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VISUAL TRANSDUCTION IN HUMAN ROD PHOTORECEPTORS

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SUMMARY

1. Photocurrents were recorded with suction electrodes from rod photoreceptors of seven humans.

2. Brief flashes of light evoked transient outward currents of up to 20 pA. With increasing light intensity the peak response amplitude increased along an exponential saturation function. A half-saturating peak response was evoked by approximately sixty-five photoisomerizations.

3. Responses to brief dim flashes rose to a peak in about 200 ms. The waveform was roughly like the impulse response of a series of four to five low-pass filters.

4. The rising phases of the responses to flashes of increasing strength were found to fit with a biochemical model of phototransduction with an 'effective delay time' and 'characteristic time' of about 2 and 800 ms, respectively.

5. Spectral sensitivities were obtained over a wavelength range from 380 to 760 nm. The action spectrum, which peaked at 495 nm, followed the template described for photoreceptors in the macaque retina. Variation between rods in the position of the spectrum on the wavelength axis was small.

6. The scotopic luminosity function derived from human psychophysical experiments was found to agree well with the measured rod action spectrum after adjustments were made for lens absorption and photopigment self-screening in the intact eye.

7. Responses to steps of light rose monotonically to a maintained level, showing little or no relaxation. Nevertheless, the relationship between light intensity and steady-state response amplitude was shallower than that expected from simple response saturation. This is consistent with an adaptation mechanism acting on a rapid time scale.

8. Flash sensitivity fell with increasing intensities of background light according to Weber's law. Sensitivity was reduced twofold by lights evoking about 120 photoisomerizations per second. Background lights decreased the time to peak and the integration time of the flash response by up to 20%.

INTRODUCTION

The characteristics of light absorption and phototransduction in retinal rods play a key role in defining our visual abilities in dim light. Direct information about the responses of human rods has been limited to corneal electroretinogram (ERG)

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recordings in which the contribution of the rods cannot be unequivocally distinguished from that of other retinal elements. This study reports the first recordings of photocurrents from single rods in the human retina. We examined the intensity dependence, kinetics, adaptational properties and spectral sensitivity of the rod response.

The spectral sensitivity of human night vision, the so-called 'scotopic luminosity function' (Crawford, 1949) is assumed to reflect the action spectrum of rods. Attempts to confirm this assumption have met with mixed results. The absorption spectra obtained from macaque and human rods by microspectrophotometry (Bowmaker & Dartnall, 1980; Bowmaker, Dartnall & Mollon, 1980; Dartnall, Bowmaker & Mollon, 1983) were too broad to be compatible with the luminosity function unless it was assumed that the distal portion of the outer segment was light insensitive. On the other hand, the human scotopic luminosity function was found to agree well with the action spectrum of macaque rods obtained from photocurrent recordings (Baylor, Nunn & Schnapf, 1984). In the present work, a comparison was made between the human rod action spectrum and the luminosity function.

The kinetics and intensity dependence of rod responses vary across species. By measuring these properties in human rods, a quantitative comparison can be made to the temporal resolution and dynamic range of human scotopic vision. Similarly, it is possible to correlate the effects of background light on rod signals to light adaptation of human vision. Steady backgrounds alter the amplitude and duration of photon signals in rods of cold-blooded vertebrates. These changes are thought to be mediated by a calcium feedback mechanism (for review see Pugh & Lamb, 1990). Similar observations have been made in mammalian rods although the properties of light adaptation are somewhat variable and species dependent (Baylor *et al.* 1984; Nakatani, Tamura & Yau, 1991; Tamura, Nakatani & Yau, 1991).

METHODS

Preparation and solutions

Suction electrodes were used to record photocurrents from thirty-two rods from seven human eye donors. Eyes were obtained from the University of California Tissue Bank. Eye donors ranged in age from 4 months to 89 years (mean 48 years). Enucleations were performed in room light within 3 h of death. Retinas were then isolated and stored in light-tight containers at 4 °C in a modified L-15 tissue culture medium (Schnapf, Nunn, Meister & Baylor, 1990). Photocurrents were measured in rods for up to 5 days after enucleation.

The visual photopigment, which was bleached during surgery and dissection, was regenerated with exogenous 11-*cis* retinal just prior to electrophysiological measurements by incubating a small piece of retina in a regeneration solution for 30–45 min at 4 °C. This solution contained a suspension of 11-*cis* retinal (Hoffman-LaRoche, Nutley, NJ, USA) incorporated in lipid vesicles (Jones, Crouch, Wiggert, Cornwall & Chader, 1989). Approximately 200 μg 11-*cis* retinal and 1 mg L-α-phosphatidylcholine (Sigma, St Louis, MO, USA) were suspended in 200 μl Locke solution.

Following regeneration the retina was chopped into fine pieces, about 100 μm on a side, and placed into a recording chamber. The chamber was continuously perfused with Locke solution and maintained at 37 °C. The Locke solution used during regeneration and chopping was saturated with O_2 and had the following composition (mM): NaCl, 140; KCl, 3·6; $CaCl_2$, 1·2; $MgCl_2$, 2·4; Hepes buffer, 3 (pH 7·4); dextrose, 10. The solution perfusing the recording chamber was saturated at 37 °C with 95% O_2–5% CO_2 and had the following composition (mM): NaCl, 120; $NaHCO_3$, 20; KCl, 3·6; $CaCl_2$, 1·2; $MgCl_2$, 2·4; Hepes buffer, 3 (pH 7·4); dextrose, 10; EDTA, 0·02; and Basal Medium Eagle vitamin and amino acid supplements (from GIBCO, Gaithersburg, MD, USA).

Light stimuli

Rods were stimulated with diffuse light incident perpendicular to the long axis of the outer segment. Light intensities are expressed as the effective intensity for a 500 nm stimulus that is plane polarized with its electric vector parallel to the outer segment discs. The dimensions of the rod outer segments were similar to those of macaque rods (20–25 μm long, 2 μm diameter). Assuming a specific axial density of 0·016 μm^{-1} (Harosi, 1975; Dartnall *et al.* 1983), the collecting area for this stimulus was estimated to be 1·7 μm^2 (Baylor *et al.* 1984). The product of the collecting area and light intensity gives the expected number of photoisomerizations per second.

Wavelength was regulated with interference filters (Ditric, Hudson, MA, USA) of 10 nm nominal half-bandwidth. Intensity was controlled with calibrated neutral density filters. Unattenuated light intensity at each wavelength was calibrated on every experimental day. In some experiments a short-wavelength blocking filter with a 600 nm cut-off (Kodak, Rochester, NY, USA, Wratten No. 29) was placed in series with interference filters to check for the possible effects of short wavelength contamination of the spectral sensitivity determinations at long wavelengths. Rods of the monkey *Macaca fascicularis*, whose action spectra are nearly identical to those of the humans reported here (Baylor *et al.* 1984), were used for this test. Methods for obtaining rods from macaques have been described previously (Schnapf *et al.* 1990). In four macaque rods, spectral sensitivities in the range 640–760 nm were found to be unaffected by the blocking filter, indicating that short-wavelength contamination was insignificant.

