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S-cone ERGs elicited by a simple technique in normals and in tritanopes

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Abstract

Purpose: to measure changes in the relative spectral sensitivities of the dark adapted and light adapted ERG and thus to establish the possible contribution of rods to the 'blue cone' ERG elicited by flashes of blue light. Background: short wavelength stimuli in the light-adapted eye evoke small rounded b-waves which have been considered to be S-cone responses. We have recorded such responses from tritanopes, which called the assumptions into question. Methods: small ERGs were recorded to blue and green flashes. The stimulus was a Ganzfeld which employed light emitting diodes. ERGs were obtained in both the dark-adapted eye and after light adaptation to intense orange light (peak wavelength 610 nm). The change in sensitivity with light adaptation and the relative spectral sensitivity was determined from the voltage/log light intensity functions, using a 10 μV criterion. Results: (1) peak times and changes in sensitivity did not help distinguish light-adapted rod from possible S-cone responses; (2) analysis of the change in the ratio of blue:green sensitivity from darkness to 4.4 log Td. 610 nm background suggests that in seven normal subjects, 90% or more of the ERG evoked by 440 nm flashes is generated by S-cones; (3) three tritanopes have insignificantly reduced S-cone responses. Conclusions: (1) clinical techniques used to isolate S-cone ERGs are appropriate; (2) there are at least two types of tritanope and in those we investigated, functional S-cones are probably displaced into the retinal periphery.

Keywords: ERG; S-cone; Tritanopia

1. Introduction

The responses of central S-cones in the ERG were first detected (Padmos, van Norren & Faijer, 1978) by using monochromatic stimulating lights flickering at 13 Hz on an intense yellow background. The very small responses had a spectral sensitivity similar to that of the blue mechanism. In a more complex 'silent substitution' method (Sawusch, Pokorny & Smith, 1987; Swanson, Birch & Anderson, 1993) two lights (e.g. 450 and 520 nm) of equal intensity for L- and M-cones were interchanged at 5 Hz against a yellow adapting background; considered to eliminate any rod response. The ERGs evoked by interchanges were thus considered to be S-responses, and some were as large as 20 μV, The spectral sensitivity function however suggests that there may be rod components, as does the peak time of the response, which at 85 ms is much later than that found by other workers. Most clinical ERG measurements have been made with simple flashes on bright white backgrounds (Gouras & Mackay, 1990; Gouras, Mackay & Yamamoto, 1993; Gouras, Mackay, Roy & Yamamoto, 1993). A 1 μV or less inflection on the ERG has been identified as a S-cone response. If the adapting background light contains only wavelengths >550 nm, rods and medium and long wavelength cones will become light adapted and the relative contri-

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bution of S cones should increase. (Miyake, Yagasaki & Ichikawa, 1985; Horiguchi, Miyake, Kondo, Suzuki, Tanikawa & Koo, 1995; Simonsen & Rosenberg, 1996). Although rod sensation ceases for practical purposes at about 500 Td., and Aguilar & Stiles (1954) were unable to detect any sensation mediated by rod vision above 2000 Td., it is possible that the rod *ERG* continues, at a reduced level, at higher backgrounds and clinical techniques supposed to isolate S cones might be artefactual. We have found normal 'S-cone' ERGs in three tritanopes, which further emphasised this possibility. Accordingly we have designed experiments to determine the degree of rod intrusion (by measuring change in spectral sensitivity on adapting to light). These experiments have lead to better understanding of the abnormality in the three tritan observers.

2. Methods

2.1. Light source

A 'mini Ganzfeld' photostimulator was constructed. light emitting diodes (LEDs) were mounted in a tube which was internally silvered and contained diffusing baffles. The light, passing forward, transilluminated a diffusing plastic meniscus in the shape of a hemisphere which was the equivalent of the 'ping-pong ball' stimulator used in small animal experiments and appeared evenly illuminated by the LEDS. Internally, a thin fibre optic light guide provided a fixation spot of controllable intensity. The plastic hemisphere was placed against the brow, the maxilla and the root of the nose, illuminating approximately 180° of solid angle, i.e. more of the nasal field but somewhat less of the temporal field than in a standard Ganzfeld. The fixation spot was arranged so the subjects looked slightly upward: the elevated upper lid did not occlude the dilated pupil.

The diodes emitted light at peak wavelengths 445, 530, 610 and 660 nm. The 610 nm light was used chiefly as a light adapting background field for rods, long wavelength (L) and medium wavelength (M) cones. but contained almost no energy at <550 nm The LEDS light outputs were regulated by fixed attenuators (nominal values of 1, 3, 10….1000). Further alteration in intensity was achieved by changing the flash duration by small steps in the range 10 μs–6 ms.

2.2. Calibrations

The intensities were measured in situ using both a Minolta CS100 and a computer-controlled tele-spectrometer. The relative intensities and spectral variations in output of all the stimuli were determined on two occasions during the experiments. Light intensities are given as Trolands (Td.), assuming 7 mm diameter pupils.

