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# Response Univariance in Bull-Frog Rods with Two Visual Pigments

MICHAEL L. FIRSOV,\* VICTOR I. GOVARDOVSKII,\* KRISTIAN DONNER†

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Rods in the bull-frog retina contain varying proportions of rhodopsin ( $\lambda_{\max} = 502$  nm) and porphyropsin ( $\lambda_{\max} = 527$  nm) in a dorso-ventral gradient from the porphyropsin-rich dorsal rim to the virtually pure rhodopsin fields of the central and ventral retina. We investigated if quantal excitations in the same rod are different depending on whether they are initiated by isomerization of a rhodopsin or a porphyropsin molecule. Current photoresponses were recorded from dark-adapted rods by sucking the outer segment into a recording pipette. The relation between pigment composition and spectral sensitivity was established by comparison with microspectrophotometrically measured absorbance spectra of rods from the same neighbourhood. Rods with suitable porphyropsin: rhodopsin mixtures (ideally between 1:4 and 1:2) were stimulated with flashes of red (608 nm) and blue (465 nm) light, whereby the red light will isomerize porphyropsin much more often than rhodopsin, and the reverse will be true of the blue light. The amplitude and shape of the single-photon response were found to be identical for the "red" and "blue" flash series to within measurement error (*ca* 10%). This indicates that the quantal responses initiated by the two pigments are identical.

Phototransduction Rhodopsin Porphyropsin Retinal Chromophore Bull-frog

## INTRODUCTION

Response univariance means that the shape and amplitude of the photoreceptor response depends only on the number of effectively absorbed photons but not on their wavelength. The "principle of univariance" (Naka & Rushton, 1966) has been found to hold generally, as is expected if a photon irrespective of its energy produces the same activated form of the visual pigment molecule. In the case of photoreceptors with mixed pigment content, it is not obvious that this should be the case. In particular, rods and cones in many species of fish and amphibians contain mixtures of pigments that differ in chromophoric group, based on either vitamin A<sub>1</sub> (retinal) or vitamin A<sub>2</sub> (3-dehydroretinal), and thereby in spectral sensitivity (Bridges, 1970; Reuter, White & Wald, 1971; Semple-Rowland & Goldstein, 1981; Crescitelli, 1958, 1990; Donner, Firsov & Govardovskii, 1990).

The key product in the visual pigment bleaching sequence is metarhodopsin II (MII) which sets off the excitation cascade. Both the amplitude and the kinetics of the photoresponse depend on the kinetics and efficiency of the interaction between MII and transducin as well as the kinetics of MII inactivation by phosphoryl-

ation and arrestin binding (reviewed by Pugh & Lamb, 1990). In MII the chromophore is still bound to its original site, so the nature of the chromophore might influence the properties of the molecule and hence the photoresponse. Recently, Corson, Derguini, Nakanishi, Crouch, Macnichol and Cornwall (1991) have reported that substitution of the native chromophore by the artificial analogue 9-desmethyl retinal makes response shape dependent on wavelength in salamander rods. Conceivably, the different native chromophores of the rod pigments rhodopsin (retinal) and porphyropsin (3-dehydroretinal) could also be associated with such differences.

In this work we investigate the possibility that single-quantum responses (SQRs) initiated by rhodopsin and porphyropsin in the same cell could be different, using rods of the bull-frog (*Rana catesbeiana*) containing various proportions of the two pigments. Porphyropsin absorbs long wavelengths better than rhodopsin, so (partially) selective excitation can be achieved by use of short-wavelength ("blue", here 465 nm) or long-wavelength ("red", here 608 nm) stimulus flashes. Current photoresponses were recorded from single rods drawn into suction pipettes, and the amplitudes and kinetics of "blue" and "red" responses were determined. To within measurement error (*ca* 10%), no differences were detected, which implies that rhodopsin- and porphyropsin-initiated SQRs are identical.

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## MATERIALS AND METHODS

### *Animals and preparation*

Adult bullfrogs, *Rana catesbeiana*, were obtained from KONS Scientific Co., Inc. (Germantown, Wis.) and kept on a 12:12 hr light–dark cycle at 12–14°C in basins with a white floor. These conditions favour the development of the dorsal porphyropsin field (Tsin & Beatty, 1980). Prior to the experiment, frogs were dark-adapted overnight at room temperature. A frog was killed by decapitation and double-pithed, and the eyes were removed under dim red light. Small segments of the eyecup were excised from the desired region, usually midway between the horizontal meridian and the dorsal border (to obtain pieces of retina with mixed pigment content). The retina was isolated in Ringer solution containing (mM): NaCl, 90; KCl, 2.5; MgSO<sub>4</sub>, 1.0; CaCl<sub>2</sub>, 1.0; NaHCO<sub>3</sub>, 5.0; glucose, 10; HEPES, 10 (pH 7.5–7.7) and sodium aspartate, 10 (to eliminate possible synaptic feedback from other rods).

### *Microspectrophotometry*

The pigment composition of a rod can be determined from its absorption spectrum. For about half of the rods studied electrophysiologically, other rods from the same small neighbourhood were subjected to microspectrophotometric examination as described by Govardovskii and Zueva (1988) and Donner *et al.* (1990). Averaged absorption spectra from 20–40 single rod outer segments in each case were computer-fitted with sums of rhodopsin and porphyropsin nomograms and related to the electrophysiological data.