Conversion of photon density to trolands

For comparison to psychophysical and ERG studies, light intensities can be expressed in scotopic trolands (sc td). One sc td was calculated to be equivalent to a transversely incident light (500 nm, polarized) of intensity 5 photons $\mu m^{-2} s^{-1}$, or 8·5 photoisomerizations s^{-1} (Wyszecki & Stiles, 1982, eqn (20) (2.4.4.); Schnapf *et al.* 1990, eqns (1)–(3)). This calculation assumes 0·69 for preretinal transmittance (Baylor *et al.* 1984), 0·98 for scotopic luminosity (Wyszecki & Stiles, 1982, p. 256), 0·35 for the optical density of the rod outer segment to axial illumination (Baylor *et al.* 1984) and 2 for the factor by which the rod inner segment focuses light on the outer segment (Schnapf *et al.* 1990).

The trolands conversion is relatively insensitive to the assumed constants with the exception of the focusing factor (Schnapf *et al.* 1990). A value of 2 was chosen from the relative cross-sectional areas of inner to outer segments in primate rods. While this estimate of the focusing factor has not been experimentally verified, its validity is supported by the human retinal densitometry of Pugh (1975). In those experiments the apparent photosensitivity of rhodopsin in axially illuminated rods was 10^{-7} (sc td s)$^{-1}$. Using the above conversion, it can be calculated that the corresponding photosensitivity of rhodopsin in free solution based on the Pugh measurements is $1{\cdot}3 \times 10^{-8}$ μm^2. This value is similar to the range of values obtained from spectrophotometric measurements of solubilized rhodopsin (Dartnall, 1972). In previous work on monkey rods, it was assumed that there was no focusing effect of the inner segment (Baylor *et al.* 1984).

Electrical recording and filtering

Photocurrents were measured with an Axopatch 1C patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Prior to analog–digital conversion for computer analysis, signals were filtered by a 4-pole Bessel filter on the Axopatch and by an additional 8-pole Bessel filter (Frequency Devices, Haverhill, MA, USA). Signals were sampled above the Nyquist rate to avoid aliasing. The Bessel filters introduced a constant time delay (in the pass-band of the filter) which was inversely related to the cut-off frequency of the filter. These delays were taken into account in the analysis of the kinetics of the photocurrents by shifting the responses on the time axis by the appropriate amount. For some figures, records were further smoothed by a digital 4-pole Butterworth filter with zero phase delay.

Additional details of the surgery, dissection and recording techniques are given elsewhere (Baylor *et al.* 1984).

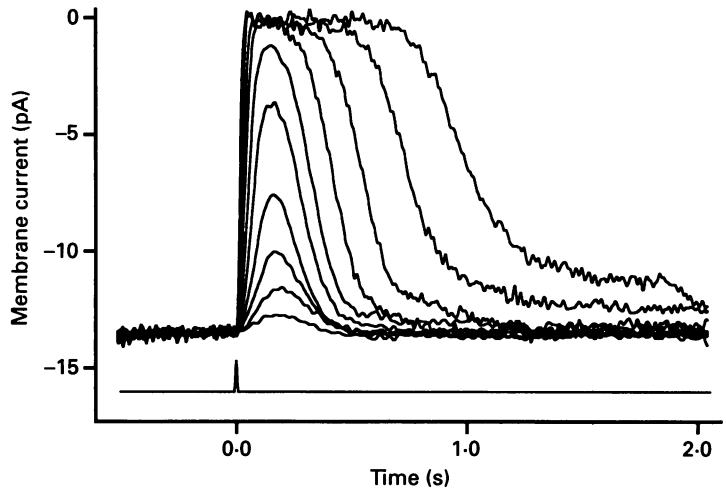

Fig. 1. Superimposed responses to 10 ms flashes of increasing strength in a human rod. Traces are averaged responses of 2–21 flashes. Membrane current is plotted as a function of time. Flash photon densities increased by roughly factors of 2 from 7 to 3060 photons μm^{-2}. Flash monitor shown below current traces. Bandwidth, DC–50 Hz.

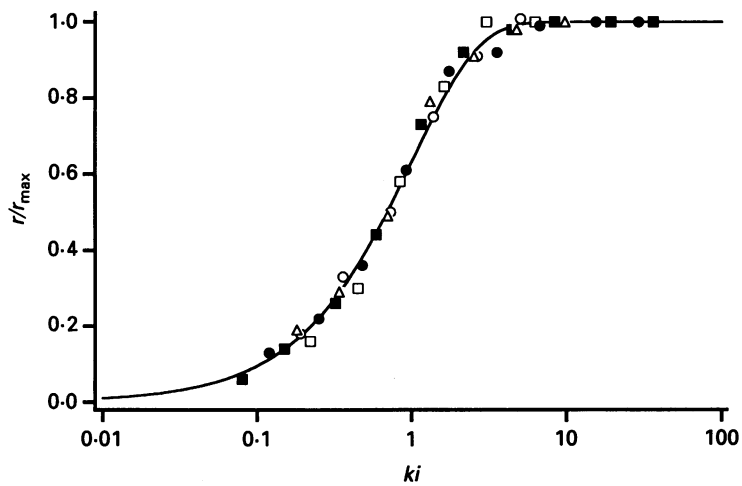

Fig. 2. Relationship between peak response amplitude and flash photon density on normalized axes. Symbols plot peak response amplitude measured in 5 rods; each rod represented by a different symbol. Smooth curve is eqn (1) in the text. Values of r_{max} ranged from 9 to 14 pA; values of k were in the range 0·016–0·028 photons^{-1} μm^2.

RESULTS

Responses to flashes

A family of superimposed responses to 10 ms flashes of light is shown in Fig. 1. In successive traces flash intensity was increased by factors of about 2. Traces are average responses to multiple flashes. In this cell, a saturating flash evoked a

maximal response of 14 pA. Maximal responses of up to 20 pA were recorded in other rods. With increasing flash strength the time to the peak of the response decreased slightly.

