects placed in the different color categories. Data were interpolated at 50% probability of seeing.

sations because subjects in our earlier experiments reported a significant fraction of blue responses with foveally presented flashes (560 nm and 580 nm) and yellowish fixation targets (560 nm and 580 nm) as well as no fixation target. (These observations did not require the presence of a laser beacon because a static aberration correction was used.) A possible systematic hue bias also does not affect the wide variety of responses each subject used, nor the differences in response across subjects with different L to M cone ratios.

Model of small spot detection

We created a simple model of detection to gain insight into why we observed such large variability in color responses to monochromatic flashes. Previous models of small spot detection (Cicerone & Nerger, 1989; Vimal et al., 1989; Wesner et al., 1991) relied on the assumption that the stimulus always illuminates an integral number of cones uniformly on each presentation. In actuality of course, the retinal light distribution of the stimuli used in these experiments is nonuniform and broadened by diffraction and aberrations, and thus on any given presentation will illuminate some cones more strongly than others. In addition, the number of cones expected to absorb enough quanta to elicit a response will vary from flash to flash depending not only on quantal fluctuations as previous investigators have assumed, but also on where the flash lands, for example, near the center of a cone or in between cones. The model we constructed incorporates the measured point-spread functions and the measured cone mosaics of our subjects to estimate quantum catches in the cones resulting from randomly distributed test flashes. All calculations were performed using custom MatLab software.

Stimulus light distribution on the retina

Point-spread functions were calculated from the residual aberration recorded by the adaptive optics system's wave-front sensor for each subject (HSPSF500.txt, HSPSF550.txt, HSPSF600.txt; YYPSF500.txt, YYPSF550.txt, YYPSF600.txt; APPSF500.txt, APPSF550.txt, APPSF600.txt; MDPSF500.txt, MDPSF550.txt, MDPSF600.txt; BSPSF500.txt, BSPSF550.txt, BSPSF600.txt; these files are 100 x 100 matrices written as tab delimited text files. The scale for each point-spread function is the same as that specified in the cone location file cones.txt.). These point-spread functions included the effects of diffraction and the uncorrected aberrations of both the optical system and the subject's eye, but did not include ocular scatter, which is not captured by wave-front sensors. The point-spread function was then convolved with a 0.3-arcmin circular function, representing the small spot stimulus, to generate the retinal profile of the stimulus after diffraction and blur by residual aberrations.

Though our model does not include scattered light, we believe its effects can be safely ignored. Scattered light forms a dim, diffuse halo or skirt around the core of the point-spread function generated by aberrations and diffraction. The contribution of the scatter is not well known close to the peak of the point-spread function. However, Vos et al. (1976) estimated that for a 5.8-mm pupil, similar to what we used, the amount of scattered light 5 arcmin from the peak is a thousand times smaller than the height of the point-spread function. Our use of adaptive optics increases the peak height by an additional factor of 10, implying that scattered light is roughly 10,000 times dimmer than the point-spread function peak.

Retinal mosaics

The model incorporated the trichromatic cone mosaics of each subject, obtained with adaptive optics retinal imaging (Hofer et al., 2005) (cones.txt). One problem with this was that for some subjects, not every cone in the patch of retina could be characterized, which would have distorted the model due to locations of artificially low sensitivity. In the case where there were no large patches of contiguous cones that could be successfully characterized, as occurred for HS, cone locations from a patch of a different subject's retina were used (scaled to reflect the cone spacing of the original subject), and cone identities were assigned randomly based on the observed proportion in the retina of the subject of interest. This is justified because cone pigment assignment is generally random (Roorda & Williams, 1999; Roorda et al., 2001; Bowmaker et al., 2003; Hofer et al., 2005).

Figure 5 shows an example of a stimulus light distribution and a retinal sensitivity map. Maps of retinal sensitivity were constructed by convolving arrays of subjects' cone locations with a Gaussian cone aperture function (MacLeod, Williams, & Makous, 1992; Chen, Makous, & Williams,

