

# Thermal Activation and Photoactivation of Visual Pigments

Petri Ala-Laurila,\* Kristian Donner,<sup>†</sup> and Ari Koskelainen\*

\*Laboratory of Biomedical Engineering, Helsinki University of Technology, Helsinki, Finland; and <sup>†</sup>Department of Biosciences, University of Helsinki, Finland

**ABSTRACT** A visual pigment molecule in a retinal photoreceptor cell can be activated not only by absorption of a photon but also “spontaneously” by thermal energy. Current estimates of the activation energies for these two processes in vertebrate rod and cone pigments are on the order of 40–50 kcal/mol for activation by light and 20–25 kcal/mol for activation by heat, which has forced the conclusion that the two follow quite different molecular routes. It is shown here that the latter estimates, derived from the temperature dependence of the rate of pigment-initiated “dark events” in rods, depend on the unrealistic assumption that thermal activation of a complex molecule like rhodopsin (or even its 11-*cis* retinaldehyde chromophore) happens through a simple process, somewhat like the collision of gas molecules. When the internal energy present in the many vibrational modes of the molecule is taken into account, the thermal energy distribution of the molecules cannot be described by Boltzmann statistics, and conventional Arrhenius analysis gives incorrect estimates for the energy barrier. When the Boltzmann distribution is replaced by one derived by Hinshelwood for complex molecules with many vibrational modes, the same experimental data become consistent with thermal activation energies that are close to or even equal to the photoactivation energies. Thus activation by light and by heat may in fact follow the same molecular route, starting with 11-*cis* to all-*trans* isomerization of the chromophore in the native (resting) configuration of the opsin. Most importantly, the same model correctly predicts the empirical correlation between the wavelength of maximum absorbance and the rate of thermal activation in the whole set of visual pigments studied.

## INTRODUCTION

Sensors designed to be activated by light energy will necessarily have some propensity for activation by thermal energy alone. In the visual system, randomly occurring thermal activations of visual pigment molecules constitute an irreducible noise that sets an ultimate limit to the detection of weak light (Autrum, 1943; Barlow, 1956; Aho et al., 1988; Donner, 1992). Thermal activations of the rod visual pigment are very sparse, but can be studied by the electrical signals they generate in the photoreceptor cell. Discrete “bumps” in the receptor current of single rods, recorded, e.g., by the suction-pipette technique, report single-molecule events in a population of  $>10^9$  rhodopsins. Thus, Baylor et al. (1980, 1984) were first able to show that the rate constant for thermal activation of toad and macaque rhodopsin ( $\sim 10^{-11} \text{ s}^{-1}$  at 37°C) was of the right magnitude to account for the intrinsic “dark light” invoked to explain the statistics of light detection by dark-adapted humans (Barlow, 1956; cf. Hecht et al., 1942).

Baylor et al. (1980) determined the Arrhenius activation energy of the thermal process from the temperature dependence of the rate constant in toad rhodopsin, obtaining  $\sim 22$  kcal/mol. This is only about half of the energy needed for activation by light (Lythgoe and Quilliam, 1938; St. George, 1952; Cooper, 1979; Barlow et al., 1993). Because there can be no reasonable doubt that the discrete

dark events in the rod current do arise from spontaneous activation of single molecules of visual pigment (cf. Firsov et al., 2002), the current interpretation of this discrepancy is that the photic and thermal modes of activation follow different pathways, the activation energies of which bear no necessary relation.

This view, however, fails to address the striking and biologically important empirical relation between spectral and thermal properties of visual pigments. There is a significant inverse correlation between the dark-event rate and the wavelength of maximum absorbance ( $\lambda_{\text{max}}$ ) (Donner et al., 1990; Firsov and Govardovskii, 1990; Fyhrquist, 1999; see Figs. 2 and 3 below). The correlation is qualitatively consistent with the hypothesis that a pigment maximally sensitive to long-wavelength light (i.e., low-energy photons) has a comparatively low-energy barrier for activation and thus a high probability for thermal activation, whereas short-wavelength sensitive pigments have a higher activation energy and are therefore thermally more stable (de Vries, 1949; Barlow, 1957).

We are faced with a fundamental dilemma: if the processes of activation by light and by heat are uncoupled, the observed correlation between  $\lambda_{\text{max}}$  and thermal noise remains a mystery. To resolve this conflict, we propose a new model, drawing on insights already expressed by St. George (1952) and Lewis (1955). The crucial point is that the thermal behavior of a complex molecule (rhodopsin, or even the 11-*cis* retinaldehyde chromophore) must be described by Hinshelwood (1933) rather than Boltzmann statistics. This is because thermal activation of a molecule composed of many

Submitted October 7, 2003, and accepted for publication February 25, 2004.

Address reprint requests to Petri Ala-Laurila, Dept. of Physiology and Biophysics, Boston University School of Medicine, 715 Albany St., Boston, MA 02118-2526 USA. Tel.: 617-638-5336; Fax: 617-638-4273; E-mail: pal@iki.fi.

© 2004 by the Biophysical Society

0006-3495/04/06/3653/10 \$2.00

doi: 10.1529/biophysj.103.035626

atoms may be supported by the energy present in a large number of vibrational modes, in addition to the kinetic energy in the relative motion of molecules (translational degrees of freedom). Arrhenius-type estimates for the thermal activation energy will decisively depend on how many modes are assumed to be involved, i.e., on the complexity of the activation process. The estimates of Baylor et al. (1980) for toad rhodopsin and subsequent estimates for other visual pigments (e.g., Matthews, 1984) rely on the arbitrary assumption that the activation process is a very simple one, involving only two (translational) degrees of freedom ( $n = 2$ ). This undermines the evidence for a large difference between the energies of activation by heat and by light.

Here we test the model under the assumption that the thermal activation energy of each pigment has the same value as its photoactivation energy, this being the simplest possible alternative to the current notion that the two are very different. Some justification for assuming that they may be at least rather close comes from recent work suggesting that the gap between the minimum of the excited-state and the maximum of the ground-state energy surfaces for chromophore isomerization could be as small as 5–6 kcal/mol (Mathies, 1999).

When thermal and photic activation energies are set equal, the model predicts the temperature dependence of dark-event rates observed by Baylor et al. (1980) if the number of vibrational modes involved in the thermal activation process is  $\sim 39$ . Most importantly, with the same number of vibrational modes, the model gives a good fit to the collected data on  $\lambda_{\text{max}}$  versus light-like thermal noise in rods as well as in cones. The model by no means requires that thermal and photic activation energies be identical, but they must show some correlation, and we suggest there are good reasons to expect they do (see Discussion).

## THE MODEL

Photoactivation of a visual pigment molecule requires absorption of a photon with sufficient energy to bridge the gap ( $E_a$ ) between the ground state and the first excited state. This gap corresponds to the photon energy at some wavelength  $\lambda_0$  ( $E_a = hc/\lambda_0$ ). Stiles (1948) proposed that the reason why spectral sensitivity exhibits no sharp drop at  $\lambda_0$  is that photon energy may be supplemented by thermal energy of the visual pigment molecule. If a photon at  $\lambda > \lambda_0$  encounters a molecule that can contribute enough thermal energy ( $\geq hc(1/\lambda_0 - 1/\lambda)$ ) to the process, activation may occur. He assumed that the Boltzmann distribution gives the relevant probabilities for encountering visual-pigment molecules at given thermal energy levels. The fraction of molecules with thermal energy greater than some value  $E$  is then

$$\frac{N_1}{N} = e^{-\frac{E}{RT}}. \quad (1)$$

Obviously, the fraction with at least the energy  $E = hc(1/\lambda_0 - 1/\lambda)$  needed to supplement a photon of wavelength  $\lambda > \lambda_0$  decreases proportionally to  $\exp(1/\lambda)$ . This would explain the fact that the final slope of absorbance spectra at very long wavelengths, plotted on logarithmic ordinates against wavenumber ( $1/\lambda$ ), approximates a straight line (Stiles, 1948).