The relationship between peak response amplitude and flash strength for the rod in Fig. 1 and four other rods in the same retina is shown in Fig. 2 on normalized axes. Results from each rod are plotted with a different symbol. The peak response amplitude, r, is normalized by the maximal response amplitude r_{max} for each cell. Flash strength, i, in photons μm^{-2}, is normalized by a scaling constant k which is the normalized sensitivity characteristic of each cell (Schnapf *et al.* 1990). The smooth curve drawn is an exponential saturation function (Lamb, McNaughton & Yau, 1981; Baylor *et al.* 1984; Lamb & Pugh, 1992):

$$r/r_{max} = 1 - e^{-ki}. \quad (1)$$

In a collection of sixteen cells with large responses (mean r_{max}, 11 pA) the average value of k was $0{\cdot}018 \pm 0{\cdot}013$ photons^{-1} μm^2 (mean $\pm$ S.D.). The peak amplitude of the single photon response was extrapolated from flash responses in the linear range. In this collection of rods the mean estimated peak amplitude was 0·1 pA, assuming a collecting area of 1·7 μm^2 (see Methods). The corresponding photon density that evoked a response of half-saturating amplitude was 39 photons μm^{-2}, or 66 photoisomerizations.

The intensity dependence seen here was similar to that described previously for rods of the macaque monkey (Baylor *et al.* 1984). However, the human rods on average required twice the number of photoisomerizations to elicit a half-saturating response, and the estimated photon signal was five times smaller. These differences might be explained by incomplete photopigment regeneration in the rods of our preparation, which would be expected to reduce the amplitude of the response to single photons (Corson *et al.* 1990). The half-saturating intensities found in human and macaque rods are similar to the value of 36 photoisomerizations (4·3 sc td s) estimated by Adelson (1982) for the minimal flash strength at which transient saturation appears in human rod vision.

Waveform of responses to dim flashes

The response to a dim flash of light is shown for one rod in Fig. 3. The points plot the average of responses to flashes of 8 photons μm^{-2}. The time to the peak of the response was 156 ms in this rod, and 189 ± 37 ms (mean $\pm$ S.D.) in a collection of seventeen rods. The smooth curve is the impulse response of a series of buffered low-pass filters of the form:

$$j(t) = r[\bar{t}e^{(1-\bar{t})}]^{n-1}, \quad (2)$$

(Baylor, Hodgkin & Lamb, 1974), with $j(t)$ the change in membrane current with time, r the peak amplitude, n the number of filter stages, $\bar{t} = t/t_p$ and t_p the time to the peak of the response. For the rod in Fig. 3, n was 4 and t_p was 156 ms. In six cells with good signal-to-noise characteristics, the best-fitting values were in the range $n = 4$–5 and $t_p = 145$–190 ms. The fits of eqn (2) to the measured responses were only fair, the deviations mainly in the falling phase of the response. In some rods, a better fit was obtained to the independent activation model of Baylor *et al.* (1974) with the

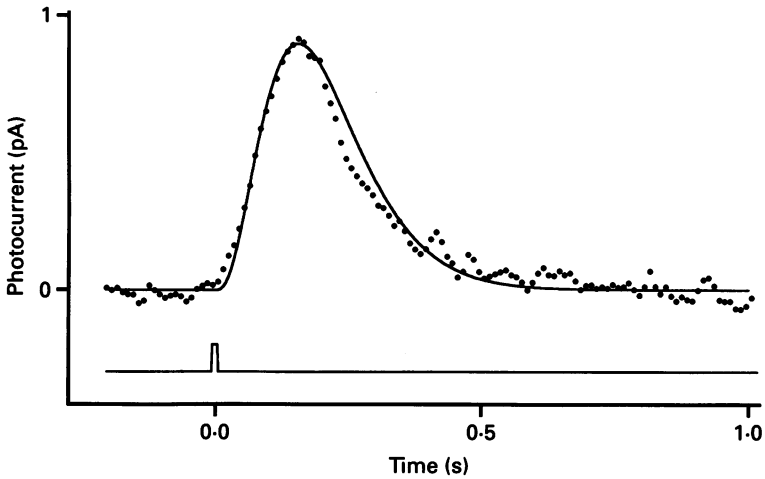

Fig. 3. Average dim flash response. Points give the response to an average of 99 flashes, 10 ms duration. Bandwidth, DC–20 Hz; r_{max} = 13 pA. The points were shifted on the time axis to take into account analog filtering delays. Zero time taken as the centre of the 10 ms flash; stimulus monitor below. Flash photon density 8 photons μm^{-2}. The continuous curve is eqn (2) with $n = 4$ and $t_p = 156$ ms.

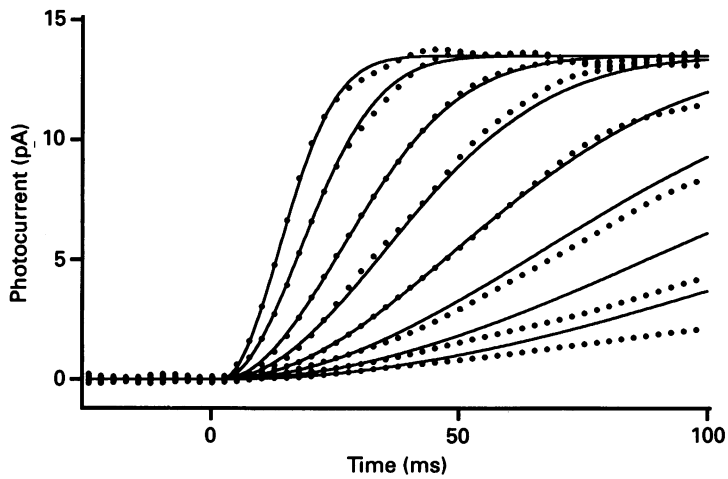

Fig. 4. Rising phase of the flash response. Points plot the photocurrent in response to a series of flashes of increasing strength. Bandwidth, DC–50 Hz; 2·5 ms sampling interval. The current responses were shifted on the time axis to take into account analog filtering delays. Zero time taken as the centre of the 10 ms flash. Flash photon densities increased by roughly factors of 2 from 20 to 2250 photons μm^{-2}. The curve is eqn (3) with t_{eff} = 2·2 ms and τ_ϕ = 705 ms.

same number of stages. One less filter stage was required to fit responses in human rods as compared to rods from the macaque (Baylor *et al.* 1984). This is due in part to a failure in the earlier macaque work to allow for delays introduced by analog filtering (see Methods).

The integration time, τ_i, is a measure of the effective duration of the photon response. It is obtained from the time integral of the dim flash response normalized to a peak height of one. In five of the six rods above with sufficiently stable recordings, the value of τ_i ranged from 204–443 ms with a mean of 316 ± 111 ms (mean $\pm$ S.D.).