In fitting nomograms we basically used the eighth-order polynomial expressions of Dawis (1981) for rhodopsin (A<sub>1</sub>-based pigment) and porphyropsin (A<sub>2</sub>-based pigment). However, the rhodopsin curve was modified to give somewhat lower absorbance in the long-wavelength end (substituting Dawis' coefficients  $b_7 = -789.371$  and  $b_8 = -60749.2$  by  $b_7 = 5211.534$  and  $b_8 = -95544.79$ ). The modified nomogram corresponds closely to the rhodopsin template published by Partridge and de Grip (1991). Further, as suggested to us by Maksimov (1988),  $(\lambda_{\max}/\lambda - 1)$  was used as parameter, which makes the curves shape-invariant on a relative frequency scale (Mansfield, 1985; MacNichol, 1986).

In a previous study (Donner *et al.*, 1990), we assumed  $\lambda_{\max} = 501.5$  and 523 nm for bull-frog rhodopsin and porphyropsin (essentially following Reuter *et al.*, 1971). Our present re-examination indicates that fits to mixed spectra improve perceptibly if a larger value for porphyropsin  $\lambda_{\max}$  is assumed. Here we use  $\lambda_{\max} = 527$  nm, which is that expected for the porphyropsin paired with rhodopsin<sub>502</sub> according to Dartnall's and Lythgoe's (1965) rule  $\lambda_1 = 0.60\lambda_2 + 186$ . The discrepancy in relation to Reuter *et al.* (1971) may be due at least in part to the presence of non-negligible fractions of rhodopsin even in the dorsalmost porphyropsin extracts of these authors (their Fig. 2). This conjecture is supported by the fact that the estimated porphyropsin content of the most porphyropsin-rich dorsal rods was never higher than

70–88% even when  $\lambda_{\max} = 523$  nm was assumed (Donner *et al.*, 1990). It is worth emphasizing, though, that our main conclusions do not depend on whether the porphyropsin peaks at 523 or 527 nm. Assuming too high porphyropsin  $\lambda_{\max}$  would mainly lead us to underestimate the porphyropsin percentage in cells with mixed pigment composition.

### *Current recording*

The current of single dark-adapted rods protruding from the edge of small pieces of retina was recorded by the suction-pipette method as described earlier (Baylor, Lamb & Yau, 1979a; Donner *et al.*, 1990). The signal was amplified (bandpass 0.016–10 Hz, in some cases 0–10 Hz; four-pole active Butterworth filter), digitized at 20–100 msec interval and stored on hard disk. Low-pass filtering at 10 Hz does not significantly influence response waveforms, as they are dominated by much lower frequencies (<1 Hz; as exemplified in Figs 3 and 5 dim-flash responses typically peak around 1.5 sec at room temperature).

### *Stimulation*

The light came from two stabilized sources with interference and neutral density filters interposed in the beam. Stimuli were 50 msec full-field flashes controlled by an electromagnetic shutter. The unattenuated intensity of light incident on the preparation was measured with a calibrated photomultiplier. A first estimate of average numbers of photoisomerizations per rod per flash was obtained from rod collecting areas and neutral filter densities (see Donner *et al.*, 1990). The actual number of photoisomerizations during each particular stimulus session was estimated from response statistics. Let the mean amplitude of responses to repeated presentations of nominally identical flashes be  $m$  and the variance  $\sigma^2$ . If variability is predominantly due to random variation of the numbers of photoisomerizations produced on individual trials, then (from Poisson statistics) the mean number of isomerizations per flash ( $N$ ) is

$$N = m^2/\sigma^2. \quad (1)$$

### *Selective excitation by different wavelengths*

Rods in the dorsalmost retina of the bull-frog contain predominantly porphyropsin whereas rods in the ventral retina contain almost pure rhodopsin (Reuter *et al.*, 1971; Makino, Kuzuo & Suzuki, 1983). Cells from the intermediate zone contain both pigments mixed in various proportions. Porphyropsin is more red-sensitive than rhodopsin, but the absorption spectra of the two pigments largely overlap. Therefore, selective absorption in one or the other can be only partly achieved, by use of wavelengths that are absorbed with different probabilities by the two pigments. Any pair of "short" (<500 nm) and "long" (>580 nm) wavelengths will basically do. In this work, 465 and 608 nm were used throughout.



The proportions of quantal responses initiated by rhodopsin or porphyropsin depends critically on the proportions of the two pigments in the rod. For best differentiation, there should be more rhodopsin (to increase the rhodopsin advantage in absorbing short-wavelength light) but not too much (not to offset the advantage of porphyropsin in the long-wavelength region). Figure 1 summarizes how absorption in the two pigments depends on the relative porphyropsin content of the cell: (1) the *thin continuous curve* gives, for 465 nm photons, the ratio of absorptions in rhodopsin to those in porphyropsin molecules; (2) the *dotted curve* gives, for 608 nm photons, the ratio of absorptions in porphyropsin to those in rhodopsin molecules; (3) the *bold continuous curve* gives the ratio of 465 and 608 nm intensities that will produce equal *total* numbers of absorptions (normalized to 1 for pure rhodopsin). All are shown as functions of the fraction of porphyropsin of pigment total in the cell. For example, if there is <10% porphyropsin, almost all 465 nm isomerizations will be in rhodopsin molecules. Such cells are not, however, advantageous for differentiating between the pigments, because even 608 nm photons will then isomerize rhodopsin more often than porphyropsin. Similarly, in cells with very high porphyropsin content (>60%) almost all 608 nm isomerizations, but also the majority of 465 nm isomerizations will be in porphyropsin molecules. Optimal differentiation is achieved in an intermediate range (from 20 to 35%). For example, with 35% porphyropsin and 65% rhodopsin, 465 nm photons will isomerize rhodopsin 2.5 times more often than porphyropsin, but 608 nm photons will isomerize porphyropsin 5 times more often than rhodopsin.

