Is colour vision possible with only rods and blue-sensitive cones?

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AT night all cats are grey, but with the approach of dawn they take on colour. By starlight, a single class of photoreceptors, the rods, function, whereas by daylight, three classes, the blue-, green- and red-sensitive cones, are active and provide colour vision. Only by comparing the rates of quantal absorption in more than one photoreceptor class is colour vision possible. Although the comparisons generally take place between the cones, they can involve the rods as well[1–4]. Here we investigate the wavelength discrimination of an extremely rare group of individuals, blue-cone monochromats, who have only rods and one class of cones[5–8]. We find that these individuals can distinguish wavelengths (440 to 500 nm) in the twilight region where the rods and blue-sensitive cones are simultaneously active.

To determine whether colour vision is possible with only rods and a single class of cones, we chose as observers five males with the rare genetic disorder known as blue-cone monochromacy or X-chromosome-linked incomplete achromatopsia[5–9]. The vision of all five of the observers can be fully described by the participation of normally functioning rods under scotopic conditions and normally functioning blue-sensitive cones under photopic ones[5,7,8]. Peripheral green and/or red cone function, which may be residual in many of the affected males in pedigrees of blue-cone monochromacy[10], was excluded by colour matching, and by spectral sensitivity[7,8] and transient tritanopia[7,11,12] measurements, made under chromatic adaptation conditions.

The psychophysical absence of the green- and red-cone sensitivities accords with the molecular genetics. Individual DNA blot hybridization patterns (J. Nathans, personal communication) reveal that the five observers have alternations in the red and green visual pigment gene cluster of their X chromosomes. The pattern in four of the observers, F.B., S.B., M.P. and K.S., corresponds to the more common of two recently reported[9], in which only one of the tandem array of red and green visual pigment genes remains and is rendered nonfunctional by a point mutation. In the fifth observer, P.S., the pattern is of the second type: both the red and green visual pigment genes are nonfunctional owing to the deletion of DNA.

Despite the loss of the green- and red-cone spectral sensitivities, all five blue-cone monochromats claim to have some colour vision, but as we have already shown[8], their self reports are unreliable. Further, their apparently normal performances on some portions of traditional colour discrimination tests at photopic levels can be explained by luminosity judgements mediated by their blue-sensitive cones[8]. The question of colour vision can be settled, however, by wavelength discrimination experiments, in which the observer is required to find the minimal or just noticeable difference (JND) in wavelength that allows him to distinguish two spectral stimuli.

As in typical wavelength discrimination experiments[13], our five blue-cone monochromat observers viewed a bipartite field, one half of which was filled with light of a standard wavelength, the other with light of a comparison wavelength (see inset to Fig. 1*c*). To ensure that the observers could not discriminate the half-fields on the basis of brightness, the luminances of the half-fields were equated according to each observer's spectral sensitivity function measured beforehand by heterochromatic brightness matching under the same stimulus conditions[14].

The results for an adapting standard of 8 trolands (td) are given in Fig. 1. Panel *a* shows the average heterochromatic brightness matching functions obtained for the five blue-cone monochromats and for 8 normal trichromats. The normal observers' function has a broad peak near 550 nm, corresponding to the maximum of the Commission Internationale d'Eclairage (CIE, 1978) average luminous efficiencies obtained by direct heterochromatic brightness matching (dotted line)[15] (which is dominated by the green- and red-sensitive cones). That of the blue-cone monochromats has a peak near 520 nm, corresponding to the maximum of the CIE (1951) scotopic luminosity function (large dashed line)[15]. At long wavelengths the two curves diverge in accordance with the photopic and scotopic functions. But, at short wavelengths, both are more sensitive than either the photopic or scotopic functions. The cause of this is revealed in Fig. 1*b*, which displays the individual brightness curves of the five blue-cone monochromats. At wavelengths shorter than 500 nm, one of the curves (F.B.) deviates strikingly from the function, indicating the emergence near 450 nm of a second peak, which is well fitted by the blue- (or short-wavelength-sensitive) cone spectral sensitivity function of Smith and Pokorny[16] (short dashed line). Thus, at this intensity level, some blue-cone monochromats are primarily using rods to generate their brightness matching function (even though their blue-cones are also active), whereas others are using both the blue-cones and rods to do so.