Lewis (1955) noted that spectra are in fact gently concave in the wavenumber domain of interest. He pointed out that for a complex molecule

with many vibrational modes (many degrees of freedom), the fraction of molecules with thermal energy  $> E$  is given by an expression derived by Hinshelwood (1933):

$$\frac{N_1}{N} = e^{-\frac{E}{RT}} \left[ \frac{1}{(\frac{n}{2} - 1)!} \left( \frac{E}{RT} \right)^{\frac{n}{2} - 1} + \frac{1}{(\frac{n}{2} - 2)!} \left( \frac{E}{RT} \right)^{\frac{n}{2} - 2} + \dots + 1 \right], \quad (2)$$

where the parameter  $n$  is the total number of quadratic energy terms (potential energy and kinetic energy) in the vibrational modes involved in activation (see below). Lewis in fact used the parameter  $m = n/2 - 1$  for the same purpose and we have previously followed his notation (Ala-Laurila et al., 2002, 2003); thus the number of modes involved is  $m + 1$  or  $n/2$  depending on notation. For values of  $n > 2$ , this produces spectra with gently accelerating slope in the domain  $1/\lambda < 1/\lambda_0$ . At any given temperature  $T$  and for any energy limit  $E$ , the Hinshelwood distribution (Eq. 2) yields a larger fraction of molecules that have thermal energy  $> E$  than does the Boltzmann distribution (Eq. 1). Lewis showed that the shape of spectra is best described with  $n$ -values between 8 and 14. It is to be expected, however, that the number depend on the reaction considered. The smaller the required thermal supplement to the photon energy, the fewer modes are likely to be involved. St. George (1952) notes: "As to the portion of the molecule that is involved in activation, the increase in  $n$  toward longer wavelengths fits the idea that an increasingly larger part of the molecule comes into play as the thermal component of the activation energy becomes larger." The limiting case is activation by thermal energy alone.

The total (maximal) number of quadratic energy terms of a molecule, the number of "internal degrees of freedom," is given by:

$$n_{\text{max}} = 6N - 12, \quad (3)$$

(see, e.g., Moore, 1962), where  $N$  is the number of atoms of the molecule. For 11-*cis* retinaldehyde,  $N = 49$  (20 C + 28 H + 1 O) and  $n_{\text{max}} = 282$ . For the rhodopsin molecule as a whole, the number is obviously very large. We do not commit ourselves to any particular idea about the molecular origin of the modes, but note that any value of  $n$  up to  $\sim 282$  (i.e.,  $\sim 141$  vibrational modes) would as such be consistent with an origin in the chromophore alone.

For purely thermal activation, the rate constant  $k$  is proportional to the fraction of molecules that have energy larger than the thermal activation energy, which we denote  $E_{a,H}$ :

$$k = A_H \times \frac{e^{-E_{a,H}/RT} \left( \frac{E_{a,H}}{RT} \right)^{\frac{n}{2} - 1}}{(\frac{n}{2} - 1)!}. \quad (4)$$

$A_H$  is a proportionality constant, often referred to as the "pre-exponential factor". The above expression for  $k$  derives from the first term of Eq. 2, which is a good approximation for the full series when  $E \gg RT$  ( $\approx 0.6$  kcal/mol at 20°C) (Hinshelwood, 1933). The rate constant based on the Boltzmann distribution is obtained from Eq. 1:

$$k = A_B e^{-E_{a,B}/RT}. \quad (5)$$

In this case, we give the pre-exponential factor as well as the activation energy the subscript B to emphasize that these values based on Boltzmann statistics (associated with "conventional" Arrhenius analysis) are different from those based on Hinshelwood statistics, denoted by subscript H. For  $n = 2$ , Eq. 4 converges to Eq. 5.

When Eq. 4 replaces Eq. 5, the generally accepted values  $E_{a,B}$  derived from experimental data on the temperature dependence of dark-event rates (Baylor et al., 1980) appear as basically arbitrary. The true relation between the energies of thermal and photic activation remains unresolved. In the following, we study the predictions of the model under the simplest possible assumption (itself not essential to the model) that the two activation energies have the same value, i.e.,  $E_a = E_{a,H}$ .

## RESULTS

### The temperature dependence of dark events in *Bufo marinus* rods

The straight line in Fig. 1 shows a fit of Eq. 4 to the Baylor et al. (1980) data on the temperature dependence of discrete dark events in a total of five individual “red” rods (marked by different symbols) of the toad *Bufo marinus*. The visual pigment is a usual 503 nm rhodopsin. The purpose is twofold. Firstly, we hereby demonstrate that a good fit can be obtained even under the assumption that the thermal activation energy is equal to the photoactivation energy of the pigment, 44.3 kcal/mol (Ala-Laurila et al., 2002). Secondly, the fit provides an estimate for the parameter  $n$ , which we will use in the subsequent modeling. In view of the standardized molecular structure of the chromophore part of visual pigments, we think it is reasonable to assume as a first-order approximation that  $n$  is constant for all. We do realize that important differences between pigments may become evident at a higher level of resolution, e.g., different coupling of vibrational modes to the activation process could be one of the tuning mechanisms underlying differences in the interplay of light and heat (Koskelainen et al., 2000).

The straight line in Fig. 1 also represents the conventional Arrhenius slope for  $E_{a,B} = 21.9$  kcal/mol, the mean of the individual  $E_{a,B}$  values determined by Baylor et al. (1980) from each of the five rods separately. Our rationale for fitting this model (Eq. 4) has been to require that the predicted temperature dependence with  $E_{a,H} = E_a = 44.3$  kcal/mol should coincide with this “mean” line. (Obviously, the line

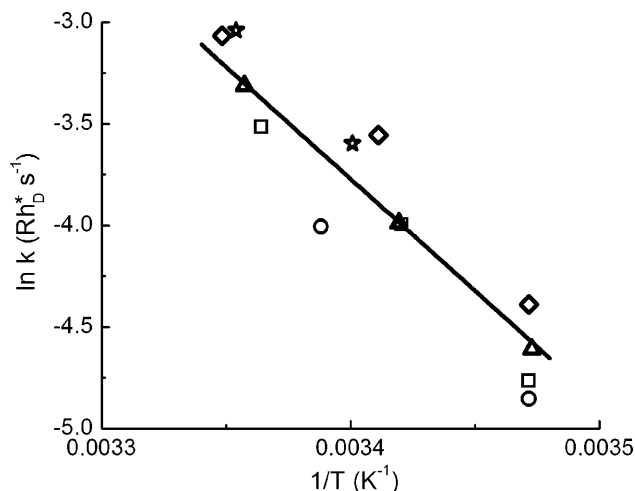


FIGURE 1 The temperature dependence of the rate of thermal dark events per rod ( $\text{Rh}\cdot\text{s}^{-1}$ ) in “red” rods of the toad *Bufo marinus*. The data are from Baylor et al. (1980, their Table 2); each symbol type denotes data from one rod. The Arrhenius plot shows the natural logarithm of the rate constant ( $\ln k$ ) as a function of the inverse value of the absolute temperature ( $1/T$ ). The straight line represents both the conventional Arrhenius slope for the value  $E_a = 21.9$  kcal/mol (the mean of the values obtained from the five rods studied) and the slope given by the “Hinshelwood” model for parameter values  $E_{a,H} = 44.3$  kcal/mol and  $n = 79$ .