In the *electrophysiological* recordings, a rough estimate of the porphyropsin content was obtained by determining the ratio of 465 and 608 nm intensities needed to evoke the same response amplitude ( $I_{465}/I_{608} \equiv$  the ratio of sensitivities at 608 and 465 nm,  $S_{608}/S_{465}$ ). This ratio was scaled by setting the mean ratio of the most short-wavelength-sensitive rods from the ventral retina equal to 1; the normalized sensitivity ratio will be referred to as the R/B ratio. The electrophysiologically determined R/B ratio cannot of course *a priori* be taken as a measure of the pigment composition: if the amplitudes of responses initiated by rhodopsin and porphyropsin are different, the ratio of intensities that produce equal response amplitudes will differ from the ratio of absorbances. However, once a correspondence between R/B and absorbance ratios has been established, pigment composition can be estimated from the R/B ratio alone, as was the case in about half of the rods analysed (i.e. for these cells no independent microspectrophotometric pigment determination is available).

#### Protocol

To avoid spurious differences due to sensitivity drift during long recording sessions, 465 and 608 nm flashes were interleaved in blocks of 20 flashes. Dim-flash blocks were alternated with occasional blocks of stronger flashes and with single saturating flashes to monitor changes in the saturating response amplitude. For the analysis, the recordings were chopped and reassembled into series of responses to flashes of one wavelength (see Fig. 2).

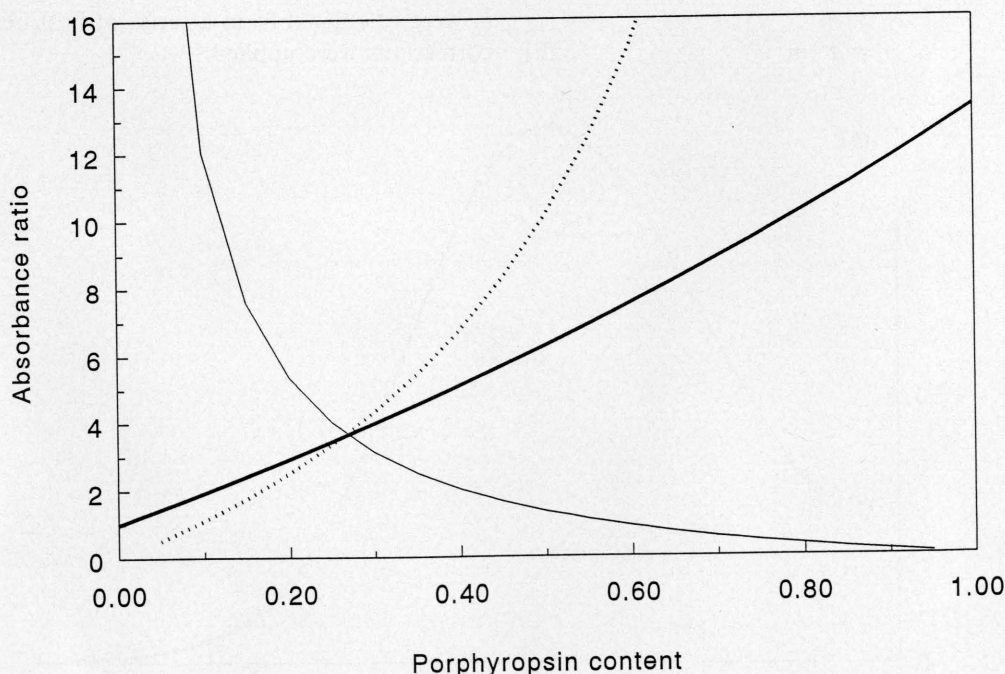


FIGURE 1. Relative probabilities that blue (465 nm) and red (608 nm) photons are absorbed in rhodopsin or porphyropsin molecules, as functions of fraction porphyropsin in the cell. Thin solid line: ratio of rhodopsin absorptions to porphyropsin absorptions for 465 nm photons. Dotted line: ratio of porphyropsin absorptions to rhodopsin absorptions for 608 nm photons. Bold solid line: ratio of total absorption of 608 nm photons to total absorption of 465 nm photons, scaled so that the ratio = 1 for pure rhodopsin.