Figure 1*c* shows the average wavelength discrimination functions of the blue-cone monochromat and normal observers obtained at the same intensity level (8 td). JNDs in wavelength are plotted as a function of the standard wavelength. The average normal curve has the same general appearance as reported classic curves[13]: a relative maximum is observed at about 530 nm, two relative minima at approximately 490 and 590 nm. The average curve for the blue-cone monochromats, on the other hand, has only one minimum between 460 and 480 nm, and wavelength discrimination completely collapses for wavelengths longer than 520 nm. Within the narrow range between 440 and 500 nm, however, their discriminability compares favourably with that of the normal observers. The individual wavelength discrimination functions of the five blue-cone monochromat observers are shown in Fig. 1*d*.

Figure 2 shows the heterochromatic brightness matches and wavelength discrimination functions of observer P.S. at three mesopic adaptation levels below rod saturation: 0.8, 8 and 80 td. Clearly, wavelength discrimination extends to longer wavelengths as the field intensity is increased, but the best performance (that is, the smallest JND) is obtained at the intermediate intensity of 8 td. The change can be related to his spectral sensitivity functions. As the rod peak declines in magnitude relative to the blue-sensitive cone peak, the sensitivities of the two receptor types first converge then diverge and the spectral region where the sensitivities are most similar shifts from the short to the middle wavelengths. At higher light levels (>800 td), where the rods saturate, his spectral luminosity function (and those of the other blue-cone monochromats) becomes unimodal, with a peak near 450 nm, and, wavelength discrimination accordingly breaks down.

Thus, the answer to the question posed by the title is yes, within a limited intensity range, colour vision is possible with only rods and blue-sensitive cones. This finding is interesting for two reasons. First, previous demonstrations of rudimentary colour vision in alleged blue-cone monochromats[17,18] have relied on the assumption that they have a third receptor type—a rhodopsin cone, which is used in conjunction with the blue-sensitive cones to discriminate colours. The presence of such receptors has not been verified psychophysically in the five blue-cone monochromats investigated here[7,8]. This accords with the original explanation of blue-cone monochromacy[5], and with the molecular genetics[9]. Second, it has been proposed that rod signals travel in pathways carrying signals from the blue-sensi-

tive cones[19,20], largely because rod vision at dusk and dawn takes on a bluish tinge. If a common neural pathway exists then it suggests that colour vision with only rods and blue-sensitive cones should not be possible; because the two types of signals are being summed rather than compared. We find the contrary, at least in the blue-cone monochromat. This implies that some rod and cone signals travel by separate pathways to the visual processing stage where wavelength discrimination takes place.

We conclude that vestigial colour vision is present in blue-cone monochromats, at intensity levels where both their rods and blue-cones are simultaneously active. This raises the issue of whether the rods generally interact with cones to contribute to colour vision. Previously, it has been demonstrated that at mesopic levels, when the field of view is enlarged to include the parafoveal retina, human colour vision is tetrachromatic: to maintain colour matches at more than one light level, four independent variables are required instead of the usual three[2,4,21]. (At any one level, colour vision is never more than trichromatic.) Further, it has been demonstrated that both protanopic and deuteranopic dichromats become trichromatic under similar conditions[22]. A plausible interpretation of these findings as well as our own is that rod signals travel along the