Using the data of the calibrations, the relative theoretical effectiveness of the stimuli were calculated from rod spectral sensitivity (V'_λ) and the analogous spectral variation of the blue mechanism (Estevez, 1979).

$$\sum_{\lambda=380}^{\lambda=700}(V'_\lambda)(B_\lambda) \quad \text{and} \quad \sum_{\lambda=380}^{\lambda=700}(V'_\lambda)(G_\lambda)$$

where B_λ and G_λ are the emissions of the blue and green LEDS, and

$$\sum_{\lambda=380}^{\lambda=700}(S_\lambda)(B_\lambda) \quad \text{and} \quad \sum_{\lambda=380}^{\lambda=700}(S_\lambda)(G_\lambda)$$

where S represents the published spectral sensitivity data for the blue mechanism. From these calculations, the green light should be more effective in stimulating rods than the blue light, by a factor of 4.2. Similarly, the blue light should be more effective in stimulating blue cones than the green light, by a factor of approximately 11.1.

2.3. ERG techniques

The experiments were performed with pupils fully dilated ($\geqq$7mm) by tropicamide 1%. Either DTL fibre electrodes or gold foil electrodes were employed. The earth electrode was placed on the forehead, and the reference electrode near the lateral canthus. The recording amplifier had a bandpass of 0.3–300 Hz, and a gain of 10000. The responses were digitised, displayed and averaged by a 'Windows TM' system, and stored in a database. With small ERGs, up to 200 repetitions were averaged. The subjects were fully dark adapted and 1–30 μV ERGs obtained to weak blue and green flashes of different intensities. The experiment was repeated with intense orange backgrounds.

2.4. Psychophysical techniques

A computer graphics system was employed to demonstrate variation in colour vision with retinal eccentricity (Arden, Gunduz & Perry, 1988; Tak Yu, Falcao-Reis, Spileers & Arden, 1991). The stimuli were subsets of Sloan optotypes, which appeared in equiluminous colour contrast viewed at ca. 30 cd m^{-2}. The thresholds are given as a percentage of the maximum possible chromatic swing.

2.5. Subjects

Experiments were carried out on seven normal subjects and three tritanopic observers. Two of which were, a mother aged 37 and her 14 year old daughter whose male sibling (although not examined) is reported to have the same colour vision as his sister. The two seen have complete loss of discrimination along the tritan axis, and no other colorimetric defect, including stan-

