*J. Physiol.* (1983), **337**, *pp.* 723–734
*With 8 text-figures*
*Printed in Great Britain*



# TEMPERATURE EFFECTS ON THE MEMBRANE CURRENT OF RETINAL RODS OF THE TOAD

BY D. A. BAYLOR, G. MATTHEWS* AND K.-W. YAU†
*From the Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305 U.S.A.*

(*Received 29 July 1982*)

## SUMMARY

1. Thermal effects on the visual transduction mechanism of toad rods were examined by recording the membrane current of a single outer segment while changing the temperature within the range 15–30 °C.

2. Warming increased the amplitude $r_{max}$ of the saturating flash response. This effect had a $Q_{10}$ of about 1·8 and may result from an increase in the light-sensitive conductance.

3. The flash sensitivity decreased with increasing temperature, while the half-saturating flash intensity increased.

4. There was no evidence of a temperature effect on the probability that an incident 500 nm photon triggered an electrical response. Together with the results in (2) and (3) this indicates that at higher temperature a successfully absorbed photon blocked a smaller fraction of the light-sensitive conductance.

5. Upon warming, the time scale of the flash response shortened but the characteristic wave form was preserved. The speed of the dim flash response, measured by the reciprocal of its time-to-peak, had a $Q_{10}$ of 2·7 and an apparent activation energy of 16·8 kcal mole$^{-1}$.

6. The power spectrum of the continuous component of the dark noise could be predicted at different temperatures by assuming that the underlying event was shaped by two of the four delays required to fit the light response. This behaviour is consistent with the notion that the continuous noise arises within the cascade of processes controlling the internal transmitter concentration of the outer segment.

## INTRODUCTION

A previous paper (Baylor, Matthews & Yau, 1980) described the strong effect of temperature on the frequency of occurrence of the spontaneous events comprising the 'discrete' component of the dark noise of toad rods. In the course of the experiments, observations were made on the temperature dependence of several other

* Present address: Department of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794, U.S.A.
† Present address: Department of Physiology and Biophysics, The University of Texas Medical Branch, Galveston, TX 77550, U.S.A.

parameters of the transduction mechanism. The present paper describes these results, including the effects of temperature on the dark current, the light response, and the 'continuous' component (Baylor *et al.* 1980) of the dark noise. Some of the results confirm previous findings of Penn & Hagins (1972) on rat rods and Baylor, Hodgkin & Lamb (1974) on turtle cones. Lamb (1982) has recently performed similar measurements on toad rods with a system permitting rapid temperature changes.

## METHODS

### *Preparation and recording*

A detailed description of the preparation and the method of recording outer segment membrane current has been given previously (Baylor, Lamb & Yau, 1979*a*; Baylor *et al.* 1980). The outer segment of a 'red' rod in a piece of isolated retina from the toad *Bufo marinus* was drawn by suction into the tip of a Ringer-filled glass micropipette. Membrane current was recorded with a current-to-voltage transducer connected to the inside of the pipette. The leakage resistance between the pipette and the outer segment, which determined the fraction of membrane current recorded, showed no obvious dependence on temperature when measured by applying small voltage steps at the positive input of the current-to-voltage transducer (Baylor *et al.* 1979*a*). The transducer output was recorded on an FM tape recorder and subsequently digitized and analysed in a PDP 11/34 computer. Power spectra were computed as described in Baylor *et al.* (1980).

### *Ringer fluid*

The Ringer solution contained (mM): NaCl, 111; $MgCl_2$, 1·6; KCl, 2·5; $CaCl_2$, 1·0; D-glucose, 10; HEPES, 3, buffered to pH 7·8 with NaOH. The temperature dependence of the $pK_a$ of HEPES is 0·014 $°C^{-1}$ (Good, Winget, Winter, Connolly, Iyawa & Singh, 1966); the changes in hydrogen ion concentration with temperature would be relatively small in most of the experiments reported here.

### *Temperature changes*

The temperature of the Ringer solution containing the pieces of isolated retina was varied by circulating water from a Peltier heat exchanger through a channel in the walls of the experimental chamber. Temperature in the chamber was measured by a calibrated thermistor mounted within 2 mm of the tip of the suction electrode. A change in temperature required roughly five minutes to reach steady state. Most of the electrical measurements were made after the temperature had reached steady state. Saturating response amplitude, however, was usually monitored periodically while the temperature was changing.

### *Light stimuli*

The dual beam optical stimulator supplied infrared light ($\lambda > 850$ nm) for viewing manipulations with an infra-red/visible image converter attached to the inverted microscope, and a beam of visible light for stimulating the cells. The visible beam passed through a narrow-band interference filter (centre wave-length 500 nm) and a series of calibrated neutral density filters for controlling intensity. The stimuli were plane-polarized with the electric vector transverse to the long axis of the outer segment and were applied diffusely on the entire retinal piece. Stimulus intensities are given as incident 500 nm photon densities and were calculated from the measured irradiance of the unattenuated 500 nm light, the neutral filter attenuations, and the flash duration, which was 20 msec. The unattenuated light was measured at the end of each experimental day by placing the probe of a United Detector Technology irradiance meter (Model 111A) at the position of the experimental chamber.