With dim brief flashes of light, the peak response amplitude scaled linearly with flash duration. At the other extreme, for very long pulses of dim light, the peak response amplitude was independent of flash duration. For a cell with kinetics like that shown in Fig. 3, the pulse duration at which these two asymptotic behaviours intersect has a value of approximately τ_i. Thus τ_i is analogous to the psychophysical parameter of 'critical duration'. The critical duration of dark-adapted human vision is approximately 100 ms (Barlow, 1958). This value is three times lower than the average τ_i measured in single rod outer segments, suggesting that rod signals may be high-pass filtered by subsequent neural processing.

Equation (2) is an empirical expression which has been used extensively to characterize the waveform of photon signals in retinas of many species. From the biochemical reactions known to underlie the process of phototransduction, Lamb & Pugh (1992) have derived analytical expressions to fit the rising phase of the flash response in rods of cold-blooded vertebrates. Figure 4 plots the rising portion of a family of responses from a human rod along with eqn (6.10) of Lamb & Pugh (1992), given here for convenience:

$$j(t) = r_{max}\{1 - \exp\left[-\frac{\Phi}{2}\left(\frac{t - t_{eff}}{\tau_\phi}\right)^2\right]\}, \quad (3)$$

where Φ is the number of photoisomerizations per flash, t_{eff} is the effective delay between the flash and the onset of the response, τ_ϕ is the characteristic time constant of phototransduction and $j(t)$ and r_{max} are as above. In Fig. 4, r_{max} is 13·5 pA, t_{eff} is 2·2 ms and $\tau_\phi = 705$ ms. The constants were determined by minimizing the squared error between the points and the curve for all the responses over the time period of 0–75 ms. In five rods, eqn (3) gave similarly good fits with the value of t_{eff} ranging from 0 to 4 ms and τ_ϕ from 705 to 924 ms. These values are about five times smaller than those derived from model fits to amphibian rods. The theoretical curves diverge from the measured responses for times greater than 75 ms. This divergence is expected because the model ignores all inactivation processes.

In this model, the theoretical light-evoked change in phosphodiesterase (PDE) activity as a function of time and intensity can be calculated from a mathematical transformation of the measured photocurrents (eqn (6.18) of Lamb & Pugh, 1992). In human rods, as in salamander rods, the theoretical estimate of PDE activity was found to rise linearly with time (after a delay of t_{eff}) and was proportional to the number of photoisomerizations. From the slopes and intercept of the transformed responses, estimates for t_{eff} and τ_ϕ were derived which were similar to those obtained directly from fits of the photocurrent to eqn (3).

Equation (3) dictates that during the rising phase of the response the relationship between response amplitude and flash strength should follow an exponential saturation function. We found that this relationship continued to be a fair approximation for times at least up to the peak, well past the time when the

measured and theoretical responses diverge. This finding suggested that PDE activity is linearly related to flash strength for times up to the peak (eqn (6.6) of Lamb & Pugh, 1992).

To test this idea we attempted to fit families of flash responses by scaling the linear range response by intensity and then compressing the scaled waveform exponentially (eqn (9) of Schnapf *et al.* 1990). For small signals this procedure accurately predicted the entire time course, but for larger signals there was good agreement only in the rising phase. A related manifestation of this failure is that the time to peak of the measured responses decreased with increasing flash strength (Fig. 1). These deviations suggest that at later times the response is shaped by more complex processes which might include non-linear activation of PDE or guanylate cyclase (reviewed in Pugh & Lamb, 1990). The fact that an exponential saturation relationship holds at the peak of the response remains unexplained and may simply be a fortuitous result of the combined effects of these or other non-linearities.

Spectral sensitivity

Action spectrum

Flash responses were measured with lights in the wavelength range 380–760 nm. The intensity dependence on response amplitude was similar at all wavelengths except for the sensitivity constant k which varied with wavelength. Responses of comparable sizes evoked by different wavelengths were indistinguishable from one another. These results support the idea (Naka & Rushton, 1966) that the flash response depended only on the number of photons absorbed and not the wavelength of the absorbed photons.

TABLE 1. Spectral sensitivity of human rods

λ (nm)	$\log S$	S.E.M.	n	λ (nm)	$\log S$	S.E.M.	n
379	−0·888	0·055	3	540	−0·328	0·004	4
390	−0·602	—	1	550	−0·477	0·028	3
398	−0·784	0·012	3	560	−0·637	0·013	3
410	−0·605	0·010	2	571	−0·949	0·017	4
418	−0·496	0·007	4	581	−1·150	0·006	5
430	−0·380	0·003	2	591	−1·517	0·005	4
440	−0·375	0·034	3	601	−1·822	0·003	5
449	−0·196	0·005	2	621	−2·527	0·012	6
460	−0·195	0·010	4	639	−3·272	0·023	4
467	−0·068	0·011	2	660	−3·849	0·010	7
480	−0·051	0·027	3	678	−4·675	0·013	4
491	−0·027	0·007	2	700	−5·172	0·010	7
499	0·000	0·003	12	720	−5·854	0·037	5
510	−0·061	—	1	740	−6·457	0·022	3
520	−0·090	0·008	3	760	−6·862	0·034	3
531	−0·302	0·012	3				

λ, wavelength; $\log S$, mean value of the $\log_{10}$ normalized sensitivity; S.E.M., standard error of the mean; n, number of rods tested at each wavelength.

Spectral sensitivity was determined at each wavelength from the value of k that gave the best fit of the peak amplitudes to eqn (1). The sensitivity at 500 nm was

obtained repeatedly throughout the experiment in order to correct, if necessary, for changes in the rod's quantal sensitivity. Sensitivities at each wavelength were calculated relative to the value at 500 nm from the ratio of $k(\lambda)/k(500)$.

Measurements were obtained from twelve rods from four donors (2 males, 2 females). The average spectrum and standard errors were calculated after individual