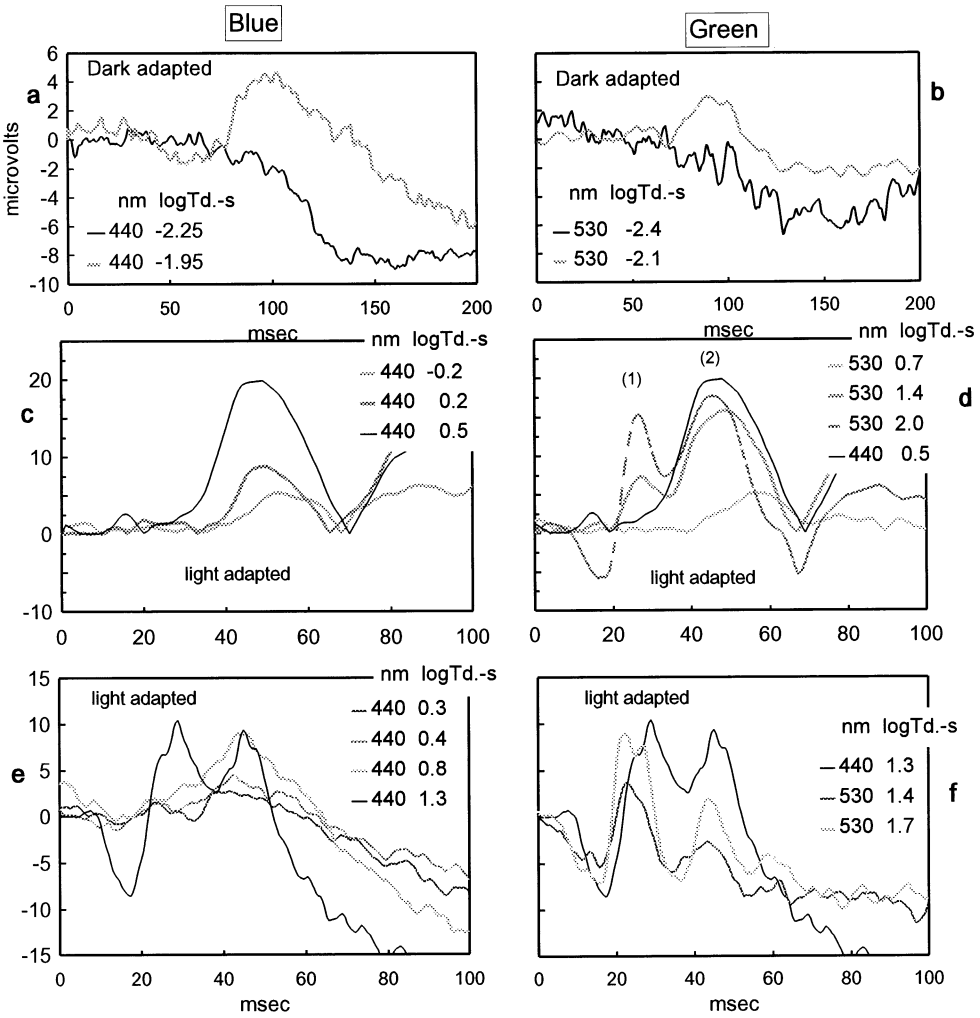

Fig. 1. Illustrative ERGs produced by two normal subjects in these experiments. The conditions of the experiments are indicated in the insets. Note, light intensities altered by changing flash durations. a, b, e, f: from one subject aged 47 years. c, d: from another 22 year old subject. a and b: in the dark adapted eye, shortest flashes (full line) produce scotopic threshold responses. Minimal b-waves appear when the flash duration is doubled. c, d: responses after adaptation to orange 610 nm light of maximal intensity (18900 Td.). Note change in time base. 440 nm flashes (c) evoke rounded b-waves, peaking at 55 ms, without preceding a-wave. When green 530 nm flashes are used (d) an initial a-wave-b-wave peak is (1) and is followed by a second peak (2). Note the full line in d is transferred from c, to demonstrate that peak 2 is the major component evoked by blue light. e,f: responses to same experimental conditions as c and d, but in an older subject. e: for 440 nm flashes of log 0.3 0.4 and 0.8 Td.-s, a rounded 'blue cone' b-wave can be seen without a preceding a-wave. When the flash is further increased to log 1.3 Td.-s, an a-wave develops, and a double b-wave can be seen. The early peak occurs at about 30 ms, typical of light adapted M- and L-cone b-waves. f: 530 nm flashes with the same adapting background all produce double peaked ERGs. The full line, transferred from e, shows that the second peak is at the same time for 530 and 440 nm light, but the 'blue cone' b-wave is relatively reduced when green light is used.

dard anomaloscopy. There is visual acuity of 20/20, and no abnormality in the fundus. The other subject is an unrelated male aged 42, who has previously been investigated exhaustively (Barbur, 1980). His vision is 6/5, he has no fundal ocular abnormality, and spectral sensitivity, measured with a 2° bipartite field and a matching technique, shows an absence of blue photopic sensitivity near the fovea. In all three, all other clinical testing is normal including the standard ERG. All the normal had visual acuity $\geqq 6/6$ and no general or systemic eye disease. All subjects gave informed consent. All procedures complied with the Declaration of Helsinki.

3. Results

3.1. General features of the normal response

In dark adaptation very weak green and blue flashes produce a scotopic threshold response (Frishman, Reddy & Robson, 1996). Slightly more intense flashes evoke small b-waves which are similar in waveform for both blue and green light. (Fig. 1a, b).

When the eye is light adapted by an intense orange background, the amount of blue and green light required to evoke small ERGs increases greatly, and the waveform changes. (Fig. 1c, d) The b-waves peak ear-

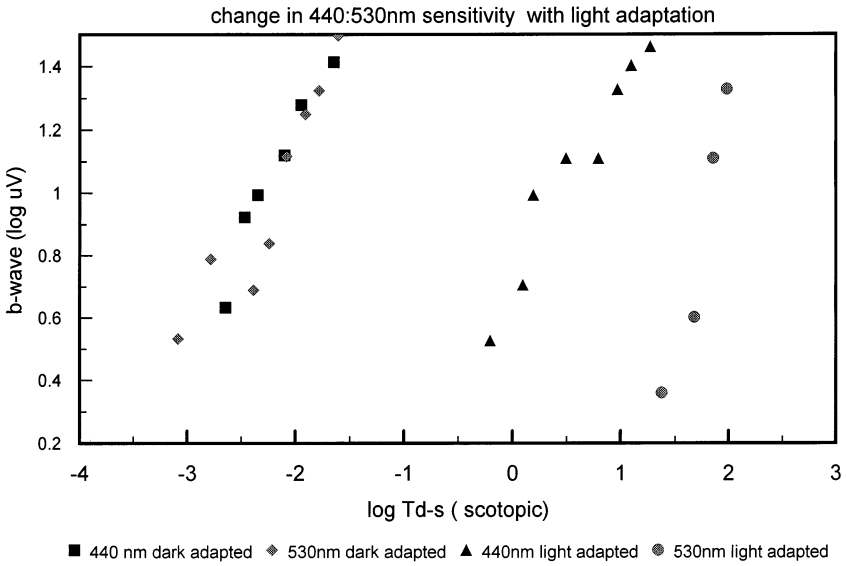

Fig. 2. Amplitude/intensity functions from a typical experiment in a third subject, for 530 and 440 nm light in dark adaptation and after light adaptation to log 4.4 photopic Td. Note the x axis is in scotopic units.

lier (note change in time base). In two young subjects, blue flashes (c) which produce minimal ERGs evoke a rounded b-wave, peaking at 45–55 ms. This has been ascribed to S-cones. When green flashes are employed (d) they evoke, in addition, an a-wave b-wave complex peaking at about 27 ms (labelled (1) in d) as well as the slower response (labelled (2)). The first peak is characteristic of the responses of L and M cones. As the green flash intensity rises, the initial L and M peaks increase more than the later S-cone peak. In panel d, the blue flash response from panel c (solid line) is shown again, to emphasise the similarity of peak 2 in the green flash response to the ERG evoked by the blue flashes. The waveforms in light adaptation differed in detail from subject to subject. An example is shown in Fig. 1e, f. The weakest blue flash in panel (e) evokes peak 2 only, but if the intensity is increased by increasing the flash duration, peak 1 appears in addition. With green flashes (panel f) first and second peaks are prominent for all flash intensities. The response to the 1.3 log Td.-s blue flash from panel e (full line) is reproduced in panel f to show the presence of peak 2 in the two responses to green light. The M-cone b-wave has a large negative 'tail'. Sometimes, when the L- and M-cone response was large, the trough between the M-cone and S-cone b-wave was negative. The amplitude of the following S-cone peak was measured from this negative value, so by comparison with the blue flashes the amplitude was overestimated. To minimise such complexities, the analysis concentrated on small responses. The specimen ERGs shown by Simonsen & Rosenberg (1996) are similar to those in panels (e) and (f), but the colour filters used in those authors experiments were broader than the emissions of the LEDS in our work.