deviates somewhat from the outcome of simply applying linear regression analysis to the set of data points.) The fitting entails optimizing the value of the parameter  $n$ , which was done as follows. The rate of change of  $\ln k$  with temperature,  $d \ln k / dT$  according to the “conventional Arrhenius” model (based on Eq. 5) is:

$$\frac{d \ln k}{dT} = \frac{E_{a,B}}{RT^2}. \quad (6)$$

The corresponding rate of change according to the “Hinshelwood” model (based on Eq. 4) is:

$$\frac{d \ln k}{dT} = \frac{E_{a,H} - \left(\frac{n}{2} - 1\right)RT}{RT^2}. \quad (7)$$

Obviously, when  $n$  is large, a given empirical temperature dependence is indicative of a larger activation energy according to Eq. 7 than according to Eq. 6, the difference being  $E_{a,H} - E_{a,B} = (n/2 - 1)RT$  (cf. St. George, 1952). To determine  $n$ , we set this expression equal to the difference between the Baylor et al. (1980) estimate 21.9 kcal/mol and our present postulate 44.3 kcal/mol:

$$(n/2 - 1)RT = (44.3 - 21.9) \text{ kcal/mol} = 22.4 \text{ kcal/mol},$$

which at  $T = 294.15$  K (21°C) gives

$$n = 2 \times [(22.4 \text{ kcal/mol}) / RT + 1] = 78.7 \approx 79.$$

The straight line in Fig. 1 represents the identical predictions of, on one hand, the “Arrhenius-Boltzmann” model for  $E_{a,B} = 21.9$  kcal/mol and on the other hand the “Hinshelwood” model for  $E_{a,H} = 44.3$  kcal/mol and  $n = 79$ . As explained above in connection with Eq. 2, this number of quadratic energy terms (kinetic + potential energy) would arise from half that number of vibrational modes, which (taken as the smallest sufficient integer, i.e., the first integer  $\geq 79/2$ ) would be 39.

We now fix the value  $n = 79$  and study whether the “Hinshelwood” model with this parameter value can account for the observed correlations between  $1/\lambda_{\max}$ ,  $E_a$ , and thermal noise in visual pigments.

### Two rod pigments differing only in chromophore

As a first example, we consider the rhodopsin<sub>502</sub>-porphyropsin<sub>525</sub> pigment pair in rods of the adult bullfrog, *Rana catesbeiana* (Reuter et al., 1971; Firsov et al., 1994). This pair offers some clear advantages for our purpose. Firstly, the A1  $\rightarrow$  A2 chromophore shift in the same opsin is a comparatively simple molecular mechanism for shifting  $\lambda_{\max}$ , and it may be assumed that the pre-exponential factor  $A_H$  in Eq. 4 does not change (see Discussion). Secondly, for these two pigments we have direct knowledge of the photoactivation energies  $E_a$  as well as the thermal activation rates. Donner et al. (1990) report the rate constant  $k_{A2} = 1.2 \times 10^{-11} \text{ s}^{-1}$  for the A2 pigment at 18°C and estimate that the rate in the

(remarkably stable) A1 pigment is  $\sim 10$  times lower. The respective photoactivation energies are  $E_{a,A2} = 44.2$  kcal/mol and  $E_{a,A1} = 46.5$  kcal/mol, consistent with the inverse proportionality between  $E_a$  and  $\lambda_{\max}$  hypothesized by Barlow (1957) (Ala-Laurila et al., 2003, 2004). Entering these values into Eq. 4 with  $n = 79$ ,  $T = 291.15$  K, and assuming that  $A_H$  does not change, we obtain the prediction  $k_{A2}/k_{A1} = 7.6$  for the ratio of rate constants. Thus, there is a fair agreement between theory and experiment. The corresponding ratio  $k_{A2}/k_{A1}$  based on the Boltzmann distribution (Eq. 5) would be 53.

### The correlation between $\lambda_{\max}$ and dark-event rate in rod pigments

We now turn to the whole sample of rod pigments from which estimates of the dark-event rate as well as the wavelength of maximum absorbance  $\lambda_{\max}$  are available. Table 1 gives the identity and numerical values of the pigments considered and the data have been plotted in Fig. 2. All dark-event rates have been referred to the same temperature, 21°C, assuming the temperature dependence measured in *Bufo marinus* rhodopsin by Baylor et al. (1980). Further, some of the original articles report only event rates per photoreceptor cell, and in these cases we have

recalculated the values to rates per pigment molecule using values for outer-segment volume and pigment density of the respective species, drawn from other sources as indicated by the references in connection with Table 1. We have included not only results from suction-pipette recordings on single rods, but also estimates based on noise analysis in retinal bipolar cells (dogfish rods) and on the statistics of light detection measured in human psychophysics. A significant correlation between dark-event rate and  $1/\lambda_{\max}$  is evident ( $r^2 = 0.59$ ).

The photoactivation energy  $E_a$  is known only for some of the pigments. To relate  $\lambda_{\max}$  to  $E_a$  for the whole data set, we use the empirical equation obtained by Ala-Laurila et al. (2004) by linear regression of  $E_a$  on  $1/\lambda_{\max}$  in 12 visual pigments:

$$E_a = 7.10 \text{ kcal mol}^{-1} + 19,800 \text{ nm kcal mol}^{-1} \times \frac{1}{\lambda_{\max}} \text{ (nm}^{-1}\text{)}. \quad (8)$$

Equation 8 is a useful statistical relation, although it should be noted that no tight physical connection exists between  $E_a$  and  $\lambda_{\max}$ . Two pigments with the same  $\lambda_{\max}$  may have quite different  $E_a$  and vice versa (Koskelainen et al.,

**TABLE 1** Characteristics of the rod visual pigments included in the analysis

Photoreceptor	$\lambda_{\max}$ (nm)	$1/\lambda_{\max}$ $10^6 \text{ m}^{-1}$	$k$ ( $\text{R}^*\text{s}^{-1}$ ) (21°C)	$-\log k$ ( $\text{R}^*\text{s}^{-1}$ ) (21°C)
Macaque ( <i>Macaca fascicularis</i> ) rods	491*	2.037	7.451E-12*	11.13
Dogfish ( <i>Scyliorhinus canicula</i> ) rods	496 <sup>†</sup>	2.016	1.365E-11 <sup>‡</sup>	10.87
Human rods	496.3 <sup>§</sup>	2.015	7.30E-12 <sup>¶</sup>	11.14
Bullfrog ( <i>Rana catesbeiana</i> ) rhodopsin rods	501.7 <sup>  </sup>	1.993	2.211E-12**	11.66
Common toad ( <i>Bufo bufo</i> ) red rods	502.6 <sup>††</sup>	1.990	5.868E-12 <sup>‡‡</sup>	11.23
Cane toad ( <i>Bufo marinus</i> ) red rods	503.9 <sup>§§</sup>	1.985	1.179E-11 <sup>¶¶</sup>	10.93
Larval tiger salamander ( <i>Ambystoma tigrinum</i> ) (A2) rods	521 <sup>    </sup>	1.919	4.697E-12***	11.33
Clawed frog ( <i>Xenopus laevis</i> ) rods	521.6 <sup>†††</sup>	1.917	2.00E-11 <sup>‡‡‡</sup>	10.70
Bullfrog ( <i>Rana catesbeiana</i> ) porphyropsin rods	525.2 <sup>§§§</sup>	1.904	1.769E-11 <sup>¶¶¶</sup>	10.75
Hybrid sturgeon ( <i>Huso huso</i> X <i>Acipenser nudipectus</i> ) rods	538 <sup>     </sup>	1.859	7.00E-11 <sup>     </sup>	10.15
Sturgeon ( <i>Acipenser baeri</i> ) rods	549 <sup>     </sup>	1.821	1.07E-10 <sup>     </sup>	9.97

Normalization of the thermal dark-event rates to 21°C is based on the temperature dependence measured in *Bufo marinus* rods by Baylor et al. (1980).