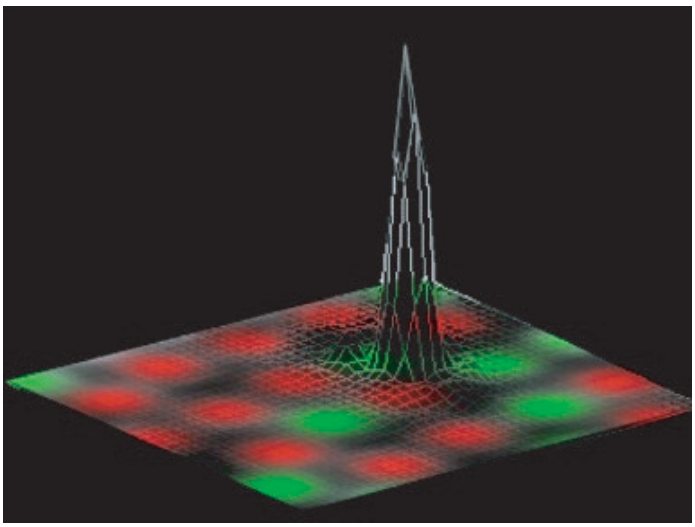

Figure 5. Example of a retinal sensitivity map and retinal stimulus profile used to model the microstimulation of the mosaic. L and M cones have been colored red and green to aid in their identification. The full width at half maximum of the retinal profile of the spot imaged with adaptive optics is smaller than the radius of individual cone inner segments near 1 deg.

1993; Qi, 1996; He & MacLeod, 1998). The actual number used in the model for the full width at half maximum of the Gaussian cone aperture function was 0.615 times the inter-cone spacing. Each cone's aperture function was then weighted by the appropriate relative quantal sensitivity for L, M, or S cones using the Smith and Pokorny cone fundamentals (Smith & Pokorny, 1975). It was assumed that the L, M, and S cones have equal quantal sensitivity at their respective peak wavelengths.

Generating cone quantum catches

Monte-Carlo simulations were performed in which the computed stimulus light distribution was allowed to fall randomly throughout the retinal patch. The stimulus was restricted from falling within a buffer zone near the edge of the sensitivity map to ensure that the entirety of the stimulus light distribution landed within the retinal area considered. On each presentation the average number of photons absorbed by each cone was computed by integrating the product of each cone's sensitivity profile and the stimulus light distribution. The actual number of photons absorbed by each cone was computed from these averages by assuming that a random Poisson process governs absorption. This process generated the number of photons absorbed for each cone in the array for each trial.

Detection rules

The results of the model depend on the minimum number of quanta required for detection to occur. In accordance with previous estimates (Cicerone & Nerger, 1989; Wesner et al., 1991; Marriot, 1963; Williams et al., 1981), a minimum number of quanta required for detection in the range of 1-10 photons was considered. The results of the model also depend on rules for pooling signals across cones prior to detection. Detection was modeled under two different scenarios, independent cone detection and spatial summation of all cone signals. In the case of independent cone detection, detection occurred when any cone absorbed at least the requisite number of photons for threshold, and all cones absorbing at least this number of quanta participated in detection. In the case of spatial summation of all cone signals, detection was assumed to occur if the sum of quanta received by all cones exceeded the minimum number of quanta required for detection. All cones receiving quanta in trials where detection occurred were assumed to participate in the detection process. The minimum number of quanta required for detection that provided the best match between the slopes of the model's and subjects' psychometric functions was 10 or more quanta if cones detect independently, and 6-7 quanta if signals are summed over all cones.

Figure 6 shows the model's results for the percentage of flash detections at 550 nm that will be mediated by individual cones at 50% probability of seeing for both spatial pooling and independent cone detection. Results for the other wavelengths in the study were similar. As can be seen, the detection rule dramatically influences the number of cones participating in the psychophysical task. If cones are independent detectors, the model predicts that more than 90% of test flash detections will be due to excitation of individual cones at 50% probability of seeing. However, if cones pool their signals across the entire retina, fewer than 3% of test flash detections will be due to individual cone excitation, with detection of most test flashes mediated by two or three cones. The curve representing spatial pooling was generated under the assumption that there is complete summation across the entire patch of retina. Psychophysical estimates of spatial pooling in foveal vision are substantially smaller, not more than three cones (Davila & Geisler, 1991; Sekiguchi et al., 1993). However, the size of the summation pool assumed has very little effect on the spatial pooling curve in Figure 6. This is because if cones pool their signals even modestly, it is unlikely that one cone alone will reach the requisite number of quanta for threshold without the surrounding cones also absorbing some quanta.