3.2. Change in sensitivity to blue with light adaptation

As the 610 nm background intensity increased, the amplitude of the ERGs evoked by blue light fell and the peak time decreased, but by inspection no difference could be seen which could mark the transition from a rod-generated to a S-cone generated b-wave. Intensity-response functions were generated for a variety of backgrounds. All were parallel, but displaced to higher flash intensities as the adapting brightness was raised. From such data the change of sensitivity for a criterion b-wave of 10 μV was determined.

Fig. 5 shows the derived relation between blue flash intensity for a criterion ERG and background intensity: there is no indication that the ERG is saturating at these higher background intensities unlike rod psychophysical responses (Aguilar & Stiles, 1954).

3.3. Change of relative spectral sensitivity from dark and light adaptation

Voltage/Log I curves were obtained for 530 and 440 nm flashes, in darkness and with various 610 nm backgrounds, to determine apparent changes in spectral sensitivity. Fig. 2 shows graphs of such amplitude/intensity functions in one subject (A091 in Table 1). It can be seen that in dark adaptation, the retina appears equally sensitive to blue and to green flashes in scotopic units, because the points lie on a single straight line. After intense light adaptation, the responses to blue are about 1 log unit more sensitive than to green. The relationship between log voltage and log intensity is similar for the four sets of data, giving a value of 0.7 for 'q', the exponent in the Rushton–Naka equation

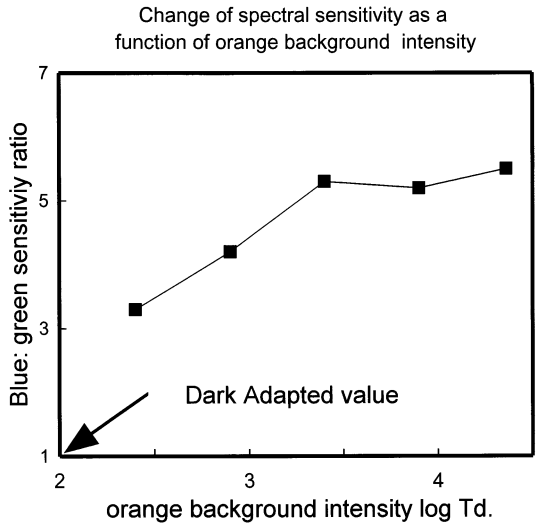

Fig. 3. Change in green:blue sensitivity ratio in a single observer as a function of adapting background intensity. The results have been scaled so that the scotopic value = 1.

(Appendix A). Data for seven subjects is shown in Table 1. In dark adaptation, the mean green light sensitivity in Td. was 0.6 log units greater than that for the blue, in satisfactory agreement with the calculations. In all seven subjects, light adaptation causes a shift in sensitivity toward the 440 nm flashes which are now 0.4 log units more effective than are 530 nm flashes. Table 1 shows this, and the overall change in relative sensitivity between 440 and 530 nm light is thus 1 log unit.

If the light adapted, slower, b-wave was entirely generated by S-cones the change predicted is 11.1/(1/4.2) = 46.3. One explanation of the difference between calculation and experimental findings is that the blue flashes evoke a mixed S-cone and rod response. The final column of the table gives the estimated S-cone to rod contribution in the response, as detailed in the Discussion. This varies from experiment to experiment.

Fig. 3 shows how the ratio of blue to green sensitivity

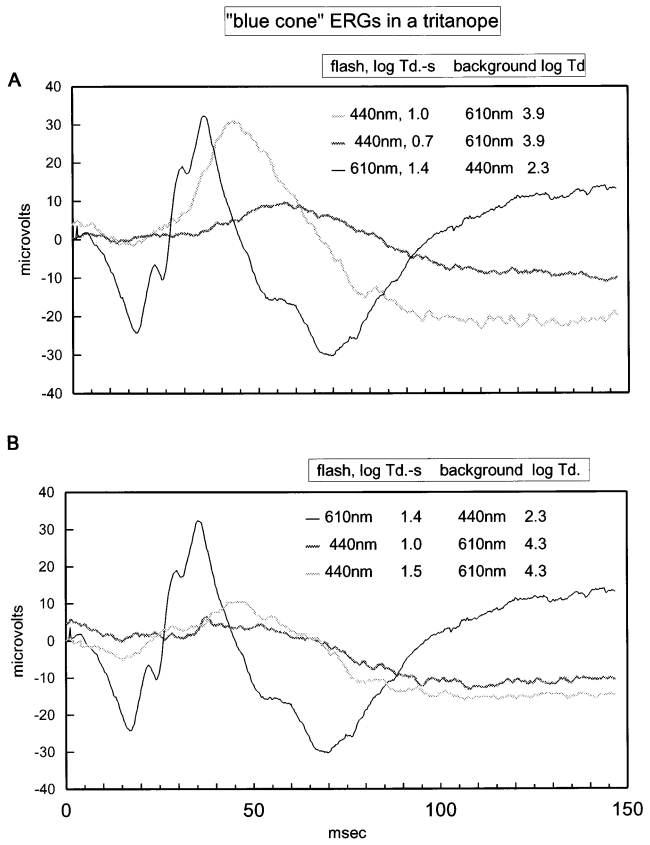

Fig. 4. Illustrative blue cone ERGs from a subject with tritanopia. In A and in B, the full line shows the ERG evoked by 610 nm flashes against a 440 nm, rod suppressing background. The half tone lines show the entirely different ERGs evoked by 440 nm flashes superimposed upon 610 nm backgrounds. A and B show two different background intensities. Note in A the 410 background that suppresses all rod response is weaker than the 610 background used in B, in both photopic and scotopic terms.

