Psychophysical Evidence for More Than Two Kinds of Cone in Dichromatic Color Blindness

Abstract. *Psychophysical evidence shows that at least some classically diagnosed dichromats have three cone types rather than two. The anomalous cones, previously thought to be absent, are less sensitive than normal cones to both spectral and temporal variations, and have spectral sensitivities like those of the abnormal cones of anomalous trichromats. These results are not consistent with either loss or replacement models of X-linked recessive color-vision defects, since some dichromats apparently have the same three photopigments as anomalous trichromats.*

Next to complete color blindness, red-green dichromacy is the most severe color vision defect. According to widely accepted loss models (*1*), a dichromat has only one of the two cone classes or photopigments sensitive to long wavelengths, not both. Although there have been some indications that some dichromats possess the "forbidden" class of long-wavelength-sensitive cones as well (*2*), we have found direct evidence for the presence of such cones and have measured their temporal and spectral sensitivity.

The principle of univariance (*3*) was used to isolate the responses of single classes of cones. The photopigment of a cone cell responds only to the number of quanta captured—not to their wavelength per se. Therefore, when the eye is presented with a mixture of two wavelengths, with magnitudes varying oppositely over time, it is possible to adjust the relative intensities of these two components to keep the number of quanta captured by a single cone type invariant. By adjusting the relative amplitudes of alternating red and green lights flickered sinusoidally in counterphase, it is possible to produce a constant response in one long-wavelength-sensitive cone type but not in another. A constant response is produced for one cone type when the ratio of intensities of the two flickering lights produces the same quantum capture from both. Thus, if dichromats have only one long-wavelength-sensitive cone type, they should see no flicker with such a stimulus (provided that its modulation is too small for other receptors to detect).

The results we obtained with six dichromats were unexpected: None of these observers was able to find any red:green amplitude ratio that eliminated perceived flicker. The color vision of each observer had been classified on the basis of several standard tests, including the AO H-R-R pseudoisochromatic plates, the Nagel anomaloscope, and neutral-point determination (*4*). Three observers were identified as protanopes (red-missing), and three as deuteranopes (green-missing).

The test stimuli were generated by a four-channel Maxwellian-view system (*5, 6*). The flickering light—red and green alternately—was viewed as a circular spot (*7*). The luminance of the red and green lights varied inversely, so that the ratio of red to green could be varied without affecting the total luminance of the combined red and green lights. This allowed us to determine the red:green ratio for minimal perceived flicker.

The six dichromats were asked to eliminate the perception of flicker by adjusting the relative amplitudes of the counterphase red and green lights when these lights were flickered at 100 percent modulation at 12 Hz. None of the dichromats were able to do this (*8*). They were also unable to completely eliminate flicker by varying the relative phases of the red and green lights once minimum flicker had been achieved (*9*). Contrary to the loss models, the dichromats seem to have more than one long-wavelength-sensitive receptor capable of detecting flicker of the red and green light.

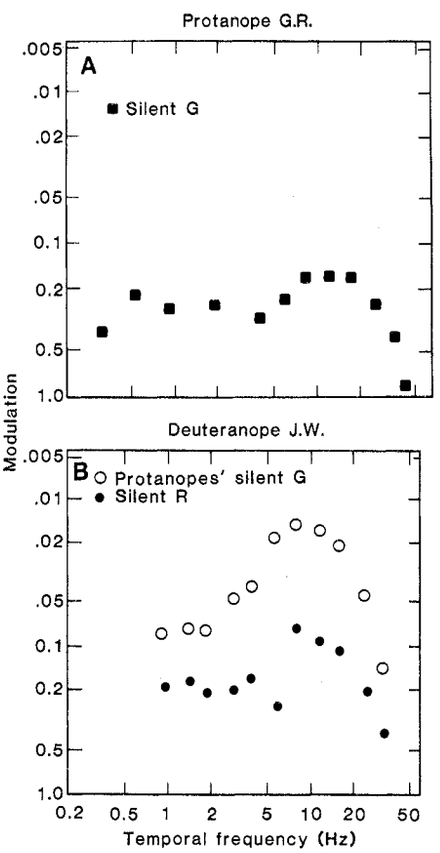

Fig. 1. (A) Flicker sensitivity of protanope G.R. when his green cones have been silenced. (B) Flicker sensitivity of deuteranope J.W. when his red cones have been silenced (closed circles) and at the protanopes' silent green ratio (open circles).

