Cone mechanisms in the electroretinogram of the cynomolgus monkey

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Three cone mechanisms have been separated with intense chromatic adapting fields in the electroretinogram (ERG) recorded at the cornea of the cynomolgus monkey. Responses were obtained to large test stimuli (45°) superimposed on a larger adapting field (68°). Peak spectral sensitivities were near 450, 530, and 580 nm. Linear summation of these three cone mechanisms, first shown by Wald's (1964) analysis of human psychophysical data, has been confirmed by the same analysis applied to the ERG spectral sensitivity data obtained from the cynomolgus monkey. The ratios derived for the three cone mechanisms in the dark-adapted cone ERG are blue:green:red = 1:6.5:5.4, and emphasize the relatively small contribution of the blue cone mechanism to the total cone ERG. Differences in the ERG spectral sensitivity of the dark-adapted eye obtained in response to 25 and 50 Hz. flickering stimuli could be explained by a linear summation of the blue cone mechanism to the red and green cone mechanisms at the lower frequency. Stimuli known to produce antagonistic responses at the level of the lateral geniculate nucleus in the monkey were shown to summate in the ERG recorded at the cornea. These data are consistent with the idea that the three cone mechanisms act independently in generating the dark-adapted cone ERG b-wave recorded at the cornea in response to a large test stimulus.

Key words: chromatic adaptation, cones, retina, monkey, electroretinogram, psychophysics, photoreceptors, color vision.

Psychophysical studies on humans have shown that at least three cone mechanisms, defined by their spectral sensitivity curves, can be revealed by measurement of thresholds to narrow-band light stimuli presented in the presence of intense chromatic backgrounds. The spectral sensitivities of these three mechanisms approximate the cone pigment absorption data obtained by microspectrophotometric measurements. The three mechanisms have sensitivity maxima in the short (430 to 450 nm.), middle (530 to 550 nm.), and long (560 to 580 nm.) wavelength portions of the spectrum and have been termed, respectively, the blue, green, and red cone mechanisms.

Selective chromatic adaptation has been used to study the blue cone mechanism in the rhesus monkey electroretinogram (ERG)
recorded from within the perifoveal retina and at the cornea. The monkey blue cone mechanism appears to have its sensitivity maximum at 450 nm, while the green and red cone mechanisms have sensitivity maxima at 540 and 580 nm, respectively. However, the contributions of the blue, green, and red cone mechanisms relative to each other and to the total dark-adapted cone ERG have not been defined. This study was done to separate the three cone mechanisms in the cynomolgus monkey ERG recorded at the cornea and to determine the way in which the three separate mechanisms contribute to the cone ERG response of the dark-adapted monkey eye.

Methods

Optical system. A two-channel Maxwellian view system was used for these studies. Light for the stimulus channel was provided by a 1,000-watt xenon arc lamp. Stimulus wavelength was selected by placing Baird Atomic narrow band (10 ± 2 nm. halfpeak bandwidth) interference filters in the stimulus beam; stimulus intensity was controlled by adjusting balanced neutral density wedges. An electronic shutter allowed presentation of single flashes (rise time 0.5 msec.); alternatively a sector disc (50 per cent duty cycle) could be used to present a flickering stimulus. Light for the adapting field or background was provided by a 50-watt tungsten-halide lamp. The color of the background was determined by interposing Corning or Wratten filters. The adapting field colors and intensities were selected for best separation of the cone mechanisms on the basis of pilot studies on both humans and monkeys. For the green cone mechanisms a purple (Wratten 35) adapting field of 5.8 log trolands intensity was used. The red cone mechanism showed best separation on a blue-green (Wratten 47) adapting field of 6.2 log trolands intensity. The blue cone mechanism was separated using a yellow (Corning 3482) field of 7.2 log trolands intensity. Calibration in trolands was made using a spectroradiometer and the CIE Vx curve.

Electroretinograms. ERG’s were recorded at the cornea with a Burian-Allen double-electrode contact lens. A 45° stimulus was needed to obtain clearly detectable ERG signals in response to narrow-band stimuli presented across the entire 400 to 700 nm. spectral range in the presence of intense chromatic backgrounds. The stimulus was centrally superimposed on a background which subtended a visual angle of 68°; this minimized the problem of stimulation of retinal areas not exposed to the background. The electrical signal was amplified by two Tektronix 122 preamplifiers in series (gain of pair = 100,000 nominal) each with a bandpass of 8 to 50 Hz. The amplified signal was monitored directly on an oscilloscope and simultaneously summed by a signal-averaging computer (Fabritek 1072).