changes as a function of orange background intensity for one observer (number 39404 in Table 1). This is the smallest change in relative spectral sensitivity for all our subjects. For convenience, the blue/ green sensitivity

Table 1
Relative spectral sensitivities of Seven normal observers to blue and green flashes in dark adaptation and when the stimuli (in luminance units) are superposed on an intense orange background

Number	Age	Dark adapted sensitivity (log blue/green)	Change in blue sensitivity on light-adapting (LU)	Light adapted sensitivity (log (blue/green)	Calculated contribution S-cones/rods
A092	21	−0.35	2.55	0.3	8.9
A091	56	−0.5	2.45	0.7	35.5
A093	21	−0.67	2.55	0.27	7.9
A094	67	−0.53	2.9	0.45	14.1
34904	37	−0.85	3.2	0.4	12.6
34313	39	−0.68	3	0.36	11.2
34903	47	−0.6	3.12	0.3	10.0
Mean		−0.60	2.82	0.40	14.32
Standard error		0.06	0.12	0.06	3.62

value in darkness has been made 1. Most of the change from dark adaptation occurs with a background 1/100 the maximum intensity: but additional change occurs as the background is raised.

3.4. S-cone ERGs in tritanopes

We have obtained ERGs from three tritan observers. In all, blue flashes against an orange background produce rounded 'S-cone' ERGs. When (Fig. 4, solid line) a 440 nm background of log 3.9 photopic Td. (620 scotopic Td.) is used, the eye was light adapted and 610 nm flashes (log 1.4 Td.-s) evoked a typical photopic ERG without evidence of any rod contribution. A higher intensity of orange background (779 scotopic Td. or 6237 photopic Td.) evokes (A) the rounded b-waves associated with the S-cones. When the flash is 0.7 Td.-s the response is about 10 μV, but increasing the blue flash intensity increases the ERG to nearly 30 μV. and it is possible that there is a rod contribution. Increasing the background intensity to 2538 scotopic or 18900 photopic Td. (B), nearly abolishes the response to blue flashes. Increasing the flash energy restores the amplitude but no more than 10 μV of b-wave develops.

Fig. 5 shows, for a normal and three tritan observers, the blue cone ERG sensitivity as a function of adapting light intensity. The Weber-Fechner relationship applies although this is the region where psychophysically the rods are saturated. The mean results from the three patients are not significantly different to the normal. The amplitudes of the ERGs from these three observers was, 7.6 μV for log 1.0 Td.-s (1 ms) blue flashes on a maximal background, and 22.5 μV for log 1.5 Td.-s flashes. For the seven normal observers, the 1 ms flashes evoked a response of 8.79 (SE 2.94) μV, but the more intense flashes were not employed for all these subjects.

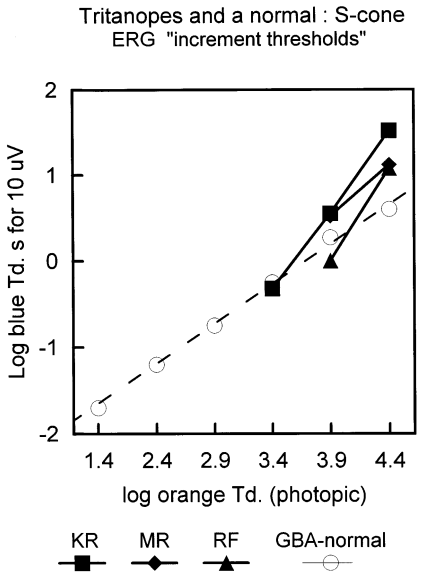

Fig. 5. b-Wave sensitivity, determined by a 10 μV criterion, as a function of orange background for a normal, and for the three tritanopic observers. The abscissal scale is in photopic Trolands. Note that the blue cone responses do not 'saturate'.

3.5. Variation of colour vision with retinal eccentricity in tritanopes

One tritanope, whose central 2° of field has been studied previously by Barbur (1980), had both a chromatic loss and a loss of sensitivity to short wavelengths consistent with S-cone loss. Fig. 6 shows how colour contrast sensitivity varies when the subtense of the target used is increased. The stimuli used in this experiment to determine colour contrast thresholds were a subset of the Sloan optotypes (the smallest subtended 3°). Foveally fixated flashed letters were employed and thus the results could not be influenced by eye movements. For the normal observer for protan and tritan axes, and for the tritanopes in the protan axis, thresholds are nearly constant and independent of letter subtense. For tritanopic colour contrast in the affected subject (open squares and dashed line, as the letter size increases from 3 to 24°, the threshold drops to similar to those found in the normal retina, when the central retina is not stimulated. (Tak Yu, Falcao-Reis, Spileers & Arden, 1991). The threshold blue letters were found by direct measurement to have the colour chromaticity co-ordinates indicated in the figure legend. By transforming these to RGB chromaticity coordinates, and then to photopic luminances (the background and target being equiluminant) we can calculate the scotopic luminance contrast is 7.1%.