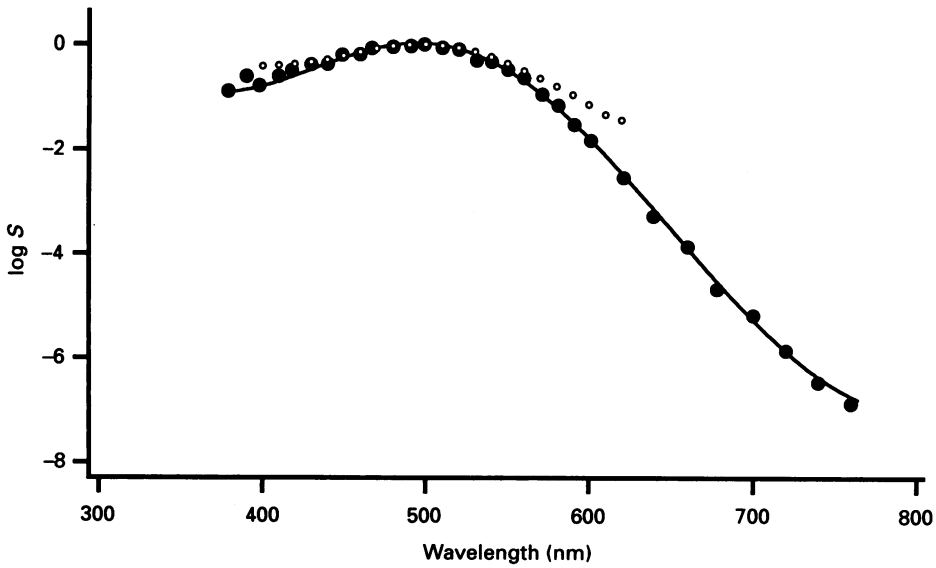

Fig. 5. Average spectral sensitivity of 12 rods. The filled circles plot the log of the normalized sensitivity as a function of wavelength (tabulated in Table 1). The continuous curve is a sixth-order polynomial (Baylor *et al.* 1987, eqn (6)) with $\lambda_m = 495$ nm. The small open circles plot the absorption spectrum obtained in human rods by microspectrophotometry (Dartnall *et al.* 1983).

spectra were shifted on the log sensitivity axis to minimize the differences between them. Average sensitivities are listed in Table 1 and plotted in Fig. 5 by the filled circles. The continuous curve near the points is a sixth-order polynomial with the same coefficients that were found previously to fit the spectral sensitivities of cones in macaque and human retinas (Baylor, Nunn & Schnapf, 1987, eqn (6); Schnapf, Kraft & Baylor, 1987). The wavelength of maximum sensitivity, λ_m, was determined by least-squares fit of the points to the curve, where the fitting errors were weighted by the number of measurements at each wavelength. The best fit was obtained for $\lambda_m = 495$ nm. Changing the value of λ_m by ± 1 nm increased the chi-squared error by 10%. The Dartnall nomogram (Wyszecki & Stiles, 1982) provided a similarly good fit to the results in the range 400–600 nm (not shown). The best fit was obtained when λ_m was set to 493 nm. The human action spectrum measured here is very similar to that previously measured in macaque rods (Baylor *et al.* 1984).

The small open circles in Fig. 5 plot the absorption spectrum obtained from microspectrophotometric measurements in thirty-nine human rods (Dartnall *et al.* 1983). The absorption curve peaks in a similar region of the spectrum ($\lambda_m = 496$ nm) but is significantly broader than the action spectrum obtained here.

On a log sensitivity *vs.* wave number plot, the long-wavelength portion of the spectrum asymptotically approached a straight line. Fitting straight lines to spectra from eight individual rods gave an average slope of 14·7 μm. This value is comparable to the value of 14·8 μm for the asymptotic slope of the human scotopic luminosity function of Crawford (1949).

Spectral variation

Variation in spectral position of curves of single rods was estimated by fitting individual spectra to eqn (6) of Baylor *et al.* (1987), and allowing λ_m to vary to minimize the differences between the theoretical curve and the measured spectra. In eleven rods from three donors (2 females and 1 male) the standard deviation of the estimated peak wavelength was found to be 1·6 nm, with a range of 5·9 nm. (One rod was not included in the analysis because its spectrum was not measured for wavelengths greater than 500 nm.)

An alternative method used to estimate the variability was to fit a straight line with a fixed slope of 14·7 μm to the descending limb of each spectrum plotted on a wavenumber scale (Baylor *et al.* 1987). For eight rods (from 3 humans) where measurements were made to sufficiently long wavelengths, the x-intercepts (at $\log S = 0$) of the fitted lines were very similar. The mean intercept was 1·786 μm^{-1}, with a range of 1·779–1·795 μm^{-1} and a standard deviation of 0·0048 μm^{-1}. On a wavelength scale this variation corresponded to a standard deviation in λ_m of 1·2 nm with a range of 3·8 nm.

The magnitude of these variations was small, consistent with the view that the differences were due to errors in experimental measurement. The number of donors was too limited to estimate intersubject variation, but there was no evidence for a clustering of spectral positions within a single eye.

Comparison to scotopic visibility

The spectral sensitivity of the standard dark-adapted human observer is indicated by the curve in Fig. 6 which plots the scotopic visibility function derived by Crawford (1949) from psychophysical experiments. The points plot the action spectrum obtained from photocurrent recordings after taking into account the estimated effects of absorption of light by the lens, and photopigment self-screening for axial illumination in the intact eye. This function gives the predicted sensitivity of single rods in the intact human eye as a function of the wavelength of light incident on the cornea.

The lens density function was calculated from tabulations in Wyszecki & Stiles (1982, Table II (2.4.6)). The form of this function is believed to be constant across subjects but to increase in absolute density with subject age. The best fit between the luminosity function and the rod action spectrum was obtained when the lens density function was scaled to an optical density ($\log_{10}$) of 0·16 at 500 nm and the peak axial density ($\log_{10}$) of a human rod was taken to be in the range 0·30–0·50. In Fig. 6, a peak axial density of 0·35 was assumed (Alpern & Pugh, 1974; Baylor *et al.* 1984).

A good match was obtained between the luminosity function and the adjusted rod action spectrum. The estimated lens density of 0·16 corresponds to the average density for a 25 year old (Wyszecki & Stiles, 1982) and is consistent with the mean

age of 22·4 years of the subjects in Crawford's study. The estimated axial photopigment density was in the range expected of a rod outer segment 20–25 μm long with a specific density of 0·016 μm^{-1} (Harosi, 1975). Similar estimates of peak axial density can be calculated from the transverse optical density measured directly

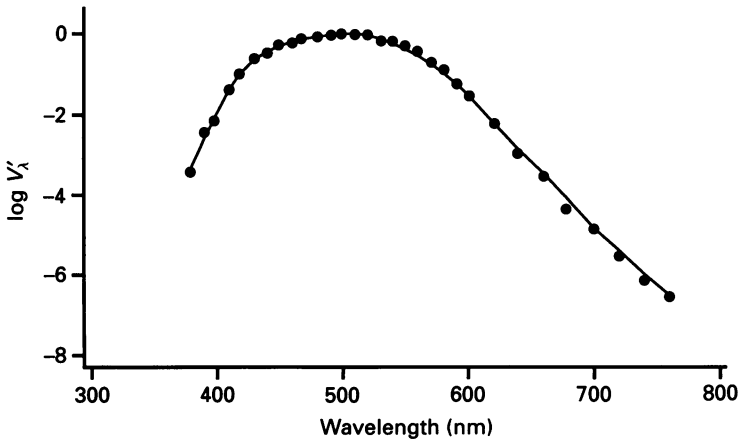

Fig. 6. Comparison of adjusted action spectrum of single rods (points) to human scotopic sensitivity function V'_λ (curve) of Crawford (1949). Adjustments given in the text.

in human rods by microspectrophotometry (Bowmaker & Dartnall, 1980; Dartnall *et al.* 1983). The shape of their absorption spectrum, however, was too broad relative to the luminosity function to allow for the additional broadening expected by photopigment self-screening. This finding led to the hypothesis that the tips of the outer segments (the region where the effects of self-screening are greatest) are electrically inactive (Bowmaker & Dartnall, 1980). No such assumption is required here in order to match the rod action spectrum to the psychophysics.