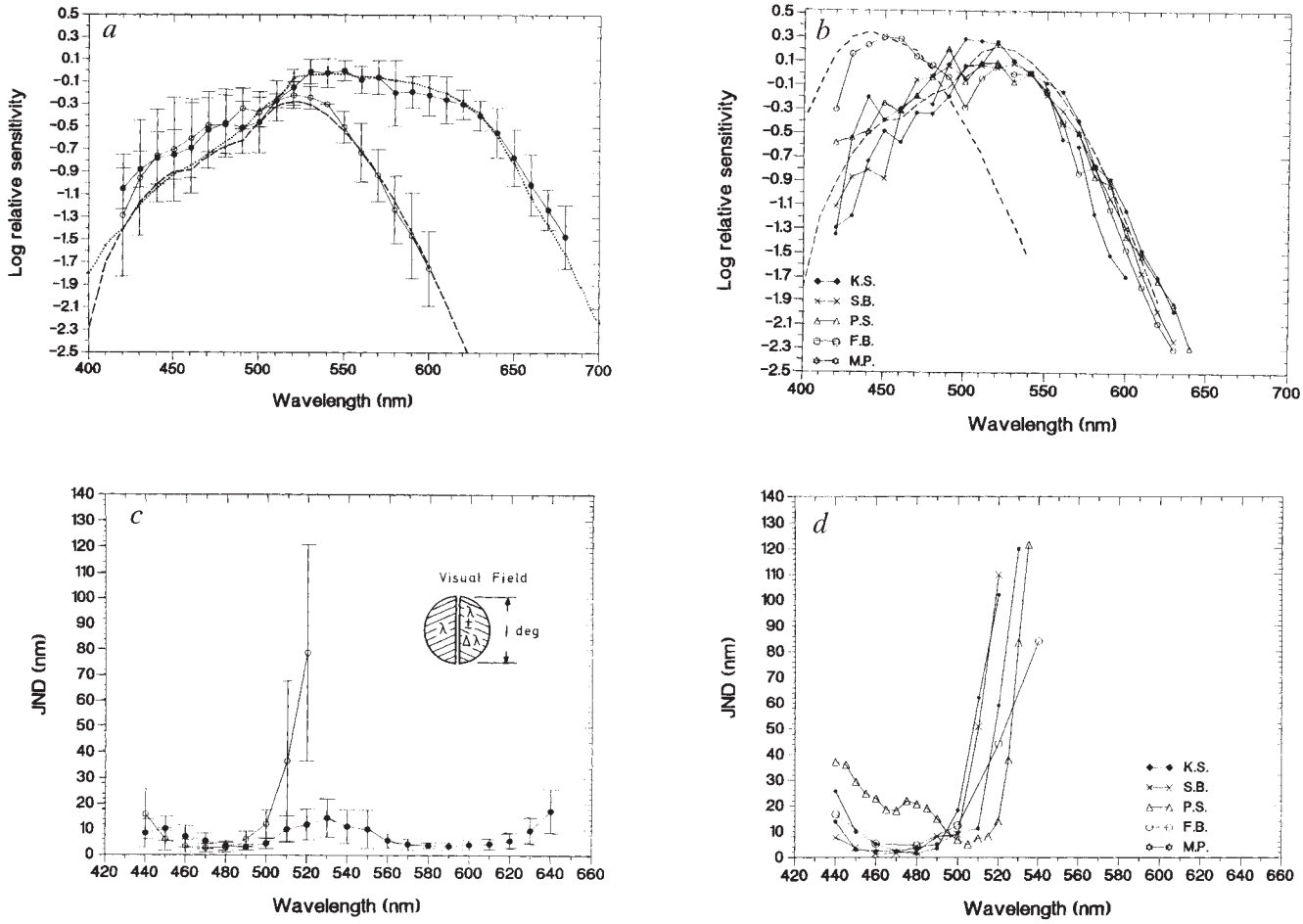

FIG. 1 *a*, The average brightness matches of 5 blue-cone monochromats (age 9–53; open circles) and of 8 normal trichromats (age 21–32; filled circles). The data points are shown with a bar corresponding to one standard deviation. The matches were made in a bipartite photometric field (see inset, Fig. 1*c*) presented in Maxwellian view. The observer was required to find by a staircase tracking procedure the luminance of the comparison half-field (as a function of wavelength) needed to match the brightness of a 540 nm standard half-field set to a retinal illuminance of 8 td. Both halves of the field, which subtended 1° of visual angle in diameter, were presented simultaneously for 1 s. The standard and comparison wavelengths were selected by means of grating monochromators (Jobin Yvon H.10 Vis), with a full-width at half-amplitude of less than 4 nm (for other details, see ref. 14). The dotted line indicates the quantized CIE (1978) average luminous efficiencies obtained by direct heterochromatic brightness matching[15]; the large-dashed line indicates the CIE (1951) scotopic luminosity function, quantized and corrected for a modest amount of macular pigment absorption[15]. The correction to the CIE scotopic function is required since it pertains to the peripheral retina, which is not screened by the short-wave absorbing macular pigment, whereas the blue-cone monochromat brightness matches pertain to the central fovea, which is. The correction for macular absorption was made according to standard mean optical density values[15], assuming a maximum density of 0.5 $\log_{10}$ unit at 458 nm. *b*, The individual heterochromatic brightness matches of the 5 blue-cone monochromats. Each curve has been separately normalized to the sensitivity value obtained at the standard wavelength (540 nm). The small-dashed line indicates the Smith and Pokorny short-wave (blue) cone sensitivity function[16]. *c*, The average wavelength discrimination curves of the five blue-cone monochromats (open circles) and of the eight normal trichromats (filled circles) measured at a retinal illuminance of 8 td. (JND, Just noticeable difference.) The inset shows the field of view, in which λ and $\lambda + \Delta\lambda$ indicate, respectively, the standard and comparison wavelengths. During an experiment, the wavelength of the comparison half-field was changed in 1-nm steps from equivalence with that of the standard half-field. The luminances of the two fields were held constant by reference to the observer's individual heterochromatic brightness functions (for details, see ref. 14). The discrimination threshold or JND in wavelength was found where the observer signalled that the two halves of the field were no longer identical in appearance. This procedure was repeated five times for a series of standard wavelength throughout the visible spectrum. Between presentations, the observer adapted for 3 s to a homogeneous white field of the same intensity as the standard and comparison half-fields. The luminance of the white adapting field was matched to that of the standard and comparison fields by means of heterochromatic brightness matching. *d*, The individual wavelength discrimination curves of the 5 blue-cone monochromats.