4. Discussion

4.1. General features of S-cone ERGs

There is some concern that (Section 1) 'S-cone' ERGs obtained by flash-on-background techniques are contaminated by rod responses, because of the similarity of their waveforms and characteristics to flicker. Again, it has been stated that S-cones do not produce off-responses, and neither do rods.

With long-wavelength backgrounds, the supposed S-cone ERG can be quite sizeable, e.g. 10 μV (Horiguchi, Miyake, Kondo, Suzuki, Tanikawa & Koo, 1995) and ca. 20 μV (Miyake, Yagasaki & Ichikawa, 1985; Sawusch, Pokorny & Smith, 1987; Swanson, Birch & Anderson, 1993, 1994; Horiguchi, Miyake, Kondo, Suzuki, Tanikawa & Koo, 1995; Terasaki & Miyake, 1995; Simonsen & Rosenberg, 1996). This is considerable, since only a few per cent. of the photoreceptors are blue cones (Kolb, 1991), and the proportion of blue

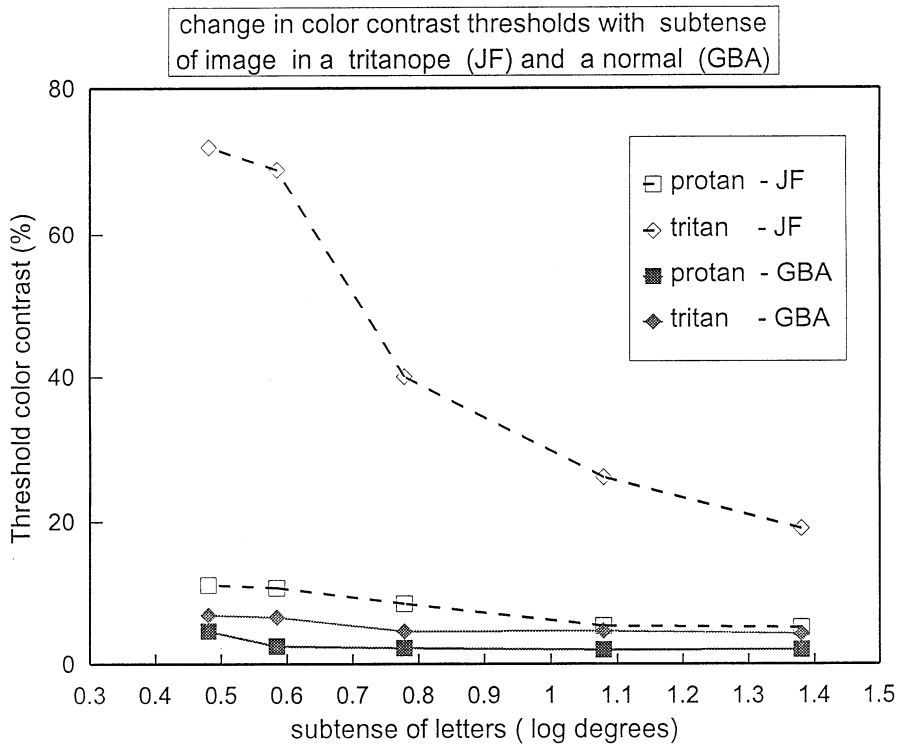

Fig. 6. Colour contrast sensitivities, measured along protan and tritan colour confusion lines, for a normal (filled symbols) and an established tritanope (open symbols). Sensitivity was tested with flashing letters, of various sizes as indicated. The fixation was at the centre of a 19 inch monitor. The smallest letter subtended 3° at the eye. Colour contrast of 100% is defined as when the separation between image and background is the maximum possible with the monitor phosphors, and 0 when the image and background are identical. By direct measurement the threshold of the large blue letters were found to have the colour chromaticity coordinates (0.3600X, 0.3105Y, 0.3295Z, with an SD of $+/-0.0002$) while the background coordinates were (0.3715X, 0.33425Y, 0.3520Z, SD $+/-0.0002$). By transforming these to RGB chromaticity coordinates, and then to photopic luminances (the background and target being equiluminant) we can calculate the scotopic luminance contrast is 7.1%.

cone bipolars and ganglion cells is similarly very small.The presumed S-cone response does not 'saturate' at background intensities thought to abolish rod activity, and the spectral sensitivity of the response is then shifted to shorter wavelengths compared to a rhodopsin-based response. From the change in relative spectral sensitivity, we calculate that (even disregarding one aberrant experiment (A091 in which a nearly 'pure' S-cone response was recorded) the average S-cone to rod contribution is 10.5:1. This means that the method of isolating S-cone ERGs, although crude, would suffice for clinical purposes, as is indeed widely assumed.