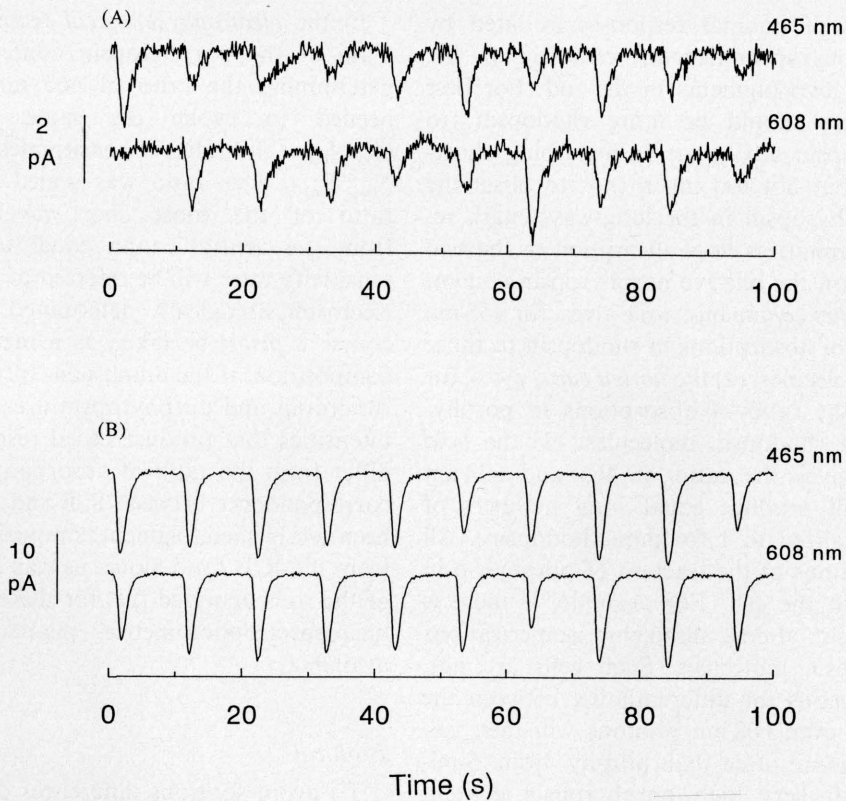


FIGURE 2. Series of individual flash responses from one rod from the dorsal retina: (A) *ca* 9 isomerizations per flash, (B) *ca* 40 isomerizations per flash. (The absorption spectrum from rods in the same neighbourhood is shown in Fig. 4 and averaged responses of the same cell in Fig. 5.)

#### Estimation of SQR amplitude

Denoting the SQR amplitude by  $a_q$ , it is self-evident that  $m = Na_q$ . Combining this with equation (1) we obtain the relation (cf. Baylor, Lamb & Yau, 1979b)

$$a_q = \sigma^2/m \quad (2)$$

which gives an estimate of  $a_q$  based on the assumption that the variation in response amplitudes is predominantly due to Poisson variation in the numbers of photoisomerizations produced on successive presentations of the same nominal flash intensity. Before  $\sigma^2$  and  $m$  were calculated from a series of flash responses, two corrections were applied.