In this study, the agreement is good out to the longest wavelengths tested (760 nm), consistent with the view that the Crawford function derives simply from rod excitation without significant input from cones. Calculations based on the action spectrum of human cones (Schnapf *et al.* 1987) indicated that anything more than about a 0·2% cone contribution in Crawford's measurements would result in a noticeable distortion of the scotopic luminosity function relative to the rod action spectrum.

Background light adaptation

Decrease in flash sensitivity

The effect of background light on flash sensitivity was assessed by presenting brief flashes of light both in the presence and absence of a steady background. Test flash intensities were kept dim so that response amplitude scaled linearly with flash strength. Sensitivity was defined as peak response amplitude divided by flash strength.

Results from experiments in five rods are shown by the symbols in Fig. 7. The

ordinate is flash sensitivity (S_F) on background of intensity I, relative to the dark-adapted flash sensitivity (S_F^D). The abscissa is background intensity scaled by the product of the normalized flash sensitivity k and integration time τ_i. The values of k and τ_i were estimated from flash responses measured in the dark-adapted state.

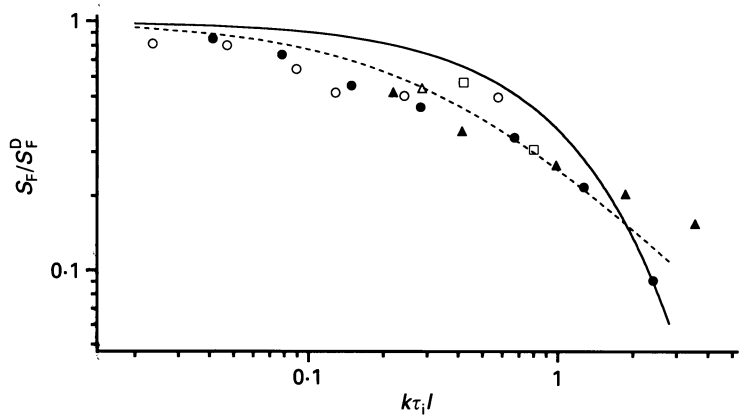

Fig. 7. Normalized flash sensitivity as a function of the scaled background light intensity in 5 rods; each rod signified by a different symbol. Flash sensitivity was normalized to the average values measured before and after presentation of the background. Background intensity I scaled by $k\tau_i$; k and τ_i were measured in the rod's dark-adapted state. The continuous curve is the expected flash sensitivity due to saturation (eqn (4)). Values of r_{max} ranged from 4 to 13 pA; values of $k\tau_i$ ranged from $2{\cdot}6\times10^{-3}$ to $7{\cdot}3\times10^{-3}$ photons^{-1} μm^2 s. The dashed curve is Weber's law (eqn (5)) with $I_0 = 0{\cdot}34/(k\tau_i)$.

Estimates of $k\tau_i$ for the five rods were in the range 0·0026–0·0073 photon^{-1} μm^2 s, with a mean value of $0{\cdot}0049 \pm 0{\cdot}0017$ photon^{-1} μm^2 s (mean ± S.D.).

The continuous curve in Fig. 7 shows the flash desensitization expected from response saturation alone. The function is given by:

$$S_F/S_F^D = e^{-k\tau_i I} \quad (4)$$

(Baylor *et al.* 1984). The points deviated from the saturation curve suggesting that a time-varying adaptational mechanism is acting to lower quantal sensitivity beyond that expected from response saturation.

This observation is similar to that obtained from adaptational studies in rods of cold-blooded vertebrates (see review in Pugh & Lamb, 1990), and in rods of several mammalian species (Tamura, Nakatani & Yau, 1989; Nakatani, Tamura & Yau, 1991; Matthews, 1991).

The sensitivity measurements fit somewhat better to Weber's law:

$$S_F/S_F^D = \frac{I_0}{I+I_0}, \quad (5)$$

where I_0 is the intensity that reduced flash sensitivity to half the dark-adapted value. The value of I_0 ranged from 40 to 122 photons μm^{-2} s^{-1} in the five rods with an average value of 71 ± 33 photons μm^{-2} s^{-1} (mean ± S.D.) or about 120 photoisomerizations s^{-1}. This is very similar to the half-desensitizing intensity found in one study in macaque rods (Baylor *et al.* 1984), and about twice the average value obtained for macaque

rods in a later study (Tamura *et al.* 1991). The dashed curve in Fig. 7 shows the best-fitting Weber relationship to the collected results on a normalized abscissa with $I_0 = 0{\cdot}34/(k\tau_i)$. Slightly better fits of eqn (5) to individual cells were obtained when the value of I_0 was free to vary for each cell.