To select the amplitude ratio that "silenced" the more sensitive class of long-wavelength cones—that is, the ratio that produced a constant quantum catch, hence a temporally invariant response in these cones—the average of 20 minimum-flicker red:green ratio settings was taken for each dichromat. The flicker sensitivity and the spectral sensitivity of the "forbidden" cones were measured using this ratio.

To measure flicker thresholds for different temporal frequencies, the apparatus permitted observers to vary the modulation amplitude of the red-green light without any change in the total luminance over time (*10*). Each subject made pairs of judgments, one on each side of the point of the extinguished flicker, and a computer (PDP/11) converted the difference between the two settings into modulation amplitudes. In each run, three pairs of settings were obtained at each of 12 frequencies, for a total of 72 thresholds. The frequencies were randomized.

Temporal sensitivity functions for single forbidden cone types are shown in Fig. 1. Protanope G.R.'s flicker thresholds were measured when his red:green ratio setting had eliminated the contribution of his dominant cones, so that the only cones being stimulated were the forbidden long-wavelength cones (Fig. 1A). Comparable data for deuteranope J.W. are shown as closed symbols in Fig. 1B and were obtained for all other dichromats tested.

These data, gathered when the contribution of the dichromat's dominant cone class has been eliminated, are not unlike those that would be obtained by stimulating only one class of cones in normal trichromats. That is, they contain a composite curve of the presumed 10-Hz luminance pathways and the 2-Hz color pathways described by Kelly and Van Norren (*5*). Thus, the forbidden cones seem to contribute to both mechanisms.

The stimulus showed to deuteranope J.W. to obtain the open data points shown in Fig. 1B was the mean red:green ratio that gave minimum flicker to protanopes, the other class of dichromats. These points look like the luminance flicker curve obtained with normal observers, which Kelly and Van Norren

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have argued reflects interactions of more than one long-wavelength mechanism. Thus, the data are additional evidence for the presence of two long-wavelength receptors in a dichromat. Moreover, the deuteranope's two long-wavelength receptors cannot be the same as the protanope's because the red:green ratio that silenced the protanopes' green-sensitive cones did not silence the deuteranope's. This implies that the spectral sensitivities of the green-sensitive cones in the two types of dichromats (and by the same reasoning, those of the red-sensitive cones) cannot be identical.

We next measured the spectral sensitivity of the forbidden cones. The red:green ratio that produced minimum flicker for each of the observers was viewed as a 1° circular field imposed upon a 10° circular background field of monochromatic light. At 10-nm increments, from 430 to 670 nm, the subject increased the intensity of a monochromatic background field until flicker could no longer be detected in the 1° field and then decreased the background intensity until flicker reappeared. Both descending and ascending thresholds were obtained during traverses of the spectrum in both directions. The four threshold settings thus obtained were averaged to provide an estimate of sensitivity at each wavelength. Figure 2A shows spectral sensitivity data obtained in this way for deuteranope J.W. The closed symbols at the bottom show J.W.'s data from signals provided by his anomalous green-sensitive cones only, that is, when his red:green setting had eliminated the relevant variation over time of responses by his normal red-sensitive cones (minimal perceived flicker). The spectral sensitivity of this anomalous photopigment has the same shape and peak wavelength as the anomalous cone photopigment that has been measured in simple deuteranomalous trichromats and that is well fitted by a template curve (*11*). The data of the other two deuteranopes show similar fits to this template curve.

Having determined the spectral sensitivity of the deuteranope's anomalous photopigment, we could select a red:green ratio that would eliminate time variation of the signal from that class of cone. The ratio of radiances of the red 640-nm and green 540-nm beams was made identical to the ratio of the subject's field sensitivities at these wavelengths measured under the silent R condition just described. The data obtained with this red:green ratio (the silent G′ condition, shown as open circles in Fig. 2A) give the spectral sensitivity of J.W.'s normal, red-sensitive cones. [The smooth curve is based on data from normal trichromatic observers (*11*).]