\*Baylor et al. (1984).

<sup>†</sup>Govardovskii and Lychakov (1977, for the species *Squalus acanthias*).

<sup>‡</sup>Ashmore and Falk (1977).

<sup>§</sup>Dartnall et al. (1983).

<sup>¶</sup>Rate of events per rod: Donner (1992); number of rhodopsin molecules per rod: Rodieck (1973, p. 112), Hárosi (1982).

<sup>||</sup>Ala-Laurila et al. (2003).

<sup>\*\*</sup>Donner et al. (1990).

<sup>††</sup>Ala-Laurila et al. (2002).

<sup>‡‡</sup>Fyhrquist et al. (1998), Firsov et al. (2002).

<sup>§§</sup>Ala-Laurila et al. (2002).

<sup>¶¶</sup>Baylor et al. (1980).

<sup>||||</sup>Makino et al. (1999).

<sup>\*\*\*</sup>Rate of events per rod: Vu et al. (1997); number of porphyropsin molecules per rod: Rieke and Baylor (2000), Sampath and Baylor (2002).

<sup>†††</sup>according to the Govardovskii et al. (2000) nomogram fitted to our own unpublished MSP data.

<sup>‡‡‡</sup>Fyhrquist, 1999 (Fig. 2).

<sup>§§§</sup>Ala-Laurila et al. (2003).

<sup>¶¶¶</sup>Donner et al. (1990).

<sup>|||||</sup>Firsov and Govardovskii, 1990.

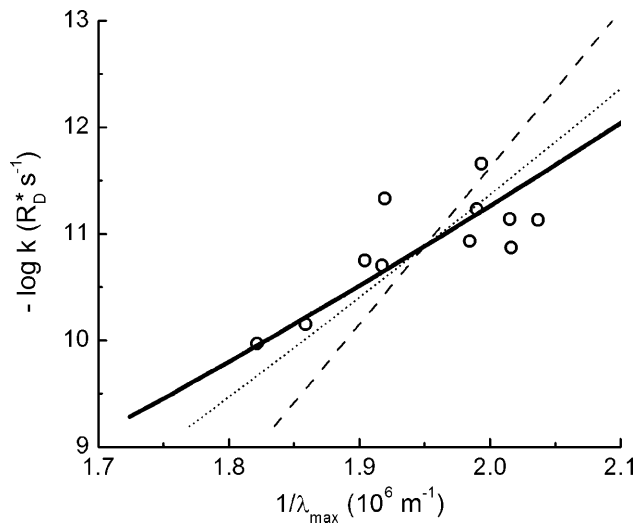


FIGURE 2 The relation between the rate of thermal dark events per molecule of visual pigment ( $R_D^* s^{-1}$ ) and the wavelength of peak absorbance ( $\lambda_{max}$ ) in rods (data from Table 1). The Briggsian logarithm of the rate constant  $k$  is plotted as a function of  $1/\lambda_{max}$ . The three straight lines show three model predictions (all vertically positioned for best fit to the data points). (Solid line) This model with  $n = 79$  (derived from the slope in Fig. 1 when thermal and photic activation energies are assumed to be equal) and the relation between  $E_a (= E_{a,H})$  and  $1/\lambda_{max}$  given by Eq. 8. (Dotted line) This model with the modification that  $E_a - E_{a,H} = 10$  kcal/mol and  $n = 44$  (the value that gives the correct slope in Fig. 1 in this case). (Dashed line) The prediction of Barlow's (1957) original formulation, based on the assumption that the distribution of visual-pigment molecules on thermal energy levels follows Boltzmann statistics. See text for details.

2000). However, the regression Eq. 8 explains 73% of the variation in  $E_a$  in the sample of visual pigments that have been studied in this respect (Ala-Laurila et al., 2004). In testing this model, we now assume that the same equation also describes the relation between the thermal activation energy  $E_{a,H}$  and  $\lambda_{max}$ .

The solid straight line in Fig. 2 traces the model prediction on this assumption (for  $n = 79$  and  $T = 294.15$  K). The line has been vertically positioned for best fit to the whole data set, which implies fixing the value of the pre-exponential factor  $A_H$  in Eq. 4. Clearly, the model fairly successfully predicts how steeply the thermal activation rate depends on  $\lambda_{max}$ . For comparison, the dashed line shows the (excessively steep) relation based on the Boltzmann distribution (Eq. 5), as originally suggested by Barlow (1957). The dotted line shows the prediction of this "Hinshelwood" model under the modified assumption that  $E_{a,H}$  is systematically somewhat smaller than  $E_a$  (see below).

### The correlation between $\lambda_{max}$ and light-like dark noise in cones

There is much less reliable data on thermal activation rates in cone pigments. One reason is that discrete dark events can be recorded only in some special cases, notably in the so-called "green rods" of amphibians (Matthews, 1984), which contain an S-cone pigment, (Hisatomi et al., 1999; Ma et al.,

2001). In three of the other four available estimates for cone pigments, thermal activation rates have been computed by the original authors (Lamb and Simon, 1977; Schnapf et al., 1990; Sampath and Baylor, 2002) from the "dark" noise power in the frequency band of photoresponses, a procedure fraught with more uncertainty than the analysis of unipolar, photon-like deflections from baseline. The fifth data point represents a human psychophysical estimate, based on the variability of light detection by dark-adapted foveal cones (Donner, 1992). The data are collected in Table 2 and dark-event rates have been plotted as function of  $1/\lambda_{max}$  in Fig. 3. Again, a significant correlation is evident ( $r^2 = 0.93$ ). The solid straight line shows the prediction of this model on the same assumptions as for the rod data (Fig. 2), and the dashed line the corresponding prediction based on the Boltzmann distribution. Both have been vertically positioned for best fit to the whole data set. For the cone data, this requires a value of the pre-exponential factor  $A_H$  that is some four orders of magnitude larger than for the rod data. Yet, just as for rods, the Hinshelwood model predicts the slope of the dependence of thermal activation rate on  $\lambda_{max}$  rather well, as opposed to the Boltzmann model, which produces a much too steep dependence. Like in Fig. 2, the dotted line shows the prediction of this model under the modified assumption that there is an energy offset between  $E_{a,H}$  and  $E_a$  (see below).

### Robustness of the model

Above, we have explored the predictions of the model under the assumption  $E_a = E_{a,H}$ . How sensitive are they to alterations of this assumption? Particularly, how much will they change if it is assumed that the peak of the ground-state energy barrier and the trough of the excited-state energy surface are separated by 5–10 kcal/mol, consistent with recent molecular modeling (Okada et al., 2001; Mathies, 1999)? To investigate this, we have repeated the calculations assuming that the photic and thermal activation energies are offset by a constant amount  $\Delta E = E_a - E_{a,H} = 10$  kcal/mol. For *Bufo marinus* rhodopsin we then get  $E_{a,H} = E_a - \Delta E = (44.3 - 10)$  kcal/mol = 34.3 kcal/mol. Optimizing the fit to the dark-event rate versus temperature data of Baylor et al. (1980) as in Fig. 1 now requires a different  $n$ -value, calculated in the same way as before:

$$(n/2 - 1)RT = (34.3 - 21.9) \text{ kcal/mol} = 12.4 \text{ kcal/mol},$$

which gives  $n \approx 44$ .