ERG studies were performed on three adult female cynomolgus monkeys (Macaca fascicularis) fed a standard diet (Purina Monkey Chow). Monkeys were anesthetized with sodium pentobarbital (25 mg. per kilogram) administered by the intravenous route, and then maintained with additional doses of 5 mg. per kilogram administered by the intraperitoneal route. Pupils were maximally dilated with 10 per cent phenylephrine hydrochloride and 1 per cent cyclopentolate hydrochloride.

ERG spectral sensitivity data were based on the log relative quantum flux at the retina necessary to elicit a criterion amplitude in the ERG for narrow-band stimuli presented under dark-adapted conditions or under conditions of chromatic adaptation. The stimulus was either single light flashes of 10 msec. duration repeated at one second intervals, or a 25 Hz. flickering light. ERG criterion amplitudes varied with the adaptation conditions. At 25 Hz., 5.0 µv was used as the criterion for the green cone mechanism (purple background), 1.0 µv for the red cone mechanism (blue-green background), and 0.5 µv for the blue cone mechanism (yellow background). For the single flash stimulus, response amplitude vs. stimulus intensity curves were obtained at each wavelength in the presence of chromatic adapting backgrounds. The spectral absorption of the macaque lens was used to convert quantum fluxes to the retinal level.

Summation of responses to stimuli of different wavelengths was studied by modifying the apparatus in the following way: the stimulus beam was divided into two beams, each of which passed through a different narrow-band filter and set of neutral density filters before being recombined at the eye. First, the relative intensity of the two beams was adjusted so that when presented separately each yielded the same response amplitude. Then the absolute intensity of the matched pair was adjusted such that when the intensity of each beam was doubled, the response amplitude elicited by that beam doubled; this insured that the response to each light was in the linear (dynamic) range of the stimulus intensity vs. response amplitude function. When the relative and absolute intensities of the two stimuli had been adjusted to meet these two criteria, the response of the dark-adapted eye to each stimulus presented by itself was compared with the response of the dark-adapted eye to the
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Fig. 1. Monkey dark-adapted cone ERG spectral sensitivity data (open circles). Stimulus (25 Hz.) subtended a visual angle of 45°. Sensitivity in this and subsequent figures is on a quantum basis at the retina (see Methods). Average of three animals. Vertical line = ± 1 standard deviation (average). Curve: Wald's human dark-adapted extrafoveal cone spectral sensitivity, determined by psychophysical threshold technique.18

paired stimuli presented simultaneously. Wavelength pairs across the spectrum were tested by pairing a 660 nm. stimulus with other stimuli at 20 nm. wavelength intervals to 420 nm. The stimulus pair of 530 and 600 nm., previously reported to be maximally antagonistic at the level of the monkey lateral geniculate nucleus,14 was also tested.

Since the blue mechanism appears to have a lower critical flicker fusion frequency than either the red or green cone mechanisms,15-17 the spectral sensitivity of the ERG of the dark-adapted monkey eye was measured in response to a stimulus flickering at a low (25 Hz.) and a high (50 Hz.) rate. The sensitivities obtained at the two rates were compared at each wavelength to determine the contribution of the dark-adapted blue-cone mechanism.

Results

Fig. 1 shows ERG spectral sensitivity data from the dark-adapted eye of the cynomolgus monkey in response to a 25 Hz. flickering stimulus. The smooth curve is Wald's18 human extrafoveal dark-adapted cone spectral sensitivity curve obtained by measurement of psychophysical thresholds.

Fig. 2 shows the data obtained with narrow-band flickering (25 Hz.) stimuli superimposed on intense chromatic backgrounds. The spectral sensitivity of the dark-adapted monkey eye shows a maximum near 540 nm. Stimuli presented in the presence of the yellow-adapting background (see Methods) reveal a blue mechanism, with a maximum near 450 nm. Stimuli presented on the purple background show a green mechanism with a maximum sensitivity near 530 nm. In the presence of the blue-green background, stimuli reveal a red mechanism with its peak sensitivity near 580 nm.