If foveal cones act as independent detectors, then detection of near-threshold tiny test flashes is almost always mediated by a single cone. If this were true, then the rich diversity of color sensations reported by our observers at threshold would immediately imply that stimulation of two cones of the same class will not necessarily evoke the same color sensation. This is because our observers required six-to-eight color categories in circumstances when only two classes of cones (L and M) were capable of participating in detection. On the other hand, there is little evidence that

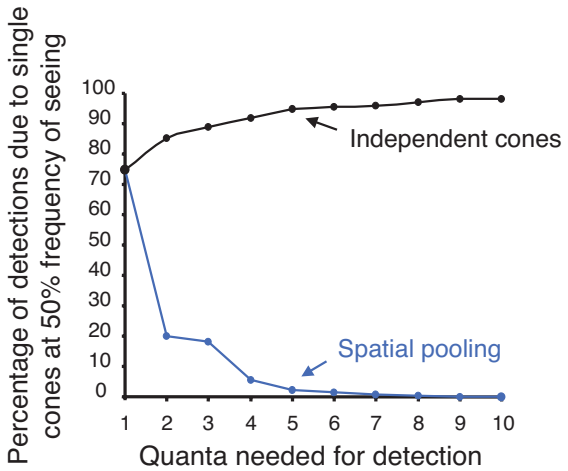

Figure 6. The model's prediction of the percentage of detections at 50% frequency of seeing that are mediated by a single cone as a function of the minimum number of quanta that must be absorbed for detection to occur. For independent cones, the *x*-axis represents the number of quanta each cone is required to absorb if it is to participate in detection. For spatial summation of all cone signals, the *x*-axis represents the number of quanta that must be absorbed by the entire ensemble of cones if detection is to occur. Each curve represents 2000 simulations at a wavelength of 550 nm. The best match to subjects' data is 10 or more photons if cones detect independently and 6-7 photons if signals from cones are summed across the retina.

cones are independent detectors, and several studies indicate that foveal cones pool their signals to some extent (Davila & Geisler, 1991; Sekiguchi et al., 1993; Hsu, Buschbaum, & Sterling, 2000; DeVries, Qi, Smith, Makous, & Sterling, 2002). If cones pool their signals at detection threshold, then multiple cones contribute to detection, even for the very tiny stimuli we used.

Can excitation of multiple cones explain the diversity of subjects' responses?

If multiple cones are involved in detection, then it might be possible to explain the diversity of color sensations experienced by subjects to variations in L and M cone quantum catches from flash to flash, without having to conclude that excitation of one particular L(M) cone can result in a different sensation than excitation of any other L(M) cone. For example, white percepts might result from the combined excitation of L and M cones (Krauskopf, 1978).

We do not believe that the diversity of color experiences our subjects reported can be completely explained by combined stimulation of both L and M cones. The fraction of white responses made by subjects with different cone ratios is not consistent with the idea that all white responses are due to excitation of mixtures of both L and M cones. This theory predicts that subjects with more equal numbers of L and M cones will report the most white responses, because these subjects have the largest fraction of their retinal mosaics made up of neighboring L and M cones. Figure 7 shows this expectation is not bourn out by the data. Subjects with very similar numbers of L and M cones report very few white responses, whereas subjects with very different numbers of L and M cones report a large number of white sensations, despite the fact that it is least likely that both L and M cones will participate in flash detection for these subjects.

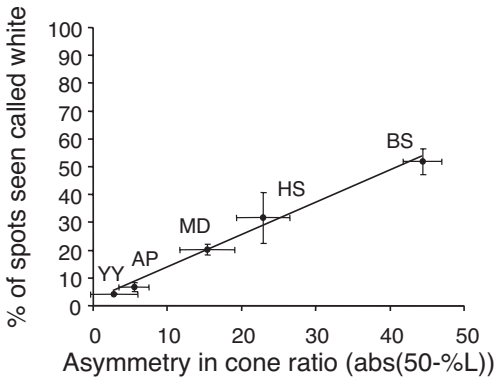

Figure 7. White responses as a function of the asymmetry in L to M cone ratio. Shown is the percentage of all spots seen that each subject reported as white, averaged from 20% to 85% probability of seeing and averaged over wavelength. Subjects with the most balanced numbers of L and M cones report the fewest white responses, whereas subjects with the most extreme ratios of L and M cones report the greatest number of white responses. The same trend is evident at each of the three individual wavelengths tested. This is contrary to expectation if all white responses could be explained by stimulation of mixtures of L and M cones. Error bars represent ±1 *SD*. Horizontal error bars represent the uncertainty in the determination of each subject's L to M ratio.