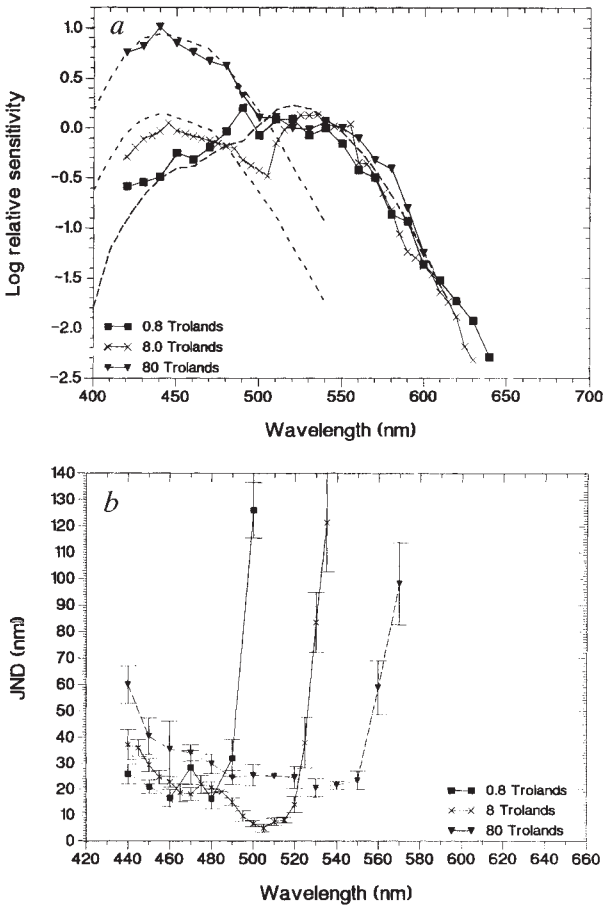

FIG. 2 *a*, The heterochromatic brightness matches of blue-cone monochromat P.S. measured at three retinal illuminances of the standard 540 nm field: 0.8, 8 and 80 td. The large-dashed line indicates the CIE (1951) scotopic luminosity function, quantized and corrected for macular pigment absorption; the small-dashed line, the Smith and Pokorny short-wave (blue) cone sensitivity function[16]. *b*, The wavelength discrimination functions of blue-cone monochromat P.S. measured at the same three levels as in *a*. The error bars indicate one standard deviation of the mean.

cone chromatically-opponent[22-24] and non-opponent[25] pathways and do not have access to an independent channel[26-29]. In normal trichromats, the input from the rods may only slightly alter the ongoing activity of these pathways, leading to tetrachromatic colour matching but causing no new or independent sensations. In dichromats and blue-cone monochromats, on the other hand, given the reduced number of their independent cone signals, such variable rod input may significantly modulate activity in the opponent pathways, permitting wavelength discrimination and leading to sensations that qualitatively differ from those attributable to their cones alone[22]. □