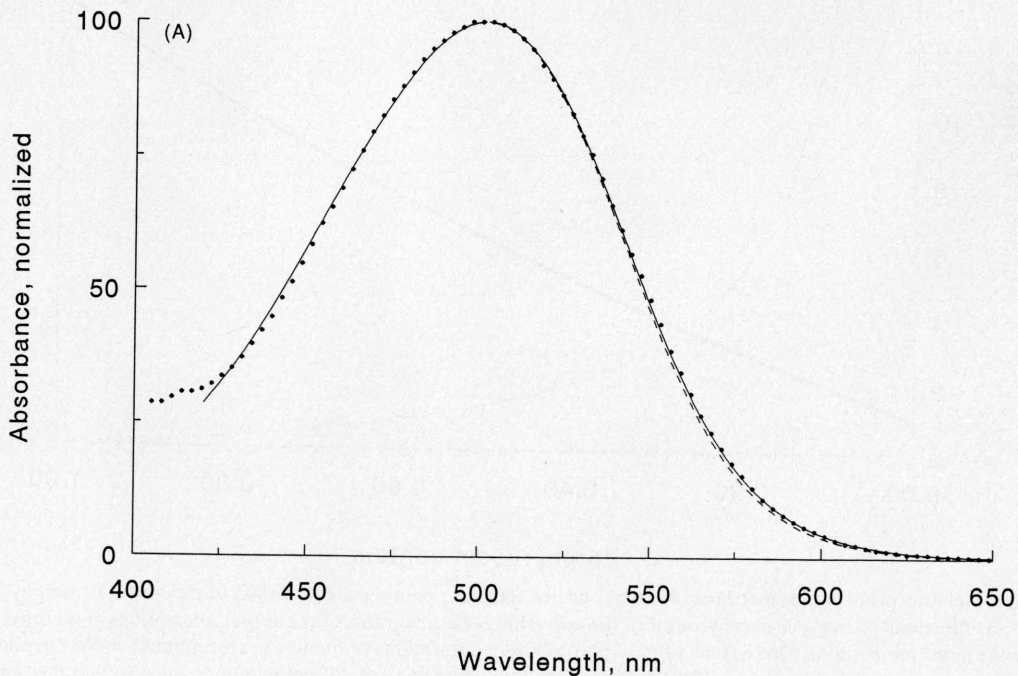


FIGURE 3(A). *Caption on facing page.*

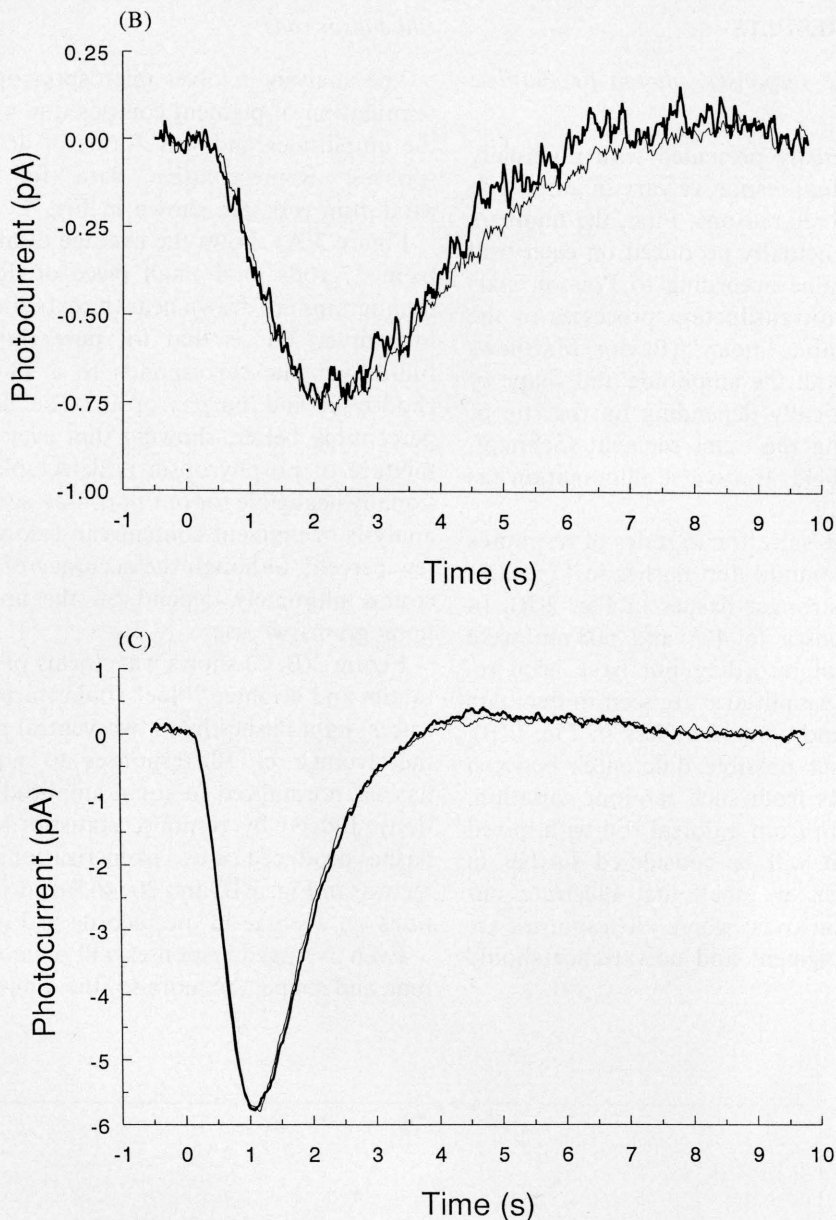


FIGURE 3. (A) Absorption spectrum averaged from the spectra of 27 single rods from one small piece of retina just dorsal to the optic disk (dots). The interrupted curve is the nomogram for rhodopsin<sub>502</sub>. The full-drawn curve, which gives the best fit, is a 98 + 2% sum of nomograms for rhodopsin<sub>502</sub> and porphyropsin<sub>527</sub>. (B, C) Averaged photoresponses from two rods from the ventral retina [(B) and (C) from different rods]. Each trace is the average of 10 responses. Bold traces mark 465 nm responses and thin traces 608 nm responses. Flash intensities estimated from response statistics were *ca* 2 isomerizations/flash in (B) and 18 (608 nm) and 20 (465 nm) isomerizations/flash in (C). The responses have been scaled to equal amplitude, which entailed multiplication of the "red" response by 0.64 in (B) and by 0.9 in (C).

(1) Correction for changes in the total light-sensitive current. If the amplitude of saturated responses,  $a_{\max}$ , changed during experiments, responses were scaled by factors  $a_{\max}/a_{\max}(t)$ , where  $a_{\max}$  is the saturating amplitude in the beginning of the experiment and  $a_{\max}(t)$  was obtained by linear interpolation between consecutive readings throughout the experiment.

(2) Correction for saturating response compression. As half-saturating intensities were typically *ca* 10–20 isomerizations, the saturating non-linearity of the rod could cause some compression of even fairly "small"

responses. The correction was based on Michaelis-type saturation:

$$a' = a \times a_{\max}/(a_{\max} - a) \quad (3)$$

where  $a$  is the recorded and  $a'$  the corrected response amplitude.

These corrections are not particularly critical for the analysis of univariance, as we took care to adjust the intensities of blue and red test flashes to produce responses of approximately the same size. Thus the two sets of responses would be affected equally by the saturating nonlinearity.

## RESULTS

*Variation between single responses: photon fluctuations and intrinsic variability*

When a rod is repeatedly presented with nominally identical flashes, individual responses vary in amplitude and shape for at least three reasons. First, the numbers of photoisomerizations actually produced on each trial vary around a mean value according to Poisson statistics. Second, the phototransduction processes in the rod are intrinsically variable, "noisy" (Baylor, Matthews & Yau, 1980). Third, both the amplitude and shape of the SQR vary systematically depending on the site of photon absorption along the outer segment (Schnapf, 1983), which with full-field, transverse illumination (as here) will vary randomly.

Figure 2 illustrates the variation in series of responses to nominally identical stimuli: dim flashes in Fig. 2(A) and about four times stronger flashes in Fig. 2(B). In both panels, the responses to 465 and 608 nm were interleaved in the actual recording but have been regrouped for the figure. Amplitudes are seen to fluctuate strongly in Fig. 2(A) and somewhat less in Fig. 2(B).

Our task is to extract possible differences between "red" and "blue" SQRs from such random variation. The records in Fig. 2 are from a dorsal rod with mixed pigment content, which will be considered further in Figs 4 and 5. However, we shall first illustrate our procedures on rhodopsin rods, where all responses are initiated by the same pigment and univariance should certainly hold.