4.2. Precision of the estimate of S-cone to Rod contribution

It is possible (Appendix A) to derive a relationship between the proportion of the ERG generated by the S-cones (plotted in Fig. 7 on the ordinate as 'n/m') and the ratio (p) of blue:green light intensities (quantum corrected) which evoke equal light adapted ERG amplitudes, plotted on the abscissa. Values of p obtained by experiments, such as those shown in Fig. 4, are summarised in Table 1, and placed on the theoretical line in Fig. 7. Associated values of n/m can then be read off and are also shown in Table 1. We believe these estimates are not greatly affected by uncertainties in the calculations or experimental methods. Thus, if the emission of the blue LEDS is assumed to be 10 nm towards shorter or longer wavelengths, the heavy curve in Fig. 7 is only trivially shifted, by less than the line thickness. Doubling the lens absorption (data from Wyszecki & Stiles (1982)) gives the lower dotted line in Fig. 7. The upper dotted line represents the expected curve if the experiments were performed on aphakes.

We have ignored the STR and other complexities in the ERG, although they may be present, and this may explain why the slope of the relationship between log blue 'threshold' and Log I in Fig. 5 is not 1.0 (Robson & Frishman, 1995). Also the light-adapted ERGs have a complex waveform, so any method of measuring the amplitude of individual is open to objection. However we have measured the S-cone b-wave from the previous trough to the 50 ms peak, which would overestimate the S-cone contribution to green flashes and thus underestimate the change in spectral sensitivity between dark and light. When the background illumination

intensity is reduced, the waveforms elicited (especially by the green flashes) are simplified (not illustrated) and the proportion of rods:S-cones that generate the response increases in a sensible way (Fig. 4)

4.3. Tritanopes with S-cone ERGs

We are the first to record S-cone responses from the entire retina. Therefore our estimate of the ratio of rod to S-cone ERG voltage suffers from fewer uncertainties than those of previous workers, and supplements the scant previous findings (Horiguchi, Miyake, Kondo, Suzuki, Tanikawa & Koo, 1995) of normal 'S-cone' responses in tritanopes. The result implies, for the first time, that some tritans have a normal number of responding blue cone units. However, in other reports the S-cone ERG has been found absent in tritanopes (Miyake, Yagasaki & Ichikawa, 1985). In one tritanope with an S-cone ERG (Padmos, van Norren & Faijer, 1978) psychophysical testing established (Pokorny, Smith & Went, 1981; Smith, O' Shea, Pokorny, Mets, Applebury & Li, 1994) that his abnormality was in the distribution of S-cones, which were present peripheral to the posterior pole. This also appears to occur in one of our cases. The rod luminous contrast sensitivity of the blue flashes on an orange background is 7%, less than the threshold contrast of 20% found by Aguilar & Stiles (1954), or the 16% found by Hofmann, Barnes & Hallett (1990), for gratings at optimal luminance (log 0.1 sc. Td.-s). Thus, it is unlikely that in tritanopes, the ERG amplitudes or psychophysical thresholds are affected by rod intrusion.

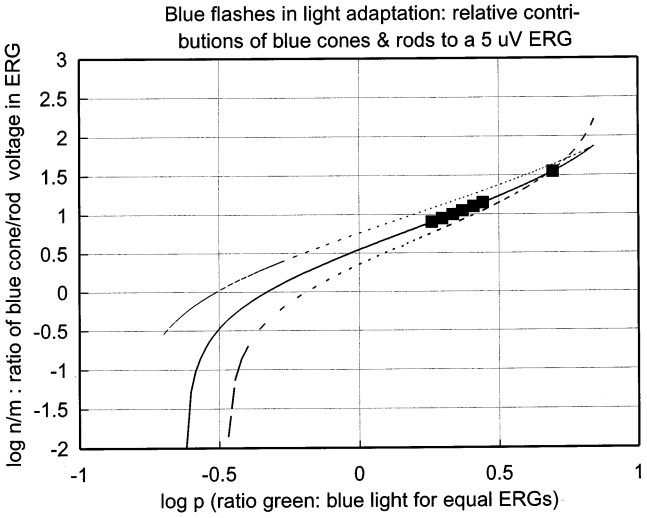

Fig. 7. Full line: the values of p (ratio of green:blue intensities for equal ERG voltages) plotted for various values of n/m (the S-cone voltage/rod voltage to blue flashes (Appendix A)). The p-values for the seven observers results have been placed on the curve, so that values of n/m can be read off. Dotted lines show how the calculated values change with differing estimates of lens absorption. For further details see text.

Two different point mutations in the gene coding for blue opsin have been found in tritanopes (Weitz, Miyake, Shinzato, Montag, Zrenner, Went & Nathans, 1992), but in one of the five families investigated, and in three of the four simplex cases, there was no abnormality at all in any of the seven PCR products which spanned the entire six exons of the gene. In rod monochromats also, a genetic heterogeneity has been found (Nathans, Maumenee, Zrenner, Salowski & Sharpe, 1993). In the cases in which no abnormality was found in the blue photopigment gene, Weitz, Miyake, Shinzato, Montag, Zrenner, Went & Nathans (1992) suggest that there may be a point mutation upstream of the regions that they analysed. A mutation that affects the distribution of S-cones in a ring around the macula (Curcio Allen & Sloan, 1991) has been described. Other such regulatory genes have been identified. In transgenic animals, disturbance to the promoter region upstream of the rhodopsin gene has caused irregular topographic distribution of expression (Kumar & Zack, 1995). This then might explain the finding of tritanopes with responding peripheral blue cones.