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1. McCann, J. J. *Science* **176,** 1255-1257 (1972).
2. Trezona, P. W. *Vision Res.* **13,** 9-25 (1973).
3. Stabell, B. & Stabell, U. *Vision Res.* **14,** 1389-1392 (1974).
4. Palmer, D. A. *J. opt. Soc. Am.* **68,** 1501-1505 (1978).
5. Blackwell, H. R. & Blackwell, O. M. *Vision Res.* **1,** 62-107 (1961).
6. Alpern, M., Lee, G. B. & Spivey, B. E. *Archs Ophthal., N.Y.,* **74,** 334-337 (1965).
7. Zrenner, E., Magnussen, S. & Lorenz, B. *Klin. Mbl. Augenheilk.* **193,** 510-517 (1988).
8. Hess, R. F., Mullen, K. T., Sharpe, L. T. & Zrenner, E. *J. Physiol.* **417,** 123-149 (1989).
9. Nathans, J. *et al. Science* **245,** 831-838 (1989).
10. Smith, V. C. *et al. Invest. Ophthalmol. Visual Sci.* **24,** 451-457 (1983).
11. Mollon, J. D. & Polden, P. G. *Phil. Trans. R. Soc.* **B278,** 207-240 (1977).
12. Hansen, E., Seim, T. & Olsen, B. T. *Nature* **276,** 390-391 (1978).
13. Wright, W. D. & Pitt, F. H. G. *Proc. Phys. Soc.* **49,** 329 (1937).
14. Reitner, A., Sharpe, L. T. & Zrenner, E. *Vision Res.* (in the press).
15. Wyszecki, G. & Stiles, W. S. *Color Science: Concepts and Methods, Quantitative Data and Formulae* 2nd Edn (Wiley, New York, 1982).
16. Smith, V. C., Pokorny, J. *Vision Res.* **12,** 2059-2071 (1972).
17. Alpern, M., Lee, G. B., Maaseidvaag, F. & Miller, S. *J. Physiol.* **212,** 211-233 (1971).
18. Young, R. S. L. & Price, J. *Invest. Ophthalmol. Visual Sci.* **26,** 1543-1549 (1985).
19. Willmer, E. N. *J. theor. Biol.* **2,** 141-179 (1960).
20. Trezona, P. W. *Vision Res.* **10,** 317-332 (1970).
21. Clarke, F. J. J. *Optica Acta* **7,** 355-384 (1960).
22. Smith, V. C. & Pokorny, J. *J. opt. Soc. Amer.* **67,** 213-220 (1977).
23. Ingling, C. R. *Vision Res.* **17,** 1083-1089 (1977).
24. Boynton, R. M. *Human Color Vision* (Holt, Rinehart and Winston, New York, 1979).
25. van den Berg, T. J. T. P. *Mod. Probl. Ophthal.* **19,** 341-343 (1978).
26. D'Zmura, M. & Lennie, P. *Vision Res.* **26,** 1273-1280 (1986).
27. Sharpe, L. T., Fach, C., Nordby, K. & Stockman, A. *Science* **244,** 354-356 (1989).
28. Gouras, P. *Science* **147,** 1593-1594 (1965).
29. Gouras, P. & Link, K. *J. Physiol.* **184,** 499-510 (1966).

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Repeat I of the dihydropyridine receptor is critical in determining calcium channel activation kinetics

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MEMBRANE depolarization causes many kinds of ion channels to open, a process termed activation[1]. For both Na^+ channels[2-4] and Ca^{2+} channels[5,6], kinetic analysis of current has suggested that during activation the channel undergoes several conformational changes before reaching the open state. Structurally, these channels share a common motif[7]: the central element is a large polypeptide with four repeating units of homology (repeats I–IV), each containing a voltage-sensing region, the S4 segment[8-11]. This suggests that the distinct conformational transitions inferred from kinetic analysis may be equated with conformational changes of the individual structural repeats[8]. To investigate the molecular basis of channel activation, we constructed complementary DNAs encoding chimaeric Ca^{2+} channels in which one or more of the four repeats of the skeletal muscle dihydropyridine receptor are replaced by the corresponding repeats derived from the cardiac dihydropyridine receptor. We report here that repeat I determines whether the chimaeric Ca^{2+} channel shows slow (skeletal muscle-like)[12] or rapid (cardiac-like)[13] activation.

Expression of cDNAs encoding chimaeras with one or more of the large, putative cytoplasmic regions of the cardiac dihydropyridine (DHP) receptor replaced by corresponding regions of the skeletal muscle DHP receptor, showed that the putative cytoplasmic region linking repeat II and repeat III is a major determinant of skeletal muscle-type excitation-contraction coupling, whereas the four repeating units of homology, constituting the putative transmembrane and adjacent regions, are important in determining channel properties[14]. To examine the functional significance of each repeat in Ca^{2+} channel activation kinetics, we constructed 15 different expression plasmids carrying chimaeric DHP receptor cDNAs (Fig. 1). These expression plasmids were injected into skeletal muscle myotubes prepared from mice with the muscular dysgenesis mutation[15]. Myotubes expressing each of the chimaeric plasmids were identified on the basis of contraction in response to electrical stimulation[12]; all the chimaeric plasmids were functionally expressed, except pSkC21. The whole-cell variant of the patch-clamp technique revealed that all cells displaying contractile responses also expressed Ca^{2+} currents, although the level of expressed current differed for the different chimaeras. Specifically, the maximal

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