*Rhodopsin rods*

The analysis involves microspectrophotometrical determination of pigment composition and comparison of the amplitudes and waveforms of 465 and 608 nm responses. Representative data for (virtually) pure rhodopsin rods are shown in Fig. 3.

Figure 3(A) shows the average of absorbance spectra from 27 rods in a small piece of dorsal retina. Two nomograms are drawn near the experimental points: the interrupted line is that for pure rhodopsin while the full-drawn line corresponds to a 98 + 2% mixture of rhodopsin and porphyropsin. The fit of the latter is perceptibly better, showing that even such a small admixture of porphyropsin is detectable (although functionally negligible for our purposes: see Fig. 1). Thus, the analysis of pigment content can resolve differences of a few percent, although the accuracy of the values will of course ultimately depend on the applicability of the nomograms we use.

Figure 3(B, C) shows waveforms of current responses to dim and brighter "blue" (bold traces) and "red" (thin traces) light flashes from two ventral rods. Each trace is the average of 10 responses to nominally identical flashes, normalized to equal amplitudes (see figure caption). Judged by response statistics [equation (1)], the flashes produced *ca* two isomerizations on average in the records in Fig. 3(B) and 20 (465) and 18 (608) isomerizations on average in the records in Fig. 3(C).

Even averaged responses will of course vary in amplitude and shape, the more so, the dimmer the flashes and

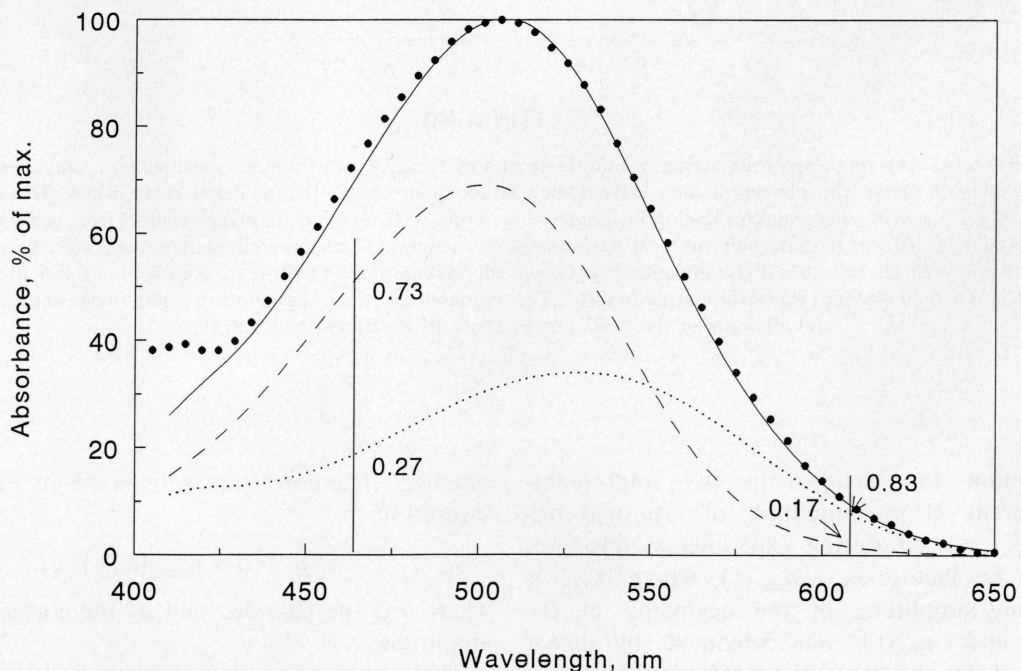


FIGURE 4. Absorption spectrum averaged from spectra of 41 single rods in one small piece of dorsal retina (solid circles). The full-drawn curve fitted to the data is a 67 + 33% sum of rhodopsin<sub>502</sub> and porphyropsin<sub>527</sub> nomograms (these components are shown as interrupted and dotted curves). The vertical lines illustrate that 73% of all isomerizations by 465 nm photons will occur in rhodopsin and 27% in porphyropsin, whereas 83% of all isomerizations by 608 nm photons will be in porphyropsin and only 17% in rhodopsin. The records in Figs 2 and 5 are from a rod from the same piece of retina as this absorption spectrum.