### The correlation between $\lambda_{max}$ and dark-event rates

The  $E_a$  values for *Rana catesbeiana* porphyropsin and rhodopsin together with  $n = 44$  in Eq. 4 yields the dark-event rate ratio  $k_{A2}/k_{A1} = 13.6$ . Compared with the experimental estimate  $k_{A2}/k_{A1} \approx 10$ , this prediction is about equally good as the value 7.6 obtained under the previous assumption  $E_{a,H} = E_a$  and  $n = 79$ .

**TABLE 2** Characteristics of the cone visual pigments included in the analysis

Photoreceptor	$\lambda_{\max}$ (nm)	$1/\lambda_{\max}$ $10^6 \text{ m}^{-1}$	$k$ ( $\text{R}^*\text{s}^{-1}$ ) ( $21^\circ\text{C}$ )	$-\log k$ ( $\text{R}^*\text{s}^{-1}$ ) ( $21^\circ\text{C}$ )
Cane toad ( <i>Bufo marinus</i> ) green rods	432.6*	2.312	$5.231\text{E-}11^\dagger$	10.28
Human L-cones	558.4‡	1.791	$1.34\text{E-}07^\S$	6.87
Macaque ( <i>Macaca fascicularis</i> ) L-cones	561¶	1.783	$5.948\text{E-}06^\parallel$	5.23
Turtle ( <i>Trachemys scripta elegans</i> ) L-cones	617**	1.621	$5.28\text{E-}05^{\dagger\dagger}$	4.28
Larval tiger salamander ( <i>Ambystoma tigrinum</i> ) L-cones	620‡‡	1.613	$9.580\text{E-}06^{\S\S}$	5.02

Normalization of the thermal dark-event rates to  $21^\circ\text{C}$  is based on the temperature dependence measured in *Bufo marinus* rods by Baylor et al. (1980).

\*Ala-Laurila et al. (2002).

†Matthews (1984).

‡Dartnall et al. (1983).

§Rate of events per cone: Donner (1992); cone dimensions: Snyder and Pask (1973); Dartnall et al. (1983); pigment concentration: Hárosi (1982).

¶Baylor et al. (1987).

||Rate of events per cone and outer segment volume: Schnapf et al. (1990); pigment concentration: Hárosi (1982).

\*\*Loew and Govardovskii, 2001.

††Rate of events per cone: Lamb and Simon (1977); outer segment volume: Liebman and Granda (1971); pigment concentration: Hárosi (1982).

‡‡Makino et al. (1999).

§§Sampath and Baylor (2002).

In Figs. 2 and 3, presenting the full sets of data on rod and cone pigments, the predictions of the “energy offset” modification ( $E_a - E_{a,H} = 10 \text{ kcal/mol}$  and  $n = 44$ ) are shown as dotted lines. The fits are slightly less good than those for  $E_{a,H} = E_a$  and  $n = 79$  (solid lines), but still much better than the Boltzmann fits (dashed lines).

Thus, the model works well with a moderate offset between  $E_a$  and  $E_{a,H}$ , as long as the two are coupled (see Discussion). On the other hand, the modification does not improve the fits obtained under the simplest assumption  $E_{a,H} = E_a$ . In the model parameters, energy offsets are traded against changes in the apparent number of thermal

modes ( $n/2$ ) involved in the activation process. Due to this trade-off, the model is not very helpful for determining the precise relation between  $E_{a,H}$  and  $E_a$ , nor the value of  $n$ , from the experimental data. Its value lies in showing that the data are consistent with the idea that photoactivation and thermal activation energies are similar or equal, and in explaining the correlation between  $E_{a,H}$  and  $\lambda_{\max}$  on this basis.

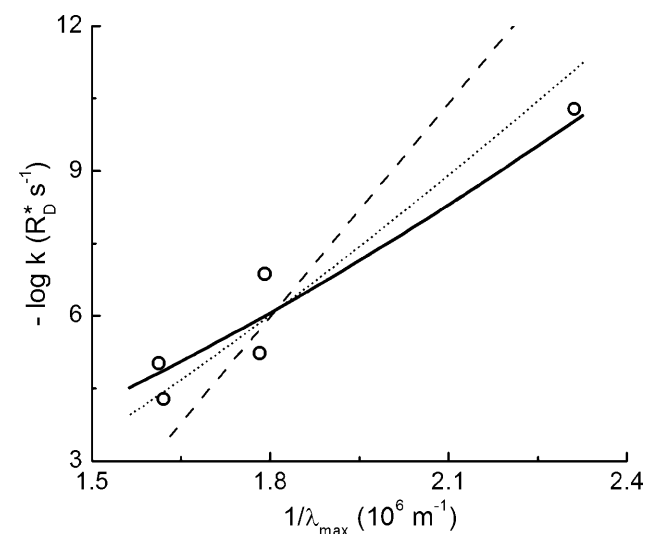
We may finally note that in the “Boltzmann” model (Eq. 5), the introduction of a constant offset  $\Delta E$ , such that  $E_{a,B} = E_a - \Delta E$ , will not at all affect the predicted ratio of dark-event rates in two pigments with different activation energies. In the ratio  $k_{A2}/k_{A1}$ , the offset  $\Delta E$  disappears by reduction: if  $k_{A1} \propto \exp[-(E_{a1} - \Delta E)/RT]$  and  $k_{A2} \propto \exp[-(E_{a2} - \Delta E)/RT]$ , then  $k_{A2}/k_{A1} = \exp[(E_{a1} - \Delta E - E_{a2} + \Delta E)/RT] = \exp[(E_{a1} - E_{a2})/RT]$ . Thus the predicted ratio  $k_{A2}/k_{A1}$  is the same as if  $E_{a,B} = E_a$ , implying that the much too steep dependence of dark-event rates on  $\lambda_{\max}$  remains unaffected.

## DISCUSSION

### Discrete dark events and the elusive “low-energy” thermal activation pathway

Ever since Baylor et al. (1980) estimated the energy for thermal activation of *Bufo marinus* rhodopsin to  $\sim 22 \text{ kcal/mol}$ , the incompatibility with photoactivation energies,  $\sim 40\text{--}50 \text{ kcal/mol}$  (Lythgoe and Quilliam, 1938; St. George, 1952; Cooper, 1979), has posed a major difficulty for any effort to understand the nature of thermal activation and its relation to the spectral light absorbance of visual pigments. This problem has been approached in different ways.

It has been suggested that the discrete photon-like events in the rod dark current might not, in fact, reflect spontaneous activation of single-pigment molecules, but arise at some later point in the transduction machinery (for a discussion,



**FIGURE 3** The relation between the rate of thermal dark events per molecule of visual pigment ( $\text{R}_D^* \text{s}^{-1}$ ) and the wavelength of peak absorbance  $\lambda_{\max}$  in cones (data from Table 2). The Briggsian logarithm of the rate constant  $k$  is shown as a function of  $1/\lambda_{\max}$ . The three straight lines show the three model predictions explained in the legend to Fig. 2. The solid line gives the prediction of this model under our “main” assumptions ( $E_a = E_{a,H}$ ,  $n = 79$ ,  $E_a$  and  $1/\lambda_{\max}$  related by Eq. 8).

see Barlow et al., 1987). At least in the case of vertebrate rods, however, it is hard to believe that the discrete events could originate elsewhere than in the visual pigment. The shape of the electrical response to photoactivation of a single rhodopsin molecule depends on serial activation of some 100 transducins by the rhodopsin and subsequent suppression of rhodopsin catalytic activity by multiple phosphorylation and arrestin binding, as well as a number of other shut-off reactions (Leskov et al., 2000; Pugh and Lamb, 2000; Fain et al., 2001). It is highly improbable that standardized bumps indistinguishable from this repeatedly arise, e.g., from concerted spontaneous activation of hundreds of transducins followed by quenching that just happens to mimic the shut-off kinetics of the light response.