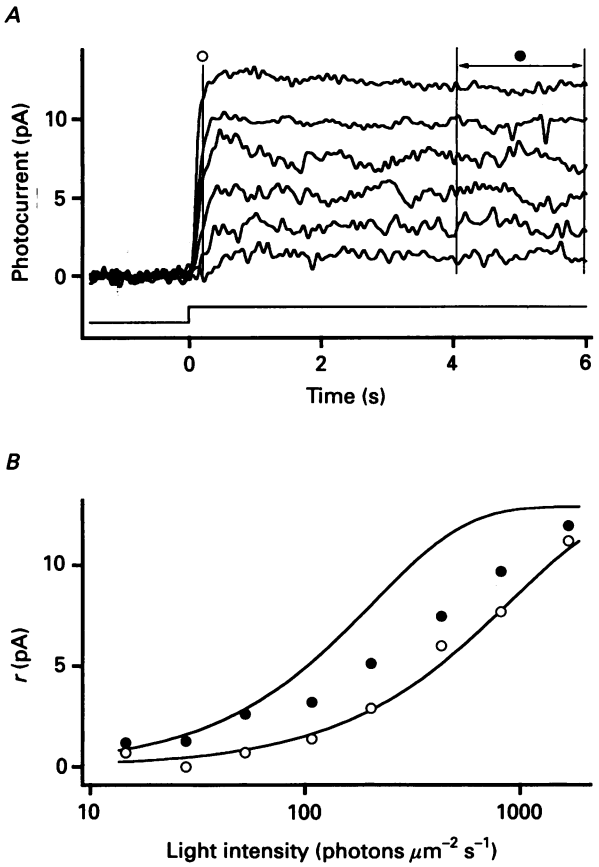

Fig. 8. Responses to steps of light from rod (●) in Fig. 7. *A*, photocurrent as a function of time after the onset of a step of light. Stimulus monitor below. Intensities were incremented by factors of 2 from 15 to 1660 photons $\mu m^{-2}\ s^{-1}$ (not all traces shown). Bandwidth, DC–10 Hz. Circles denote the times used to measure the photocurrent amplitudes plotted in *B*. *B*, filled circles plot steady-state response amplitude averaged over $t = 4$–6 s as a function of light intensity. The open circles plot the amplitudes at 200 ms. The curves plot the expected response amplitudes based on the instantaneous saturation function measured in this rod with brief flashes. They are given by eqn (6) with $r_{max} = 13$ pA and $k = 0{\cdot}011$ photons^{-1} μm^2, $\tau(t) = 424$ ms (left curve) and $\tau(t) = 123$ ms (right curve).

Responses to steps

Further evidence for this time-dependent adaptational process can be seen in the responses to the background steps themselves. An example is shown in Fig. 8 for one rod in Fig. 7 (●). While the response to the light step is essentially monotonic, rising to a maintained level of current (Fig. 8*A*), the final current level obtained in bright

light is less than that predicted on the basis of the instantaneous saturation characteristic determined for this rod. The filled circles in Fig. 8*B* plot the steady-state amplitude of the response as determined by averaging over the time window from 4 to 6 s. There were no further changes in the amplitude of the current during the

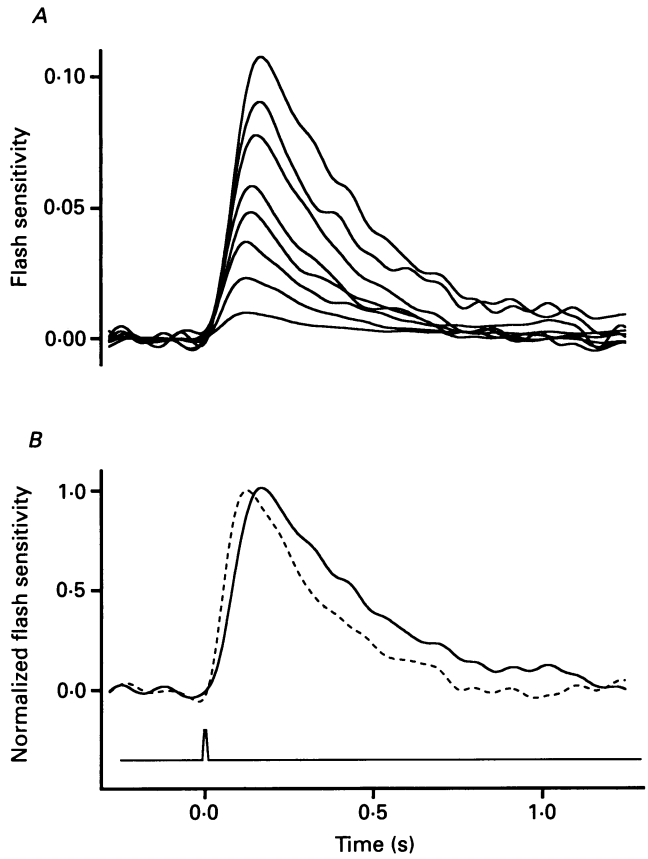

Fig. 9. Change in the kinetics of the flash response with light adaptation. *A*, flash sensitivity (change in membrane current (pA) per photoisomerization) is plotted as a function of time after the centre of the flash for the rod in Fig. 8. Flash duration, 10 ms; stimulus monitor below. Traces are superimposed averages of 25–75 responses to linear range flashes in the presence of steady background lights. The largest trace was measured in the absence of a background. Background intensities were increased by roughly factors of 2 from 8 to 485 photons μm^{-2} s^{-1}. Bandwidth, DC–10 Hz. *B*, for better comparison of the waveforms, the dark-adapted trace and the trace obtained on the brightest background are normalized to the same peak height.

remaining 9 s of light exposure (not shown). The curve on the left is the expected response amplitude in the steady state derived from the dark-adapted flash response family. The curve is given by:

$$r = r_{max}(1 - e^{-k\tau(t)I}), \tag{6}$$

where $\tau(t)$ is the time integral up to time t of the normalized flash response

($\tau(\infty) = \tau_i$) and k is fixed by the dark-adapted saturation function for flashes. The observed steady-state relationship was shallower than that predicted from the idea that photon signals summed linearly and then were compressed by exponential saturation. Similar behaviour was observed in the other four rods. The poor fit indicates that the gain of phototransduction decreased in the presence of sustained bright light. The absence of a significant sag in the membrane current suggests that this adaptational process occurred on a rapid time scale, reaching a new steady state close to the time the step response had reached its peak value.

The speed with which this process turns on was investigated by measuring the amplitude of the step response at fixed early times. The open circles in Fig. 8 plot the response amplitude measured 200 ms after step onset. The curve near the points is eqn (6) with $t = 200$ ms and k fixed as above. The close correspondence of the points and the curve indicates that the delayed desensitization had not yet been activated to a significant extent. By 300 ms, clear deviations from the expected static saturation function were evident.

A more prominent (and variable) relaxation in the step response was reported in rods of bovine, rabbit and monkey, while waveforms more comparable to those seen here were reported for rods of the rat and cat (Nakatani *et al.* 1991; Tamura *et al.* 1991).

Kinetics of flash responses on steady backgrounds

Steady background lights caused the flash response to speed up. This is illustrated in Fig. 9 for the same rod as in Fig. 8. In *A*, flash sensitivity (change in membrane current per photoisomerization) is plotted as a function of time after the flash. The superimposed traces are average responses to linear range flashes in the presence of steady background lights. Background intensities were in the range 0–485 photons $\mu m^{-2} s^{-1}$. The largest trace was obtained in the absence of a background. The reduction in duration of the flash response is illustrated more clearly in Fig. 9*B* where the dark-adapted flash response and the response on the brightest background are renormalized to the same peak. In this rod, the time to peak on the brightest background was reduced by 25% relative to the dark-adapted flash response. The integration time was reduced by a similar amount. Four other rods showed comparable changes in kinetics. On the brightest backgrounds the time to peak and integration time of the flash response were reduced 19%, on average, in the five rods tested. Changes of similar magnitude have been seen in some macaque rods (Baylor *et al.* 1984) and in rods of the guinea-pig (Matthews, 1991). More dramatic changes in kinetics have been observed in cold-blooded vertebrates (eg. Baylor, Lamb & Yau, 1979).