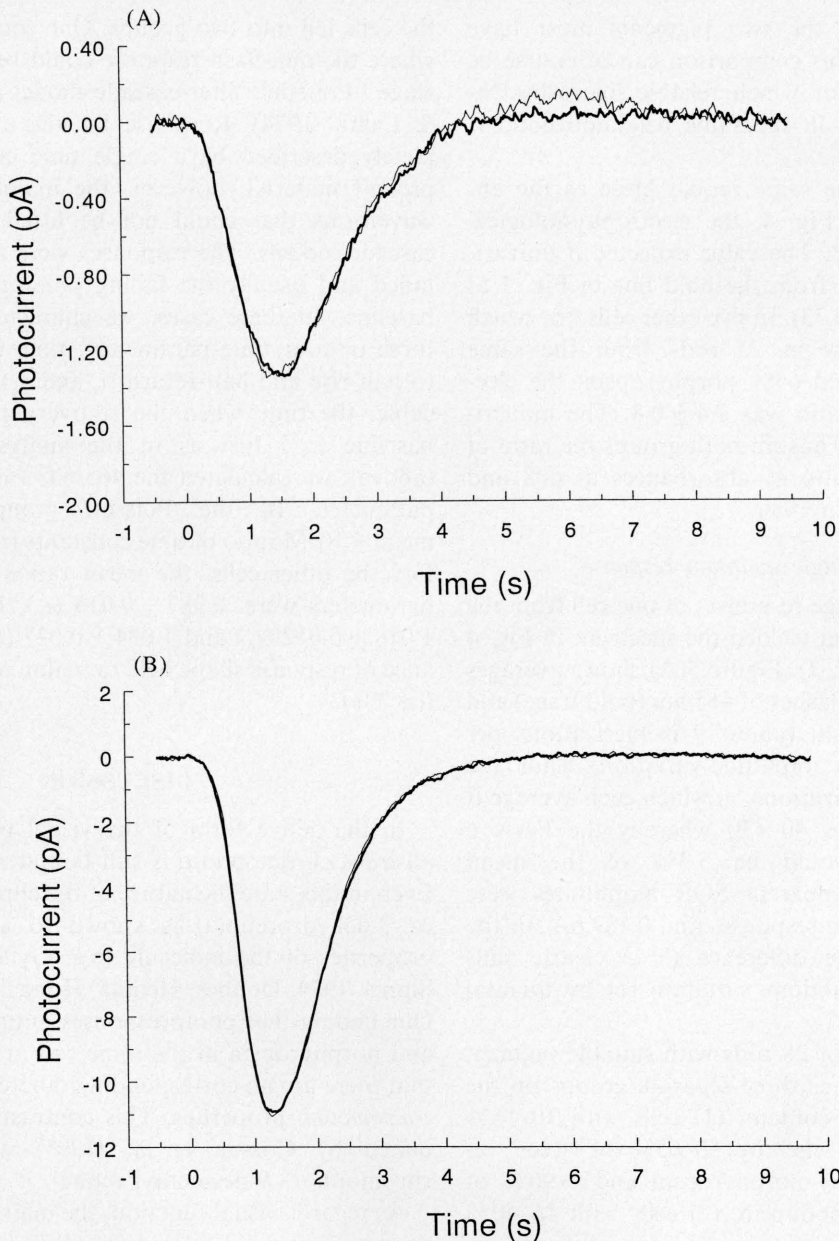


FIGURE 5. Responses from a dorsal rod of mixed pigment content (same cell as in Fig. 2). (A) Averages of 40 responses to 465 nm (bold trace) and 608 nm (thin trace) flashes; mean intensity *ca* 9 isomerizations per flash. (B) Averages of 10 responses to stronger flashes of 465 nm (bold trace) and 608 nm (thin trace) light. The responses have not been scaled in this figure. As flashes in (B) were only *ca* four times stronger than in (A), the responses in (B) appear to be too large. The reason is that they were collected near the beginning of the long experiment over which the amplitude of saturated responses fell from 24 pA to a final value of 7 pA.

the fewer the responses summed. [The averages in Fig. 3(B), for example, “contain” a total number of  $ca$   $10 \times 2 = 20$  isomerizations, so the standard deviation due to quantal fluctuations alone will exceed  $\pm 20\%$ .] In the figure, the responses have been scaled to equal amplitude to facilitate comparison of response kinetics. The larger responses, which are less sensitive to the rod’s intrinsic variation then become virtually indistinguishable [Fig. 3(C)], while clearer differences remain in the waveform of the small responses [Fig. 3(B)]. As “blue” and “red” SQRs in pure rhodopsin rods should in principle be identical, these provide a kind of visual yardstick by which to judge differences in rods with mixed pigment content.

#### *Absorbance and spectral sensitivity in rods with mixed pigment content*

The absorbance spectrum in Fig. 4 (solid circles) was averaged from the spectra of 41 rods in one small piece of dorsal retina. The nomogram fit indicates 33% porphyropsin and 67% rhodopsin, near-ideal for differentiation (cf. Fig. 1). Also shown are the component rhodopsin and porphyropsin spectra.

A straightforward test for univariance of response amplitudes is to compare the electrophysiologically measured spectral sensitivity with the absorbance spectrum. If the two give the same result in rods suitable for rhodopsin/porphyropsin differentiation, the quantal

responses initiated by the two pigments must have the same amplitude. This comparison can of course be applied only to cells for which reliable microspectrophotometry from rods in the same neighbourhood is available.

In five cells from the same retinal piece as the absorbance spectrum in Fig. 4, the electrophysiological R/B ratio was  $4.5 \pm 0.3$ . The value expected if univariance holds is 4.4 (read from the bold line in Fig. 1 at porphyropsin fraction 0.33). In five other cells, for which microspectrophotometry in 23 rods from the same neighbourhood indicated 65% porphyropsin, the electrophysiological R/B ratio was  $7.4 \pm 0.8$ . The univariance prediction is 8.0. Thus, in both groups the ratio of sensitivities and the ratio of absorbances at 608 and 465 nm coincided within 10%.

#### *The amplitude of the single-quantum response*

Figure 5 shows average responses of one cell from the same piece of retina that yielded the spectrum in Fig. 4 (the same cell as in Fig. 2). Figure 5(A) shows averages of responses to 40 dim flashes of 465 nm (bold trace) and 608 nm (thin trace) light (about 9 isomerizations per flash as estimated from amplitude variation). Thus, the total number of isomerizations on which each average is based is about 350 (*ca*  $40 \times 9$ ) whereby the Poisson standard deviation would be 5.3% of the mean ( $=100 \times \sqrt{350/350}$ ). Apparent SQR amplitudes were 0.195 pA for the 465 nm responses and 0.189 pA for the 608 nm responses. The difference (3%) clearly falls within the range of random variation set by quantal fluctuations alone.