While it is now widely accepted that the dark events originate in the visual pigment, this has led to another state of nescience: the two processes of thermal and photic activation evidently follow different molecular pathways, but nothing is known about the low-energy thermal pathway. The problem is exacerbated by the fact that 22 kcal/mol is not only radically smaller than the photoactivation energy, but too small to allow the reaction to visit the early postisomerization photoproducts of rhodopsin, notably bathorhodopsin, whose ground-state enthalpy lies  $\sim 35$  kcal/mol above that of the native pigment (Cooper, 1979; Mathies, 1999; Okada et al., 2001). The very rapid formation of bathorhodopsin (within 200 fs; Schoenlein et al., 1991; Wang et al., 1994) leaves no time for significant conformational changes in the opsin (cf. Filipek et al., 2003). Thus, entropy stays essentially constant up to the bathorhodopsin stage, and the change in free energy is approximately equal to the enthalpy change.

A cogent and testable hypothesis about the identity of the low-energy activation pathway was proposed by Barlow et al. (1993), stating that the thermal events originate in a small subpopulation of rhodopsin molecules where the Schiff base linking the chromophore to the opsin is unprotonated. The calculations of the authors indicate that Schiff-base deprotonation would lower the activation energy for chromophore isomerization from  $\sim 45$  kcal/mol to about the right level,  $\sim 23$  kcal/mol. This hypothesis, however, now seems implausible, as recent studies have failed to detect any pH dependence either of dark-event rates in rods (Firsov et al., 2002) or the dark-noise-equivalent rate of quantal activations in cones (Sampath and Baylor, 2002).

It is worth remembering that all experimental evidence for low thermal activation energies comes from measurement of dark-event rates at a few temperatures (cf. Fig. 1). The values extracted from such data depend wholly on the theory applied. We here suggest that the low estimates may be analytical artifacts, due to improper application of conventional Arrhenius analysis to a complex molecule. When the thermal energy present in the vibrational modes of the chromophore is taken into account, the data are consistent with the idea that thermal and photic activation energies are close or even

equal,  $\sim 40$ – $50$  kcal/mol. We propose that a particular “low-energy” thermal activation pathway simply may not exist.

### The pre-exponential factor

It seems remarkable that the rate of thermal activations should show essentially parallel relations to  $\lambda_{\max}$  within the groups of rod and cone pigments (Figs. 2 and 3), although the absolute levels for rods and cones are separated by some four log units. However, this may be perceived to support the notion that the systematic,  $\lambda_{\max}$ -dependent, variation within both classes is due to a similar mechanism, coupling the energy of the first excited state and the ground-state energy barrier for thermal activation. By contrast, the difference in absolute levels would indicate some global difference in the design of rod and cone opsins.

In the formalism of the model, the difference resides in the “pre-exponential factor”  $A_H$  (see Eq. 4). We can only speculate on its molecular correlate. Rod opsins evidently have a very “closed” chromophore pocket, which probably imparts high thermal stability to the chromophore. (The price is slow chromophore exchange, thus slow recovery from bleaching.) Regardless of the energy barrier, the possible molecular routes for thermal isomerization of the chromophore may be tightly restricted by the opsin. By contrast, the chromophore pocket of cone pigments is much “looser”, arguably to ensure fast chromophore exchange, thus fast recovery from bleaching. In at least some cone pigments, there is a continuous exchange of chromophore even in darkness (Matsumoto et al., 1975). This seems consistent with the idea that the opsin control is much less restrictive than in rods.

Differences in the pre-exponential factor ( $A_H$ ) within either group of pigments (rod or cone) will appear as random variation when the dark-event rate is considered as a function of  $\lambda_{\max}$ . The  $A_H$  difference is expected to be negligible between the A1 and A2 members of a pigment pair, as both are restricted by the same opsin. For rod pigments with different opsins, the “unexplained” component of experimental variation (cf. Fig. 2) may at present be the only clue to the possible range of variation in  $A_H$ . In contrast to the rod opsins, the cone opsins phylogenetically belong to several subfamilies (see, e.g., Yokoyama and Yokoyama, 2000), and one might expect even more variation, e.g., in the topology of the chromophore pocket. In the small experimental material available, however, it is not possible to assess the true degree of “random” scatter of cone dark-event rates.

### The correlation between the excited-state energy minimum and the ground-state barrier for chromophore isomerization

The model by no means requires the assumption that the photic and thermal activation energies be the same. Weaker

forms of correlation will work just as well, but of course some form of coupling is assumed. This is the essence of Barlow's (1957) original proposition that a bathochromic spectral shift always carries a cost in terms of increased thermal noise. The hypothesis has been criticized on the grounds that there are no good physical reasons why the two kinds of activation energies would have to correlate (see, e.g., Goldsmith, 1989a,b, referring to Cundall, 1964). However, there seem to be good reasons to expect they should. The all-*trans* bleaching intermediate bathorhodopsin, some 5–6 kcal/mol downhill from the peak of the ground-state energy surface for chromophore isomerization, is formed within the first 200 fs from photon absorption (Schoenlein et al., 1991; Wang et al., 1994). The rapidity of this reaction, one of the fastest photochemical reactions known, requires that the excited-state and ground-state surfaces be close, hence a fairly strict coupling between the ground-state barrier and the photoactivation energy  $E_a$  (Mathies, 1999). Although the modeling has been based on one specific rhodopsin (bovine), it may be assumed that the main features, i.e., the relation between the energy surfaces and the crossover from the excited state to the ground state, remain similar in different pigments with different photoexcitation energies  $E_a$ .

It is also instructive to apply a teleological argument. A visual pigment ought to combine a high quantum efficiency for photoisomerization with high thermal stability. Thermal stability would be maximized if the ground-state barrier for chromophore isomerization were "infinitely" high, but such a molecule would have zero quantum efficiency, because it would always drop back to the 11-*cis* ground state from the excited state. High quantum efficiency for photoisomerization requires that the ground-state barrier be equal or lower than the excited-state surface in the direction of the isomerization coordinate. In view of the stability requirement, it is optimal to have a barrier as high as possible consistent with this. To the extent that the properties can be tuned by natural selection, the result would be a close coupling of the photic excitation energy and the thermal energy barrier.

### Relation of this model to molecular theory

Our model shows that the multimodal character of thermal activation will critically affect the temperature dependence of the rate of such activations, thus undermining the assumptions on which generally accepted estimates of the activation energy are based. It is a purely statistical model, making no detailed claims about molecular processes. In this sense it is complementary to a large body of sophisticated work involving molecular orbital theory and molecular dynamic modeling (Martin and Birge, 1998; Kalé et al., 1999; Kusnetzow et al., 2001; Ben-Nun et al., 2002; Saam et al., 2002). On the other hand, our assumptions and parameters, particularly the number of vibrational modes required, must of course not conflict with what is known about the molecular events in

chromophore isomerization. It is gratifying to note that evidence from resonance Raman spectroscopy suggests that several tens of modes can contribute to isomerization (Lopponow and Mathies, 1988; Lin et al., 1998; Kim et al., 2003).