Moreover, the fraction of white responses for subjects with extreme ratios is too large to be explained by combined excitation of M and L cones. With our detection model, we also calculated the expected fraction of all flashes seen in which detection is mediated by both L and M cones. This is the upper bound on the number of white responses that is consistent with the mixture theory. Figure 8 shows this upper limit for one subject, BS, at 550 nm. Because this subject has so few M cones in his retinal mosaic, even if cone signals are summed across the entire retina, both L and M cones will be excited on fewer than a quarter of all trials in which the flash is seen. However, BS reported over 55% of all flashes seen as white. This is significantly higher than the upper bound allowed if all white responses are caused by combined excitation of L and M cones, and this discrepancy increased for longer stimulus wavelengths. This result implies that white sensations can result from excitation of cones of only one class. Apparently, then, stimulation of cones containing the same photopigment can give rise to different color sensations.

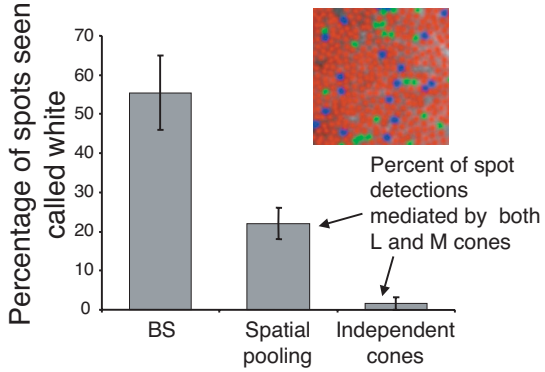

Figure 8. Percentage of spots seen called white by BS at 550 nm, 50% frequency of seeing, compared with the maximum percentage of white responses allowed if white responses are caused only by combined excitation of L and M cones. Predictions from the model are shown if all cone signals are summed and if cones detect independently. Error bars for BS are the 95% confidence limits. Error bars on the maximum percentage of white responses allowed by excitation of both L and M cones represent the range for quantal detection thresholds from 5-10 photons. The inset shows BS's retinal mosaic. Because there are so few M cones, even if cones' signals are summed across the entire retina, both L and M cones will be excited on fewer than a quarter of all trials in which the flash is seen. However, BS reported over 55% of all flashes seen as white, more than twice the maximum expected if white responses are caused only by combined stimulation of L and M cones.

Other explanations for the white response

While equal and simultaneous excitation of both L and M cones may very well cause a white percept, the actual behavior exhibited by subjects with different cone ratios indicates that this cannot explain the majority of white sensations experienced. We explored two alternative explanations for the origin of the white response. One hypothesis is that the circuitry responsible for carrying chromatic signals, whether it be the midget system or some other pathway, is highly spatially localized in the retina. If this is true, there will be some retinal regions, due to the generally random arrangement of L and M cones, where only one cone type will be present, and it will not be possible to form a spectrally opponent signal. Perhaps excitation of cones in these regions does not evoke a chromatic response. In this case the number of white responses is expected to be proportional to the fraction of cones in each subject's mosaic that are in clumps of like-type cones. Because the number of clumps will increase with the asymmetry in cone ratio, subjects with the most balanced ratios will exhibit the fewest white responses, in line with our results.

Another hypothesis is that the white responses are a consequence of the different neural weighting that must be given to signals arising from individual L and M cones in subjects with different relative L and M cone numerosity. For example, consider YY and BS. Both see a large stimulus of 580 nm as yellow, yet BS has about 16 times more L cones for each M cone than YY. This implies that somewhere in the chromatic pathway a signal from an individual M cone in BS's retina must acquire a weight about 16 times larger, relative to the weight given to an individual L cone signal, than is given to the signal from an individual M cone in YY's retina. It would be expected that an L cone in both YY's and BS's retinas would be required to absorb the same number of quanta for either of them to detect the presence of a stimulus. However, depending on where in the chromatic pathways signals from L or M cones are normalized, it could be that an L cone in BS's retina may have to absorb 16 times more quanta than an L cone in YY's retina before BS will say he saw the stimulus as colored. This essentially results in separate thresholds for detecting a stimulus and seeing a stimulus as colored, with the difference being largest in those subjects with the least balanced ratio of L to M cones. This is similar to an idea put forth by Massof (1977) to explain the variation in appearance of near-threshold stimuli as a consequence of quantal fluctuations and generalized opponent color mechanisms.