5. Note added in proof

Since submitting this manuscript we have recorded the ERGs of a woman who is a member of a three generation family with tritanopia, with male to female transmission (AD). Under conditions identical to those given in Section 2, the blue cone ERG was <2 μV. This result demonstrates the heterogeneity of tritanopia, mentioned in Section 4, and provides additional evidence that the technique can evoke S-cone ERGs largely uncontaminated by rod intrusion.

Appendix A

If an ERG was entirely produced by S-cones, we would expect to find, using published data (Wyszecki & Stiles, 1982), that our blue light was 11.1 times more effective than green. This did not appear to be the case, and therefore it appeared useful to calculate the change in spectral sensitivity that would be expected in ERGs which consisted of mixtures of S-cone and rod responses. We may assume that ERG amplitude (V) for small ERGs is linearly proportional to light intensity; this is (almost) true.

$$^{R}V_{\lambda} = {}^{R}zI_{\lambda} + {}^{R}C$$

λ indicates wavelength (of blue or green light) and R the type of receptor (in this case rods or blue cones). I is measured in units of quanta incident/second/unit area. z is a proportionality constant. It will vary with

the type of receptor stimulated. C is a constant which also depends on the type of photoreceptor.

If ${}^{R}C/{}^{R}z \ll 1$, this reduces to ${}^{R}V_{\lambda} = {}^{R}zI_{\lambda}$

and this approximation is widely held to be valid in dark adaptation (providing the b-wave is small and is in the 'linear' range, without saturation) since $V = 0$ when $I = 0$, and when the light intensity doubles, V doubles. In light adaptation, in general, several receptor types may contribute to the corneally recorded ERG, making it difficult to test the approximation, but in experimental animals where there is only one type of response, it is accepted that in light adaptation the relationship between amplitude and intensity is still determined by the Rushton–Naka relationship:

$$V = V_{\max} \cdot I^{q}/(I^{q} + k^{q})$$

where q is a constant which approximately equals 1, and k is a constant the value of which is the light intensity required for semisaturation. For $V < 0.2\ V_{\max}$ linearity will still apply. The effect of light adaptation is to decrease $V_{\max}$ and increase k.

In complete dark adaptation we can be sure that all the response to weak flashes is generated by rods, whether the light is blue or green. For small rod ERGs (${}^{rod}V$) to green (I_G) and blue (I_B), light calculation (and experiment) shows that blue light is less effective than green:

$${}^{rod}V_G = 4.2mI_G; \qquad {}^{rod}V_B = mI_B$$

$$\text{when } {}^{rod}V_G = {}^{rod}V_B, \qquad mI_B = 4.2mI_G \tag{1}$$

A necessary consequence is that the slope of the lines for ERGs generated by the green and blue flashes in the log/log plot should be similar, and this was found to be the case. For S-cone ERGs, green light is calculated to be less effective than blue and therefore when

$${}^{S-cone}V_B = nI_B; \qquad {}^{S-cone}V_G = 0.09nI_G \tag{2}$$

In our experiments, what we believe to be the S-cone responses are derived from receptors which absorb very little of the orange background light, and which therefore remain essentially dark adapted. Therefore the slope of the intensity/response relationship, on double-log coordinates should be the same for S-cones and the rods, necessary if there is a single mechanism operating. Measurements were only made for small ERGs in which this condition held.

From Eqs. (1) and (2) it is possible to deduce the relative contributions of S-cones and rods to the ERG, although an additional assumption must also be made that the ERGs evoked by the S-cones and the rods are independent. When the ERGs for blue and green light have equal amplitudes,

$${}^{S-cone}V_B + {}^{rod}V_B = {}^{S-cone}V_G + {}^{rod}V_G$$

what we want to know is:

$${}^{S-cone}V_B/{}^{rod}V_B \quad \text{or} \quad m/n$$

expanding the relation above,

$$\text{for green } {}^{S-cone}V_G + {}^{rod}V_G = 0.09nI_G + 4.20mI_G \tag{3}$$

$$\text{and for blue } {}^{S-cone}V_B + {}^{rod}V_B = nI_B + mI_B \tag{4}$$

and in dark adaptation (for the light levels used), S-cones are inactive, and thus the relation between green and blue light intensities is as given for rods. In light adaptation by an orange source, the S-cones will remain dark adapted, but the rods will light adapt and the relative rod contribution to the entire ERG will decrease. The value of m depends on the level of light adaptation. If the ERGs evoked by green and blue light are identical in amplitude:

$$0.09nI_G + 4.2mI_G = nI_B + mI_B \quad \text{or}$$

$$n(0.09I_G - I_B) = m(I_B - 4.2I_G)$$

In light adaptation, the ERGs are equal in amplitude for the combined rod and 'S-cone' component when $I_G = pI_B$,where p is an unknown constant

$$n(0.09pI_B - I_B) = m(I_B - 4.2pI_B)$$

$$n(1 - 0.09p) = m(4.2p - 1)$$

$$n/m = (4.2p - 1)/(1 - 0.09p) \tag{5}$$

$$\text{hence } \frac{{}^{S-cone}V_B}{{}^{rod}V_B} = n/m = (4.2p - 1)/(1 - 0.09p)$$

This analysis is used to evaluate the experimental results. As an additional check, we also added various proportions of V'_{λ} to tabulated values of the spectral sensitivity of the blue mechanism (Estevez, 1979), and, convolving the resultant with the emission of the green and blue LEDS, calculated their expected relative sensitivities to the mixtures. This exercise gave results very similar to the analysis above.

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