### CONCLUSION

When the internal energy present in the many vibrational modes of a visual pigment molecule is taken into account, the distribution on thermal energy levels follows Hinshelwood (1933) rather than Boltzmann statistics. The large difference between current estimates of thermal activation energies and photoactivation energies then disappears as an analytical artifact. The experimental data are consistent with the two kinds of activation energy being close or even equal. This has at least two important consequences. Firstly, it allows that the molecular pathways for activation by light and by heat may be identical from the initial event of chromophore isomerization in the native (resting) configuration of the opsin. Secondly, it makes understandable the biologically important correlation between high sensitivity to long-wavelength light and high rates of spontaneous activations in visual pigments.

We thank Drs. Robert Barlow and Robert Birge for valuable discussions and Dr. Richard Mathies for constructive comments on the manuscript.

This work was supported by the Academy of Finland (grants 49947 and 206221), the Emil Aaltonen Foundation, and the Ella and Georg Ehrnrooth Foundation.

### REFERENCES

- Aho, A. C., K. Donner, C. Hyden, L. O. Larsen, and T. Reuter. 1988. Low retinal noise in animals with low body temperature allows high visual sensitivity. *Nature*. 334:348–350.
- Ala-Laurila, P., R. Albert, P. Saarinen, A. Koskelainen, and K. Donner. 2003. The thermal contribution to photoactivation in A2 visual pigments studied by temperature effects on spectral properties. *Vis. Neurosci.* 20:411–419.
- Ala-Laurila, P., J. Pahlberg, A. Koskelainen, and K. Donner. 2004. On the relation between the photoactivation energy and the absorbance spectrum of visual pigments. *Vision Res.* In press.
- Ala-Laurila, P., P. Saarinen, R. Albert, A. Koskelainen, and K. Donner. 2002. Temperature effects on spectral properties of red and green rods in toad retina. *Vis. Neurosci.* 19:781–792.
- Ashmore, J. F., and G. Falk. 1977. Dark noise in retinal bipolar cells and stability of rhodopsin in rods. *Nature*. 270:69–71.
- Autrum, H. 1943. Über kleinste Reize bei Sinnesorganen. *Biologisches Zentralblatt*. 63:209–236.
- Barlow, H. B. 1956. Retinal noise and absolute threshold. *J. Opt. Soc. Am.* 46:634–639.
- Barlow, H. B. 1957. Purkinje shift and retinal noise. *Nature*. 179:255–256.
- Barlow, R. B., Jr., R. R. Birge, E. Kaplan, and J. R. Tallent. 1993. On the molecular origin of photoreceptor noise. *Nature*. 366:64–66.
- Barlow, R. B., Jr., E. Kaplan, G. H. Renninger, and T. Saito. 1987. Circadian rhythm in *Limulus* photoreceptors. *J. Gen. Physiol.* 89:353–378.
- Baylor, D. A., G. Matthews, and K. W. Yau. 1980. Two components of electrical dark noise in toad retinal rod outer segments. *J. Physiol. (Lond.)*. 309:591–621.