The percentage of flashes seen that should be called white, given this hypothesis, was modeled under the simple assumption that the most numerous cone type in the retina has a separate threshold for seeing color that is related to the detection threshold by the ratio of the more numerous to least numerous type of cone. This hypothesis was modeled at 550 nm, so only L and M cones were considered. For example, with a minimum number of quanta required for detection of n, we assumed, for a subject with a ratio of L to M cones of 3 to 1, that a white response would occur when only L cones absorbed quanta from the flash and the L cone excitation (either combined, in the case of spatial pooling, or for each individual L cone, in the case of cone independence) was at least as large as n but less than 3 n (for less than n quanta absorbed no detection occurs). We did not consider additional white responses that may be due to mixtures of L and M cone excitation, as the low numbers of white responses made by YY and AP, who exhibit L to M ratios near 1 to 1, indicate that these should be responsible for a very small number of white sensations. In both cases, for cone spatial pooling and cone independence, the model's prediction of the percentage of spots seen that should be called white did not depend significantly on the number chosen for minimum number of quanta required for detection.

Figure 9 replots the percentage of spots seen that each subject called white as well as the percentage of cones in each subject's mosaic that were in clumps of like-type cones (L or M cones that neighbored only other L or M cones), and the model's results for the percentage of spots seen that should be called white if white responses are caused by the normalization hypothesis, as a function of cone ratio asymmetry. For comparison, the percentage of cones in each subject's mosaic that border cones of another type, which would predict white responses if they were only due to combined L and M cone excitation, is also shown. It is

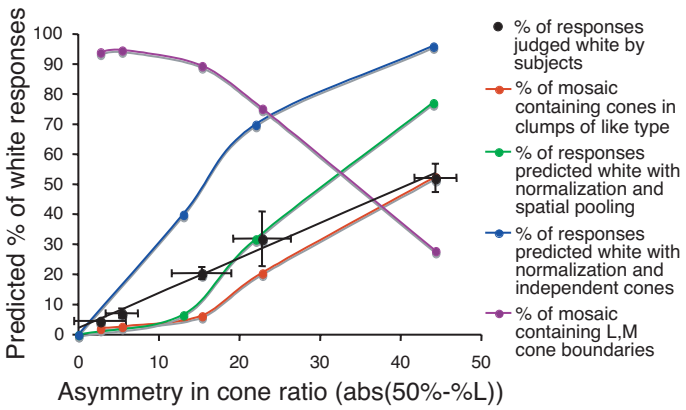

Figure 9. Comparison of the percentage of flashes seen that subjects called white (black dots) with predictions based on the hypothesis that cones within clusters of like-type cannot signal chromatic information (red curve) and the hypothesis that white responses result from differences in neural weighting given to L and M cone signals (blue and green curves). Also shown is the fraction of each subject's mosaic that contains L and M cone borders (purple curve); subjects' data should follow the shape of this curve if most white responses were caused by combined stimulation of L and M cones. Subjects' responses are best predicted by considering the fraction of cones in the mosaic within clumps of like-type. Data for the neural normalization scenarios represent 2000 simulations at each cone ratio with a 550-nm flash at 50% probability of seeing; results were averaged for detection thresholds from 5-10 quanta.

quite clear that both the neural normalization hypothesis with spatial pooling of cone signals and the hypothesis that cones within clumps produce white sensations qualitatively predict subjects' behavior. However, the hypothesis that cones within clumps produce achromatic sensations comes closest to matching subjects' data. Note that the neural normalization hypothesis with cones acting as independent detectors does a poor job of predicting subjects' behavior.

If cones do pool their signals, then both of these alternative explanations for the white response seem to qualitatively match the behavior of subjects' white responses with cone ratio asymmetry. However, if white responses are due to differing thresholds for detecting and seeing color as a result of a neural normalization, then the model also predicts, as expected, that the number of spots that appeared colored should rise with increasing probability of detection and the number of white responses should decrease. For subjects in general this is not what occurred (data not shown). In fact, only one subject, YY, generally showed an increase in colored responses as the probability of seeing increased, and because this is the subject with the most balanced cone ratio, this is the subject that would be least affected by the type of neural normalization considered here. For all other subjects the number of colored responses either decreased or remained constant as the probability of seeing increased. This makes the hypothesis that the white responses were mainly due to the effects of normalization in the chromatic pathways (at least in the simple manner considered here) somewhat less plausible than the alternative that white responses are linked to the spatial organization of the cone mosaic.