DISCUSSION

Spectral sensitivity

Comparison to absorption spectra

Wald & Brown (1958) measured the absorption spectrum of an aqueous solution of human rhodopsin and the spectrum of a suspension of human rod outer segments. The spectrum in aqueous solution was blue-shifted by 7 nm as compared to the latter

spectrum (493 *vs.* 500 nm). The human rod action spectrum of Fig. 5 fell between these two absorption spectra (comparison not shown). All three spectra are narrower than the absorption spectrum obtained from human rods with microspectrophotometry (Dartnall *et al.* 1983). A broadening of this spectrum might be expected from the light scattering and focusing problems related to microspectrophotometric measurements on small cells.

Variation in the action spectra of human rods

Based on an analysis of spectral sensitivity measurements in human observers Alpern (1987) inferred that the action spectrum of human rods had a mean λ_m of 497 nm. This agrees well with the estimate of 495 nm reported here. Alpern further suggested that λ_m varied across individuals, spanning a range of 20 nm with a standard deviation of 4 nm.

The number of human donors in the present study is not sufficient to rule out spectral variation between observers, however the observed variation was small and consistent with experimental uncertainty of the measurement. Experimental support for the notion of an invariant rod spectrum comes from the genetics of human rhodopsin. In a population of over 300 patients, variations in the coding portions of the rhodopsin gene were never encountered except for patients with retinal degenerations (Sung *et al.* 1991; Dryja, Hahn, Cowley, McGee & Berson, 1991). This is in contrast to the common polymorphisms observed in the genes coding for the cone photopigments which correlate with variations in colour vision (Winderickx, Lindsey, Sanocki, Teller, Motulsky & Deeb, 1992). If intersubject differences in the rhodopsin spectrum are in fact rare, then the results of Alpern (1987) might be explained by variations in lens absorption, photopigment density or measurement error.

Comparison to human ERGs

Hood & Birch (1990) have used the leading edge of the ERG to monitor the electrical activity of rods in the human retina. The intensity dependence was found to fit the exponential saturation function of eqn (1) and the time course was consistent with the low-pass filter model of eqn (2) with an n value of 4–5. Values for the semi-saturating flash strength and time to peak could not be evaluated independently because of the intrusion of signals from other retinal elements beyond the first 25 ms of the ERG record. The time to peak, therefore, could not be observed directly. When they assume a value of 7 sc td s for the half-saturating flash strength (or 60 photoisomerizations), Hood & Birch (1990) estimated values of 149 and 191 ms for the time to peak of the linear flash response in two subjects. These values are in excellent agreement with the present measurements.

The Lamb & Pugh (1992) model has also been fitted to the rising edge of the human rod ERG by Breton & Schueller (1992) and Hood & Birch (1992). Best fits were obtained with values of about 2·5 ms and 500 ms for t_{eff} and τ_ϕ respectively, similar to the results of the present work. In the model, the constant τ_ϕ is an indication of the time constant for change of cGMP concentration per photoisomerization, and τ_ϕ^{-2} can be thought of as the overall gain of phototransduction. The somewhat higher values of τ_ϕ obtained here (705–924 ms) as compared to that in the intact eye are consistent with the low quantal sensitivity of the rods of our preparation.

In amphibian rods, the value of τ_ϕ is 5–10 times larger than that in humans,

suggesting that the cGMP concentration changes more slowly in amphibians (Lamb & Pugh, 1992). Differences of this magnitude are to be expected from the differences in cytoplasmic volume of amphibian and primate rod outer segments (Pugh & Lamb, 1993).

Background desensitization

Comparison to other species

In background light, the size of the photon signal was reduced twofold at a background intensity of about 120 isomerizations s^{-1}. This value is roughly similar to the values observed previously in non-human primates (Baylor *et al.* 1984; Tamura *et al.* 1991). In rods of other mammalian species (rabbit, cattle, rat and cat), the half-desensitizing intensity was about 30–40 isomerizations s^{-1} (Nakatani *et al.* 1991). In cold-blooded vertebrates, considerably dimmer lights are needed, only about 1–10 isomerizations s^{-1} (e.g. Baylor, Matthews & Yau, 1980; Lamb *et al.* 1981; Copenhagen & Green, 1985). The lower value in cold-blooded vertebrates can be partly attributed to the larger amplitude and longer duration photon signals in lower vertebrates.

The precise relationship between flash sensitivity, kinetics and background light intensity in primate rods varies across species, across studies and across rods within a single study. In this study and in Tamura *et al.* (1991), the reduction in sensitivity exceeded that expected from response saturation. In Baylor *et al.* (1984), the response saturation model fitted well overall, but showed deviations from that model at high intensities. In all three studies, background illumination reduced the time to the peak of the photon response. The macaque rods of Tamura *et al.* (1991) showed large sags in photocurrent in maintained light while the macaque rods of Baylor *et al.* (1984) and the human rods here gave sustained responses.

The reason for the differences in kinetics and desensitization is not clear. Background desensitization arises from a combination of response saturation and changes in the gain of phototransduction. Differences observed between studies may reflect subtle differences in experimental conditions such as the formulation of the incubation and recording solutions, temperature, pH, and the initial state of adaptation. The delicate balance of photochemical processes that determines the kinetics and adaptational properties of a cell might be altered by any one or combinations of these parameters.

Comparison to psychophysical experiments

The half-desensitizing background intensity of 120 isomerization s^{-1} corresponds to about 14 sc td. By comparison, the background intensity needed to reduce the flash sensitivity of a human observer by a factor of 2 is only about 10^{-3} sc td; at 14 sc td, visual sensitivity as a whole is reduced by a factor of 10^2–10^4, the exact value depending upon the duration and size of the test flash used for monitoring sensitivity (Aguilar & Stiles, 1954; Barlow, 1957). Clearly, in this range of intensities, the overall reduction in sensitivity of human rod vision cannot be attributed to a reduction in the amplitude of photon signals in the rods themselves.

Psychophysical incremental sensitivity drops off precipitously at intensities exceeding 1000 sc td (Aguilar & Stiles, 1954). Referred to as 'scotopic saturation', this effect is just apparent at about 100 sc td. Saturation of the rod system would be

expected if background light significantly reduced the amplitude of photons signals relative to the noise of the visual system. At 100 sc td, the amplitude of the photon signal in human rods in this study was reduced on average by a factor of 10. This observation is consistent with the notion then that the saturation of human rod vision can be attributed to saturation in the rods themselves (Baylor *et al.* 1984).

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