For J.W., and also for the other two deuteranopes, the spectral sensitivity functions for the normal and the anomalous photopigments have different shapes and peak locations. The anomalous cones are much less sensitive than normal.

We carried out similar experiments with the three protanopes. The anomalous cones were again much less sensitive than the normal ones (Fig. 2B). As with the deuteranopes, the data for the anomalous cones (silent G condition) agree with the curve derived from the anomalous photopigment in simple-protanomalous trichromats, whereas the data for protanopes' normal, green-sensitive cones (silent R′ condition) matches the curve obtained from normal observers (*11*). However, the differences between the peaks and shapes of the normal and anomalous functions are less pronounced than those for the deuteranopes.

Our results indicate that at least some classically diagnosed dichromats have three cone types rather than two. The anomalous cones previously thought to be absent are relatively insensitive to both spectral and temporal variations and have spectral sensitivities like those of the abnormal cones of anomalous trichromats. These results are not consistent with either a loss model (*1*) or a replacement model (*12*) of X-linked recessive color-vision defects. Since some dichromats apparently have the same three photopigments as anomalous trichomats, it may be that the class of defects (protan or deutan) is determined by which normal photopigment is replaced by an anomalous analog. The severity of the defect, ranging from dichromacy (color blindness) to simple anomalous trichromacy (color weakness), may depend on the relative sensitivity of cones that contain the anomalous photopigment.

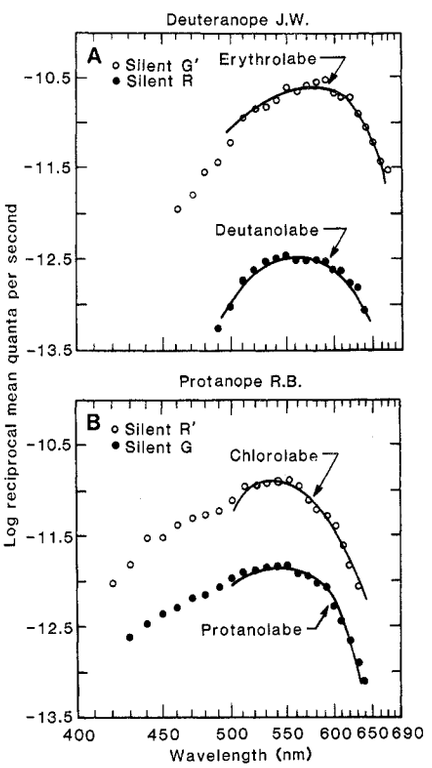

Fig. 2. (A) Exchange threshold field sensitivity of deuteranope J.W. at the silent R and silent G′ red:green ratios. (B) Exchange threshold field sensitivity of protanope R.B. at the silent G and silent R′ red:green ratios.

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References and Notes

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2. D. Farnsworth, *Vision Res.* **1**, 1 (1961); H. M. O. Scheibner and R. M. Boynton, *J. Opt. Soc. Am.* **58**, 1151 (1968); A. L. Nagy and R. M. Boynton, *ibid.* **69**, 1259 (1979).
3. The principle of univariance has been defined by W. A. H. Rushton, D. S. Powell, and K. D. White [*Vision Res.* **13**, 1993 (1973)]: "The intrinsic response of a receptor depends upon its effective quantum catch, but not upon what quanta are caught."
4. Pseudoisochromatic plates provide classifications for color observers by testing their color discrimination of figure from backgrounds in pages of differently colored printed dots. Another screening device, the anomaloscope, measures the proportion of monochromatic red that must be added to monochromatic green to provide a brightness and hue match to monochromatic yellow. Protanopes and deuteranopes can match any sufficiently bright red-green mixture to yellow, whereas normal color vision yields judgments with only small variation in the red-green ratio. A neutral point is a monochromatic light that only dichromats can match in hue and brightness to a white light. This task is impossible for normal observers. Although it is clear from our data that dichromats have three cone classes rather than two, the contribution of the forbidden cone class is not sufficient to prevent dichromats from making neutral point determinations.
5. D. H. Kelly and D. Van Norren, *J. Opt. Soc. Am.* **67**, 1071 (1977).
6. T. P. Piantanida, T. A. Bruch, M. Latch, F. D. Varner, *Vision Res.* **16**, 1029 (1976).
7. The red and green lights were produced in different ways in the two studies presented here. In the flicker study, the red and green stimuli were generated by filtering the light through Kodak Wratten filters 29 and 61, with dominant wavelengths of 633 and 534 nm, respectively. The spectral sensitivity study used Baird-Atomic 100-A° interference filters of nominal wavelengths 640 and 540 nm, respectively.
8. C. R. Cavonius and O. Estevez [*Mod. Probl. Ophthalmol.* **17**, 36 (1976)] reported that their deuteranopic subject H.S. was able to eliminate flicker at an illuminance of 800 trolands. When he visited our laboratory, however, he was unable to eliminate flicker when the level was increased to 8000 trolands.
9. In control tests, we found that flicker could be completely eliminated by any observer when this apparatus was used to flicker the same color out of phase with itself. We also found that the