The whole material of 28 rods with suitable pigment mixtures was analysed as three separate groups on the basis of porphyropsin content: (1) cells with 10–16% porphyropsin ( $n = 5$ ), whereby 50–65% of “red” responses are initiated by porphyropsin and >90% of “blue” responses by rhodopsin, (2) cells with 16–40% porphyropsin ( $n = 15$ ), whereby 70–85% of “red” responses are initiated by porphyropsin and 72–85% of “blue” responses by rhodopsin, and (3) cells with 40–65% porphyropsin ( $n = 8$ ), whereby 86–>95% of “red” responses are initiated by porphyropsin and 42–67% of “blue” responses by rhodopsin.

For each cell, the ratio of SQR amplitudes under 465 and 608 nm stimulation was estimated from response statistics. The mean  $\pm$  SEM 465/608 amplitude ratios obtained for the three groups were (1)  $1.05 \pm 0.27$ , (2)  $0.999 \pm 0.114$  and (3)  $0.81 \pm 0.17$ . Even the largest deviation from the univariance prediction 1.00, that of group (3) which amounts to 1.1 SEMs, is not significant ( $P > 0.3$  on a *t*-distribution with d.f. = 7). The conclusion for all groups is that the amplitudes of SQRs initiated by rhodopsin and porphyropsin do not differ statistically significantly.

#### *The shape of the single-quantum response*

To compare response kinetics more quantitatively than by visual inspection, characteristic temporal parameters were analysed. As regards response waveform,

the cells fell into two groups. One comprised eight cells where the dim-flash response could be fitted by a four-stage “Poisson” filter-cascade model (Baylor, Hodgkin & Lamb, 1974). Response kinetics can then be completely described by a single time constant  $\tau$ . In the present material, however, the majority of cells gave waveforms that could not be fitted by simple filter-cascade models. The responses were more sharply curtailed and usually the falling phase even overshoot the baseline. In these cases, we characterized kinetics by three or four time parameters: time to peak ( $t_p$ ), time to half-rise and half-return ( $t_r$  and  $t_d$ ), and when applicable, the time when the recovery phase crossed the baseline ( $t_0$ ). Just as in the analysis of amplitudes (above), we calculated the 465/608 ratios for each time parameter. In the Poisson group of cells, the mean  $\pm$  SEM ratio of time constants ( $\tau$ ) was  $0.99 \pm 0.02$ . For the other cells, the mean ratios of the four time parameters were:  $0.987 \pm 0.015$  ( $t_r$ ),  $1.007 \pm 0.011$  ( $t_p$ ),  $1.018 \pm 0.012$  ( $t_d$ ) and  $1.034 \pm 0.027$  ( $t_0$ ). Thus, univariance of response shape held to within measurement error (*ca* 3%).

## DISCUSSION

In the active form of the visual pigment (MII) the all-*trans* chromophore is still bound to its original site. Even in this state the nature of the chromophore (retinal or 3-dehydroretinal) is known to affect the *spectral* properties of the molecule (e.g. Gyllenberg, Reuter & Sippel, 1974; Donner, Hemilä, Hongell & Reuter, 1974). Our finding that photoresponses initiated by rhodopsin and porphyropsin in the same rod are identical implies that there are no corresponding differences in the *photo-transduction* properties. This contrasts with results obtained by Corson *et al.* (1991) with the artificial chromophore 9-desmethyl retinal.

As regards visual function, the main conclusion of the present work is related to the relative merits of rhodopsin and porphyropsin from a signal/noise viewpoint. We have previously considered the idea, essentially due to Barlow (1957), that the selection between the two is determined by two pressures that will often appear as opposite: (1) the pigment should be efficient in catching quanta in the particular light environment of the animal; (2) it should be thermally stable to minimize false “photon-like” events due to spontaneous isomerizations. The prevalence of porphyropsin in fresh-water environments (see e.g. Bridges, 1970) that are comparatively “yellowish” is qualitatively consistent with the difference in quantum catch. Similarly, the downwards-looking dorsal (porphyropsin) field in the retina of the water-loving bull-frog will most often look at under-water objects (whereas the ventral rhodopsin field looks up into the air). If quantum catch alone were important, however, porphyropsin would be superior to rhodopsin also in many other (terrestrial) light environments where it is not in fact used. We have shown that in dim light a possible photon-catching advantage of porphyropsin would be largely offset by its relative “noisiness”. The



rate of spontaneous photon-like events in porphyropsin rods is at least eight times higher than in rhodopsin rods calculated per molecule of visual pigment. As a result, porphyropsin may become really advantageous only when there is so much light that statistical fluctuation in the numbers of photons absorbed becomes a more significant source of variability than the spontaneous noise events (Donner *et al.*, 1990).

If photon fluctuation and randomly occurring photon-like events constitute the dominant noise components, the size and shape of the SQR is inconsequential for signal/noise, since real and false events will scale equally anyway. However, there are other types of noise in the retina: "continuous" transduction noise in the rods themselves (Baylor *et al.*, 1980) and more proximal neural noise. In relation to these, the size and shape of the SQR could be important—e.g. a large response at the input stage is less liable to be swamped by proximal noise. In this respect, the present work shows that there is no additional bonus nor cost associated with the use of porphyropsin rather than rhodopsin.

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