Conclusions

The spatial grain of the cone mosaic is remarkably invisible in perceptual experience (Williams, 1990). For stimuli of large spatial extent, color vision is independent of the relative numbers of cones, and color circuitry organizes itself to produce constant perception despite variations in the relative numbers of cones (Brainard et al., 2000; Neitz et al., 2002; Pokorny & Smith, 1987). But adaptive optics allows us to present stimuli on a smaller spatial scale than arises in normal perceptual experience, stimuli for which cortical circuitry had no opportunity to develop. Our experiments firmly reject the idea that excitation of all cones within the same class results in the same hue sensation. This idea has been implicit in nearly all other experiments on the appearance of small spot stimuli (Hartridge, 1954; Krauskopf, 1964; Krauskopf & Srebro, 1965; Krauskopf, 1978; Otake et al., 2000). Our results run counter to a commonly held view of the organization of color vision throughout the history of its investigation, which we refer to as the elemental sensation hypothesis. Helmholtz (1896) endorsed this view when he stated that "The eye is provided with three distinct sets of nervous fibers. Stimulation of the first excites the sensation of red, stimulation of the second the sensation of green, and stimulation of the third the sensation of violet." Though Helmholtz's view has been superseded by modern color theory in which each cone class contributes to the hue of a stimulus through two opponent mechanisms (Hurvich & Jameson, 1957), even opponent color theory explicitly links the hues perceived with stimulation of particular cone classes.

One apparent challenge to elemental sensation theory comes from a large body of literature demonstrating that excitation of cones at a distant retinal location can influence perceived color (e.g., Chevreul, 1839). Another apparent challenge is that color signals from cones can be strongly influenced by the excitation of other cone classes in the same retinal location (Knoblauch & Shevell, 2001). However, neither of these phenomena actually rejects the elemental sensation theory because both can be attributed to postreceptoral interactions among signals arising from cones with different spatial locations or photopigment. The notion survives that excitation of cones within the same class should result in the same hue sensations when stimulation of adjacent locations is precluded. Here we show that each cone class can signal multiple chromatic sensations even in the absence of changes in stimulation elsewhere in the retina or in other classes of cones. Our data indicate that even isolated stimulation of cones containing the same pigment can result in different color sensations.

Why should the number of sensations produced by excitation of individual cones exceed the number of cone classes? The visual system uses signals from single cones to derive intensity as well as spectral information, and ideally these attributes should be extractable at every retinal point. However, the cone classes are intermingled in a single mosaic so trichromatic vision is impossible on the spatial scale of a single cone. Furthermore, the cone classes are randomly arranged in the mosaic, creating clumps of cones of like type, which exacerbates the problem of collecting three spectral samples at every point. Moreover, neural circuits, such as those responsible for the receptive fields of ganglion cells, tend to draw their cone inputs from localized retinal regions. Consequently, every cone of the same class cannot possibly make the same contribution to cortical circuitry for extracting hue and brightness.

Given these organizational constraints, it may be inevitable that color sensations are not uniform within a single class of photoreceptors and reflect instead the microcircuitry of postreceptoral color mechanisms. For example, it may be that cones within clusters of cones of the same class generate achromatic sensations because the localized circuitry they serve cannot be spectrally opponent, and the task of conveying hue is left to circuits that are able to draw signals from cones of different classes. It is also conceivable that the white verses colored responses our observers frequently reported correspond to the activity of different

retinal circuits that have already been identified. For example, white responses could be mediated by the parasol ganglion cells, whereas colored responses could be mediated by the midget pathway. Coupling between cones (Hsu et al., 2000; DeVries et al., 2002; Hornstein et al., 2004) could also play a role in generating the observed diversity of color experiences.

In this first study, the use of adaptive optics allowed us to probe visual microcircuitry with much smaller psychophysical stimuli than has been possible before. It has also allowed us to characterize the optics of the eye and the trichromatic cone mosaic in the same subjects. However, a complete understanding of the topography of the functional microcircuits underlying color vision will require the ability to record which cone(s) is stimulated with each tiny probe. Our present experiments do not allow us to distinguish with certainty whether different cones of the same class evoke different sensations or whether different sensations can result from stimulating the same cone multiple times. Putnam et al. (2005) have shown that it is possible to measure the location of a stimulus on the cone mosaic with an accuracy of one-fifth of a foveal cone diameter. It may ultimately be possible to use this method to assign color experiences to specific cones in the cone mosaic.

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