shapes of the temporal sensitivity functions were the same after adaptation to a superimposed red of 678 nm as after adaptation to a blue of 470 nm, which indicates that these functions were not likely to depend on blue cone contributions. A number of less plausible optical bases for the residual flicker might be proposed, some of which we ruled out with other measurements. The role of such optical effects becomes moot, however, in view of the spectral sensitivity data.

10. A circular spot 2° in diameter and 8000 trolands was used for the flicker data. We also ran flicker studies with 10° fields and found no systematic differences in the shapes of the response curves. In the spectral sensitivity study, the circular spot was 1° in diameter and was superimposed on a 10° circular background of 8000 trolands.
11. Protanolabe and deutanolabe spectral sensitivities were measured by W. A. H. Rushton, D. S. Powell, and K. D. White [*Vision Res.* **13**, 2017 (1973)] and by T. P. Piantanida and H. G. Sperling (*ibid.*, p. 2033).
12. T. P. Piantanida, *Am. J. Optom. Physiol. Opt.* **53**, 647 (1976).
13. Supported by NIH grants EY02357 to T.P.P. and EY01128 to D.H.K. and by NIH postdoctoral fellowship EY05116 to F.S.F. This work was first reported at the 1978 Annual Meeting of the Association for Research in Vision and Ophthalmology. The abstracts appeared in *Invest. Ophthalmol.* **18** (Suppl.), 197 (1978). We thank R. M. Boynton for his help and encouragement early in the course of this work. We also thank S. L. Buck, D. Y. Teller, and especially C. S. Harris for their helpful comments on the manuscript.
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Viral Receptors on Isolated Murine and Human Ependymal Cells

Abstract. *Viruses that infect ependyma cause ependymitis in humans and hydrocephalus in experimental animals. We report that reovirus type 1 (which induces hydrocephalus in mice) binds to the surface of isolated human and murine ciliated ependymal cells. With the use of recombinant viral clones, the binding property was mapped to the type 1 viral hemagglutinin, which also determines in vivo the affinity of reovirus type 1 for ependyma. Mumps virus, measles virus, parainfluenza type 3, and herpes simplex virus type 1 bind to murine ependyma cells, whereas reovirus type 3, herpes simplex virus type 2, and poliovirus type 1 do not.*

The binding of a viral particle to the cell surface is the first obligatory step of cell infection and depends on the interaction of the virus attachment protein and the cellular receptor. The presence of a receptor for a given virus on a particular cell membrane is a primary factor in determining the affinity of that virus for the cell and the ultimate pattern of virulence. In studies of viral receptors most investigators have used continuous cell lines, and "receptor families" have been described on HeLa cells for viruses that are unrelated but may share a common receptor (*1*). Except for binding studies with lymphocytes (*2*), the binding of viruses to isolated cells known to be the target of viral infection in vivo has not been studied directly.

Infection of ependymal cells in experimental animals by certain classes of viruses, particularly those of the myxovirus group, causes hydrocephalus; in humans, cytoplasmic inclusions of viral nucleocapsid-like material have been found in ependymal cells from the cerebrospinal fluid of patients with mumps meningitis (*3*). A definitive link between viral-induced ependymitis and congenital hydrocephalus in humans has not been proved.