Fig. 3 illustrates typical ERG responses of the monkey eye to a narrow-band single-flash stimuli at or near the wavelength of maximum sensitivity of each mechanism under the adaptation conditions described in Methods. The maximum response recorded from the blue cone mechanism in
response to a single-flash stimulus under these conditions was 1.6 μV compared to 24 μV for the green and 16 μV for the red cone mechanism. Spectral sensitivity curves (not shown) derived from such responses to single flashes under the different conditions of chromatic adaptation agreed with the results obtained with flicker shown in Fig. 2.

Fig. 4 illustrates ERG responses of the dark-adapted cynomologus eye to both a 530 and a 600 nm stimulus that were presented separately and adjusted to produce equal amplitude responses (I). The lower traces show, respectively, the responses of the dark-adapted eye to the two stimuli presented simultaneously (II), and the computed sum of the top pair of traces (III). The amplitude of the response of the eye to the two stimuli together is accurately modeled by the sum of the responses to the two stimuli presented separately. Slight differences in implicit times may be attributed to the different stimulus intensities in the two cases (I and II). Similar results were obtained with the pairing of a 660 nm. stimulus with other stimuli at 20 nm. intervals across the visible spectrum.

Fig. 5 illustrates the ERG spectral sensitivity of the dark-adapted monkey eye in response to a 25 Hz. (filled circles) and to a 50 Hz. (open circles) flickering stimulus. The responses to the 50 Hz. stimulus, compared with those to the 25 Hz. stimuli, reflect a decreased sensitivity of the retina to the higher flicker frequency in the spectral region occupied by the blue cone mechanism. Subtraction, on a linear basis, of the two sets of data yields the difference points (open squares) shown here normalized on a log plot. The curve drawn through the points is the spectral sensitivity curve of the blue cone mechanism isolated in this study with flickering (25 Hz.) stimuli in the presence of a bright-yellow adapting field (see Fig. 2).

Discussion
This study shows that at least three cone mechanisms contribute to the cone electroretinogram recorded at the cornea of the cynomolgus monkey. These three cone mechanisms have been separated with the adapting background fields and criterion responses to 25 Hz. flickering stimuli given in Methods. The separation of all three mechanisms in the ERG recorded at the cornea of the cynomolgus monkey provides the basis for analyzing the relative contribution of the spectral sensitivity of each cone.
Fig. 4. Cone responses to 25 Hz. narrow-band stimuli in the monkey ERG. Top pair of traces (I): response of the dark-adapted monkey eye to 530 and 600 nm. stimuli, presented separately and matched to produce equal amplitude responses (see text). Middle trace (II): response to the combined 530 and 600 nm. stimuli. Lower trace (III): computed sum of the top pair of traces. Stimuli (25 Hz.) subtended a visual angle of 68°. Calibration symbol (lower right corner) is 5 μv vertically and 10 msec. horizontally.

The contribution of each mechanism can be evaluated by employing the method devised by Wald2 for human data obtained by psychophysical methods. For this analysis it is assumed that the dark-adapted spectral sensitivity curve $S(\lambda)$ is composed of a weighted sum of the contributions of the red cone mechanism sensitivity $R(\lambda)$, the green cone mechanism sensitivity $G(\lambda)$, and the blue cone mechanism sensitivity $B(\lambda)$. Thus

$$S(\lambda) = rR(\lambda) + gG(\lambda) + bB(\lambda), \quad (1)$$

where $r$, $g$, and $b$ are the weighting factors for the red, green, and blue sensitivity contributions. Only one more assumption must be made, namely,

$$S(\lambda) \approx rR(\lambda), \quad \lambda > 650 \text{ nm}. \quad (2)$$

The assumption of Equation 2 is borne out by examination of Fig. 2, where it may be seen that the sensitivity of the normalized green mechanism is only one-tenth that of the red mechanism for wavelengths greater than 650 nm.

Comparison of the experimentally determined spectral sensitivity of the red mechanism $R'(\lambda)$ with Equation 2 yields the weighting factor for the red mechanism:

$$\frac{S(\lambda)}{R'(\lambda)} \approx \frac{rR(\lambda)}{R'(\lambda)} = \hat{r}, \quad \lambda > 650 \text{ nm}. \quad (3)$$

Comparison of $G'(\lambda)$ with Equation 1 where $\hat{r}$ is the best estimate for $r$. yields the weighting factor for the green mechanism:

$$\frac{S(\lambda) - \hat{r}R'(\lambda)}{G'(\lambda)} \approx \frac{gG(\lambda)}{G'(\lambda)} = \hat{g}, \quad \lambda > 540 \text{ nm}. \quad (4)$$

since for wavelengths greater than 540 nm., the sensitivity of the normalized blue mechanism is less than one one-thousandth that of the green mechanism.