- Baylor, D. A., B. J. Nunn, and J. L. Schnapf. 1984. The photocurrent, noise and spectral sensitivity of rods of the monkey *Macaca fascicularis*. *J. Physiol. (Lond.)*. 357:575–607.
- Baylor, D. A., B. J. Nunn, and J. L. Schnapf. 1987. Spectral sensitivity of cones of the monkey *Macaca fascicularis*. *J. Physiol. (Lond.)*. 390:145–160.
- Ben-Nun, M., F. Molnar, K. Schulten, and T. J. Martinez. 2002. The role of intersection topography in bond selectivity of *cis-trans* photoisomerization. *Proc. Natl. Acad. Sci. USA*. 99:1769–1773.
- Cooper, A. 1979. Energy uptake in the first step of visual excitation. *Nature*. 282:531–533.
- Cundall, R. B. 1964. The kinetics of *cis-trans* isomerizations. *Prog. React. Kinet.* 2:165–215.
- Dartnall, H. J., J. K. Bowmaker, and J. D. Mollon. 1983. Human visual pigments: microspectrophotometric results from the eyes of seven persons. *Proc. R. Soc. Lond. B. Biol. Sci.* 220:115–130.
- de Vries, H. 1949. Comment to Dr. Wald's lecture. *Doc. Ophthalmol.* 3:137.
- Donner, K. 1992. Noise and the absolute thresholds of cone and rod vision. *Vision Res.* 32:853–866.
- Donner, K., M. L. Firsov, and V. I. Govardovskii. 1990. The frequency of isomerization-like "dark" events in rhodopsin and porphyropsin rods of the bull-frog retina. *J. Physiol. (Lond.)*. 428:673–692.
- Fain, G. L., H. R. Matthews, M. C. Cornwall, and Y. Koutalos. 2001. Adaptation in vertebrate photoreceptors. *Physiol. Rev.* 81:117–151.
- Filipek, S., R. E. Stenkamp, D. C. Teller, and K. Palczewski. 2003. G protein-coupled receptor rhodopsin: a prospectus. *Annu. Rev. Physiol.* 65:851–879.
- Firsov, M. L., K. Donner, and V. I. Govardovskii. 1994. Response univariance in bull-frog rods with two visual pigments. *Vision Res.* 34:839–847.
- Firsov, M. L., K. Donner, and V. I. Govardovskii. 2002. pH and rate of "dark" events in toad retinal rods: test of a hypothesis on the molecular origin of photoreceptor noise. *J. Physiol. (Lond.)*. 539:3–46.
- Firsov, M. L., and V. I. Govardovskii. 1990. Dark noise of visual pigments with different absorption maxima. *Sensornye Sistemy*. 4:25–34 (in Russian).
- Fyhrquist, N. 1999. Spectral and thermal properties of amphibian visual pigments related to molecular structure. *Dissertationes Biocentri Viikki Universitatis Helsinkiensis* 18/1999.
- Fyhrquist, N., V. I. Govardovskii, C. Leibrock, and T. Reuter. 1998. Rod pigment and rod noise in the European toad *Bufo bufo*. *Vision Res.* 38:483–486.
- Goldsmith, T. H. 1989a. Compound eyes and the world of vision research. In *Facets of Vision*. D. G. Stavenga and R. C. Hardie, editors. Springer, Berlin, Germany.
- Goldsmith, T. H. 1989b. The evolution of visual pigments and colour vision. In *Vision and Visual Dysfunction: The Perception of Colour*, Vol. 7. P. Gouras, editor. MacMillan, London, UK.
- Govardovskii, V. I., N. Fyhrquist, T. Reuter, D. G. Kuzmin, and K. Donner. 2000. In search of the visual pigment template. *Vis. Neurosci.* 17:509–528.
- Govardovskii, V. I., and L. V. Lychakov. 1977. Photoreceptors and visual pigments of Black Sea elasmobranchs. *Zh. Evol. Biokhim. Fiziol.* 13:162–166 (in Russian).
- Hárosi, F. I. 1982. Polarized microspectrophotometry for pigment orientation and concentration. *Methods Enzymol.* 81:642–647.
- Hecht, S., S. Schlaer, and M. H. Pirenne. 1942. Energy, quanta, and vision. *J. Gen. Physiol.* 25:819–840.
- Hinshelwood, C. N. 1933. *The Kinetics of Chemical Change in Gaseous Systems*. Clarendon Press, Oxford, UK.
- Hisatomi, O., Y. Takahashi, Y. Taniguchi, Y. Tsukahara, and F. Tokunaga. 1999. Primary structure of a visual pigment in bullfrog green rods. *FEBS Lett.* 447:44–48.
- Kalé, L., R. Skeel, M. Bhandarkar, R. Brunner, A. Gursoy, N. Krawetz, J. Phillips, A. Shinozaki, K. Varadarajan, and K. Schulten. 1999. NAMD2: greater scalability for parallel molecular dynamics. *Journal of Computational Physics*. 151:283–312.
- Kim, J. E., M. J. Tauber, and R. A. Mathies. 2003. Analysis of the mode-specific excited-state energy distribution and wavelength-dependent photoreaction quantum yield in rhodopsin. *Biophys. J.* 84:2492–2501.
- Koskelainen, A., P. Ala-Laurila, N. Fyhrquist, and K. Donner. 2000. Measurement of thermal contribution to photoreceptor sensitivity. *Nature*. 403:220–223.
- Kusnetzow, A., A. Dukkipati, K. R. Babu, D. Singh, B. W. Vought, B. E. Knox, and R. R. Birge. 2001. The photobleaching sequence of a short-wavelength visual pigment. *Biochemistry*. 40:7832–7844.
- Lamb, T. D., and E. J. Simon. 1977. Analysis of electrical noise in turtle cones. *J. Physiol. (Lond.)*. 272:435–468.
- Leskov, I. B., V. A. Klenchin, J. W. Handy, G. G. Whitlock, V. I. Govardovskii, M. D. Bownds, T. D. Lamb, E. N. Pugh, Jr., and V. Y. Arshavsky. (2000) The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. *Neuron* 27:525–537.
- Lewis, P. R. 1955. A theoretical interpretation of spectral sensitivity curves at long wavelengths. *J. Physiol. (Lond.)*. 130:45–52.
- Lieberman, P. A., and A. M. Granda. 1971. Microspectrophotometric measurements of visual pigments in two species of turtle, *Pseudemys scripta* and *Chelonia mydas*. *Vision Res.* 11:105–114.
- Lin, S. W., M. Groesbeek, I. van der Hoeft, P. Verdegem, J. Lugtenburg, and R. A. Mathies. 1998. Vibrational assignment of torsional normal modes of rhodopsin: probing excited-state isomerization dynamics along the reactive  $C_{11} = C_{12}$  torsion coordinate. *J. Phys. Chem. B*. 102:2787–2806.
- Loew, E. R., and V. I. Govardovskii. 2001. Photoreceptors and visual pigments in the red-eared turtle, *Trachemys scripta elegans*. *Vis. Neurosci.* 18:753–757.
- Loppnow, G. R., and R. A. Mathies. 1988. Excited-state structure and isomerization dynamics of the retinal chromophore in rhodopsin from resonance Raman intensities. *Biophys. J.* 54:35–43.
- Lythgoe, R. J., and J. P. Quilliam. 1938. The thermal decomposition of visual purple. *J. Physiol. (Lond.)*. 93:24–38.
- Ma, J., S. Znoiko, K. L. Othersen, J. C. Ryan, J. Das, T. Isayama, M. Kono, D. D. Oprian, D. W. Corson, M. C. Cornwall, D. A. Cameron, F. I. Hárosi, C. L. Makino, and R. K. Crouch. 2001. A visual pigment expressed in both rod and cone photoreceptors. *Neuron*. 32:451–461.
- Makino, C. L., M. Groesbeek, J. Lugtenburg, and D. A. Baylor. 1999. Spectral tuning in salamander visual pigments studied with dihydrorretinal chromophores. *Biophys. J.* 77:1024–1035.
- Martin, C. H., and R. A. Birge. 1998. Reparametrizing MNDO for excited-state calculations by using an ab initio effective Hamiltonian theory: application to the 2,4-pentadien-1-iminium cation. *J. Phys. Chem. A*. 102:852–860.
- Matsumoto, H., F. Tokunaga, and T. Yoshizawa. 1975. Accessibility of the iodopsin chromophore. *Biochim. Biophys. Acta*. 404:300–308.
- Mathies, R. A. 1999. Photons, femtoseconds and dipolar interactions: a molecular picture of the primary events in vision. *Novartis Foundation Symposium* 224:70–84.
- Matthews, G. 1984. Dark noise in the outer segment membrane current of green rod photoreceptors from toad retina. *J. Physiol. (Lond.)*. 349:607–618.
- Moore, W. J. (1962). *Physical Chemistry*, 3rd ed. Prentice Hall, Eaglewood Cliffs, NJ.
- Okada, T., O. P. Ernst, K. Palczewski, and K. P. Hofmann. 2001. Activation of rhodopsin: new insights from structural and biochemical studies. *Trends Biochem. Sci.* 26:318–324.
- Pugh, E. N., and T. D. Lamb. 2000. Phototransduction in vertebrate rods and cones: molecular mechanisms of amplification, recovery and light adaptation. In *Handbook of Biological Physics. Molecular Mechanisms of Visual Transduction*, Vol. 3. D. A. Stavenga, W. J. de Grip,

- and E. N. Pugh, Jr., editors. Elsevier, Amsterdam, The Netherlands. 183–255.
- Reuter, T. E., R. H. White, and G. Wald. 1971. Rhodopsin and porphyropsin fields in the adult bullfrog retina. *J. Gen. Physiol.* 58:351–371.
- Rieke, F., and D. A. Baylor. 2000. Origin and functional impact of dark noise in retinal cones. *Neuron*. 26:181–186.
- Rodieck, R. W. 1973. *The Vertebrate Retina. Principles of Structure and Function.* Freeman and Company, San Francisco, CA.
- Saam, J., E. Tajkorsheid, S. Hayashi, and K. Schulten. 2002. Molecular dynamics investigation of primary photoinduced events in the activation of rhodopsin. *Biophys. J.* 83:3097–3112.
- St. George, R. C. C. 1952. The interplay of light and heat in bleaching rhodopsin. *J. Gen. Physiol.* 35:495–517.
- Sampath, A. P., and D. A. Baylor. 2002. Molecular mechanism of spontaneous pigment activation in retinal cones. *Biophys. J.* 83:184–193.
- Schnapf, J. L., B. J. Nunn, M. Meister, and D. A. Baylor. 1990. Visual transduction in cones of the monkey *Macaca fascicularis*. *J. Physiol. (Lond.)*. 427:681–713.
- Schoenlein, R. W., L. A. Peteanu, R. A. Mathies, and C. V. Shank. 1991. The first step in vision: femtosecond isomerization of rhodopsin. *Science*. 254:412–415.
- Snyder, A. W., and C. Pask. 1973. The Stiles-Crawford effect: explanation and consequences. *Vision Res.* 13:1115–1137.
- Stiles, W. S. 1948. The physical interpretation of the spectral sensitivity curve of the eye. In *Transactions of the Optical Convention of the Worshipful Company of Spectacle Makers*. Spectacle Makers' Co., London, UK. 97–107.
- Vu, T. Q., S. T. McCarthy, and W. G. Owen. 1997. Linear transduction of natural stimuli by dark-adapted and light-adapted rods of the salamander, *Ambystoma tigrinum*. *J. Physiol. (Lond.)*. 505:193–204.
- Wang, Q., R. W. Schoenlein, L. A. Peteanu, R. A. Mathies, and C. V. Shank. 1994. Vibrationally coherent photochemistry in the femtosecond primary event of vision. *Science*. 266:422–424.
- Yokoyama, S., and R. Yokoyama. 2000. Comparative molecular biology of visual pigments. In *Handbook of Biological Physics 3. Molecular Mechanisms in Visual Transduction*. D. G. Stavenga, W. J. DeGrip, and E. N. Pugh, Jr., editors. Elsevier, Amsterdam, The Netherlands. 257–296.