Studies of reovirus type 1–induced hydrocephalus in mice have demonstrated a central role for the viral hemagglutinin. This has been shown with the use of single-segment recombinant clones between reovirus types 1 and 3. Reovirus is a segmented double-stranded RNA virus containing ten genes; clone 3.HA1 contains nine genes from type 3 and one, the S1 gene which encodes the viral hemagglutinin, from type 1; 1.HA3 is the reciprocal clone. Reovirus type 1 and clone 3.HA1 cause a nonfatal ependymal infection with no neuronal damage in newborn mice, whereas reovirus type 3 and clone 1.HA3 cause a fatal encephalitis with neuronal destruction but no ependymal cell damage (*4*). These experiments in vivo suggest that the affinities of the two reovirus serotype for ependymal or neuronal cells, and their patterns of neurovirulence, are secondary to the specific interaction of the viral hemagglutinin with a receptor on the cell surface. Thus reovirus provides an excellent model for the study of viral-receptor interactions.

To study the interactions between reovirus type 1 and ependymal cells in vitro, we prepared single-cell suspensions of viable ependymal cells from the central nervous system of adult mice by the technique described by Manthrope *et al.* (*5*). These preparations contain approxi-

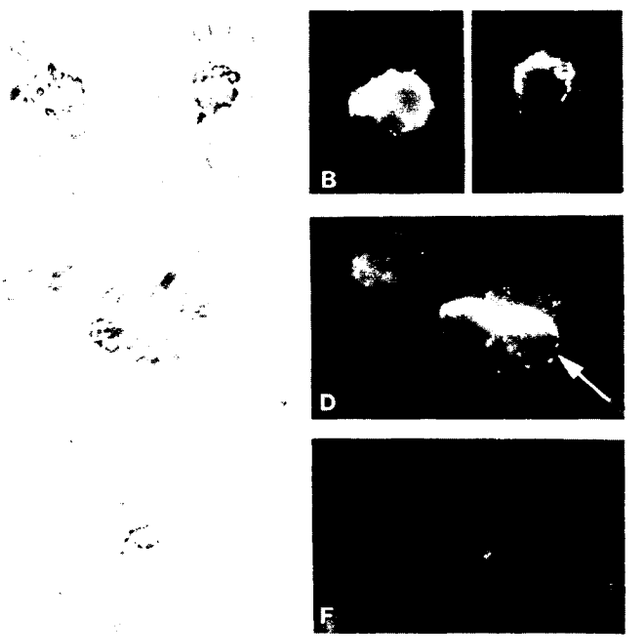

Fig. 1. The binding of reovirus to murine ependymal cells demonstrated by fluorescent staining. The cells (2×10^6 to 3×10^6, of which 50 percent were ciliated ependymal cells) were isolated by the technique of Manthrope *et al.* (*5*). The cells (5×10^5) were then incubated with 40 μl of purified reovirus type 1 or type 3 at a titer of 5×10^9 pfu/ml (the ratio of particles to plaque-forming units was 100:1; thus, 5×10^9 pfu/ml = 5×10^{11} viral particles per milliliter) for 20 minutes at 4°C, after which the cells were washed three times in F12 medium containing 25 m*M* Hepes, 1 percent bovine serum albumin, and 2.5 m*M* EGTA. The viral binding was demonstrated by indirect immunofluorescence with rabbit antibody to reovirus (*7*) and with a 1/100 dilution of FITC-conjugated goat antibody to rabbit immunoglobulin (FITC-Garig, Tago Inc., California). (A and C) Unstained ependymal cells examined by phase microscopy. (B and D) The same fields as in (A) and (C) seen by fluorescence microscopy showing bright labeling of the cell after incubation with reovirus type 1. In all instances the fluorescent dots surround the cell body; in some cases the binding is also prominent in the area of the cilia. (E and F) An ependymal cell incubated with reovirus type 3 (5×10^9 pfu/ml), stained in the same way as the cells in (B) and (D), and examined by phase (E) and fluorescence (F) microscopy. The absence of fluorescence in (F) indicates that reovirus type 3 did not bind to the cell.

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