The weighting factor for the blue mechanism is similarly obtained:

$$\frac{S(\lambda) - \hat{r}R'(\lambda) - \hat{g}G'(\lambda)}{B'(\lambda)} \approx \frac{bB(\lambda)}{B'(\lambda)} = \hat{b}. \quad (5)$$

The $\hat{b}$, $\hat{g}$, and $\hat{r}$ values calculated in this manner from the ERG data are: $\hat{b} = 0.09$, $\hat{g} = 0.59$, $\hat{r} = 0.49$, or a ratio of $b:g:r = 1:6.5:5.4$. This is in good agreement with Wald's2 data for humans, obtained by psychophysical threshold techniques ($b = 0.095$, $g = 0.58$, and $r = 0.52$, or $b:g:r = 1:6.2:5.5$).

Fig. 6 shows the results of this contribution analysis graphically. The solid lines show the curves of $\hat{r}R'(\lambda)$, $\hat{g}G'(\lambda)$, and $\hat{b}B'(\lambda)$. The dotted line is the curve of $S(\lambda)$ derived from Equation 1 using the three experimental curves. Data points describe the experimentally derived spectral sensitivities of the dark-adapted monkey.
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Fig. 5. Spectral sensitivity data describing monkey dark-adapted cone responses to 25 Hz (●) and 50 Hz (○) narrow-band stimuli subtending a visual angle of 68°. Calculated data (□) were derived from the difference in sensitivity at the two stimulus frequencies (see text). Curve describing the blue cone mechanism (Fig. 2) was positioned vertically to best fit the calculated data.

Further substantiation of the hypothesis of simple addition may be seen in the results illustrated in Fig. 4. Fig. 4 shows that the response to a stimulus composed of both 530 and 600 nm lights may be modeled by the simple algebraic sum of the responses to each light presented by itself. The response to the 530 nm light is dominated (see Fig. 6) by the green-cone mechanism \( g_G'[\lambda] = 0.57 \); \( r_R'[\lambda] = 0.36 \), whereas the response at 600 nm is dominated by the red cone mechanism \( g_G'[\lambda] = 0.14 \); \( r_R'[\lambda] = 0.37 \). Therefore the red and green cone mechanisms must be adding in a simple algebraic manner in the ERG recorded at the cornea, since any inhibitory interaction would result in a response to the combined stimulus that would be less than the sum of the individual responses to each wavelength presented separately. These same two wavelengths have been shown to produce mutually inhibitory responses at the lateral geniculate nucleus.¹¹ Similar results to Fig. 4 obtained with other spectral pairs (see Methods) further support this hypothesis.

The loss of sensitivity at high flicker rates in the short wavelength region of the spectrum (Fig. 5) can be attributed to the partial or complete fusion of the blue-cone mechanism at 50 Hz. The difference data
Fig. 6. Contributions of the three cone mechanisms to the dark-adapted monkey ERG. Solid lines describe curves derived from spectral sensitivity data obtained for the blue, green, and red cone mechanisms in the presence of an intense chromatic background (see Fig. 2). For each mechanism the data was multiplied by the appropriate weighting factor (see text). Dashed line describes the spectral sensitivity of the dark-adapted cone ERG response predicted by the linear summation of the contributions from each of the three cone mechanisms. Experimentally derived sensitivity data for the dark-adapted eye (Fig. 1) are shown by the open circles.

for the two spectral sensitivity curves approximate the spectral sensitivity of the blue cone mechanism; this approximation can be explained by the simple addition of the contribution from the blue to those of the red and green cone mechanisms.

This investigation has provided data which, within the limits of sensitivity of the ERG recorded at the cornea from a large retinal area (45°), are consistent with the idea that three cone mechanisms act independently in the generation of the cone ERG b-wave. The independence of each mechanism in relation to the other two mechanisms can explain the linear addition of each mechanism to the total dark-adapted cone response. Although b-waves are generated in cells proximal to the photoreceptors,19,29 nevertheless, the spectral sensitivities of the three cone photoreceptors can be isolated in these b-waves.

REFERENCES