## RESULTS

Fig. 1 shows the effects of a temperature change on flash responses from two rods. In the upper set of records the temperature was decreased from 22·5 to 18·8 °C and then returned, while in the lower set the change was from 19·2 to 26·3 °C. At higher temperature the amplitude of the saturating response was larger and the responses faster as indicated by a shortened time to peak amplitude and an accelerated decay.

*Saturating photocurrent*

Fig. 2 shows collected results from five cells on the variation of saturating response amplitude, $r_{max}$, with temperature. Saturating amplitudes were determined by applying bright flashes as temperature was changed continuously at a rate of 0·5–1·5 °C $min^{-1}$. Values of $r_{max}$ for each cell have been scaled to unity at 20 °C by

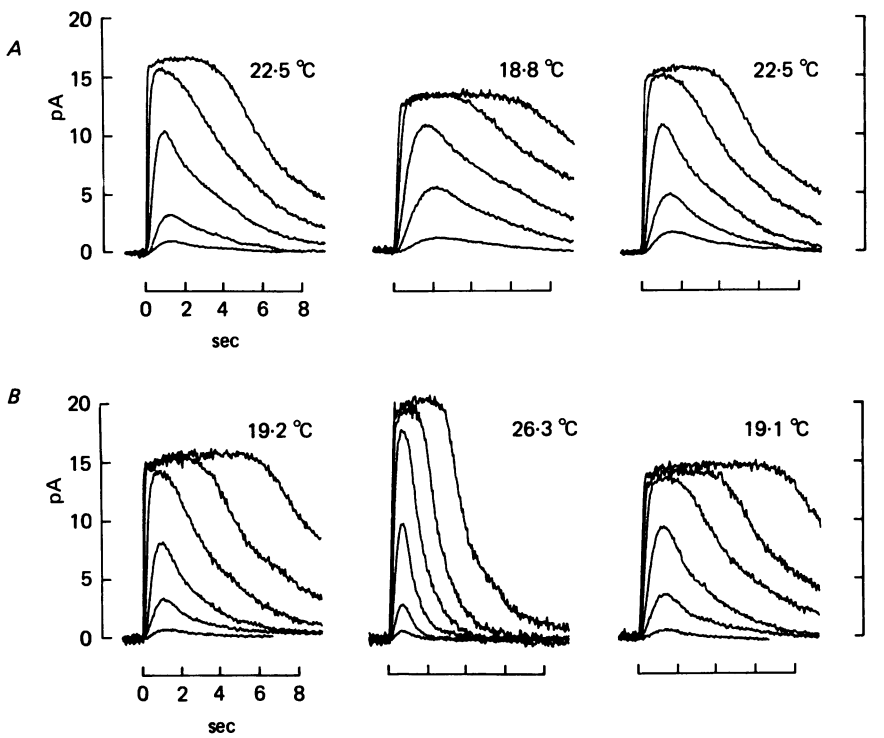


Fig. 1. Effects of a temperature change on flash responses from two rods. Superimposed traces, with the change in outer segment current relative to the level in darkness plotted as a function of time after the flash. Diffuse 20 msec flashes at 500 nm, delivered at $t = 0$. Outward change in membrane current drawn upwards. Recordings processed with a six-pole low-pass filter of cut-off frequency 6 Hz. *A*, temperature lowered from 22·5 to 18·8 °C and returned. Flash photon density was increased by factors of about 4·7 between 0·054 and 27 photons $\mu m^{-2}$. In each family the three lower traces are averages of six to forty responses while the two upper traces are single sweeps or averages of two or three responses. *B*, temperature raised from 19·2 to 26·3 °C and returned. Flash photon density increased by factors of about 4·7 between 0·051 and 140 photons $\mu m^{-2}$. In each family the two upper traces are single sweeps; from below upwards the other traces are averages of 20, 10, 5, and 2 responses respectively. Cell 4 of Table 1.

interpolation. In the linear plot on the left $r_{max}$ increased approximately linearly with warming between 14·1 and 25·4 °C. On the right is an Arrhenius plot of the same results; the slope of the linear regression line gives an activation energy of 10·5 kcal $mole^{-1}$, corresponding to a $Q_{10}$ of 1·8 between 15 and 25 °C.

Previous work (Penn & Hagins, 1972; Baylor *et al.* 1979*a*) has shown that the saturating photocurrent represents complete suppression of the steady inward 'dark current' of the outer segment. On this basis warming increased $r_{max}$ by increasing

the dark current. The records of Penn & Hagins (1972, fig. 2) show a similar effect in rat rods.

The change in a rod's saturating response was often not completely reversible after prolonged (> 15 min) temperature changes of over 5 °C (see Table 1). This may be due to long term changes in intracellular sodium concentration or some other deleterious effect on the cell. Effects associated with changes in intracellular sodium concentration might be only very slowly reversible (see Yau, McNaughton & Hodgkin, 1981).

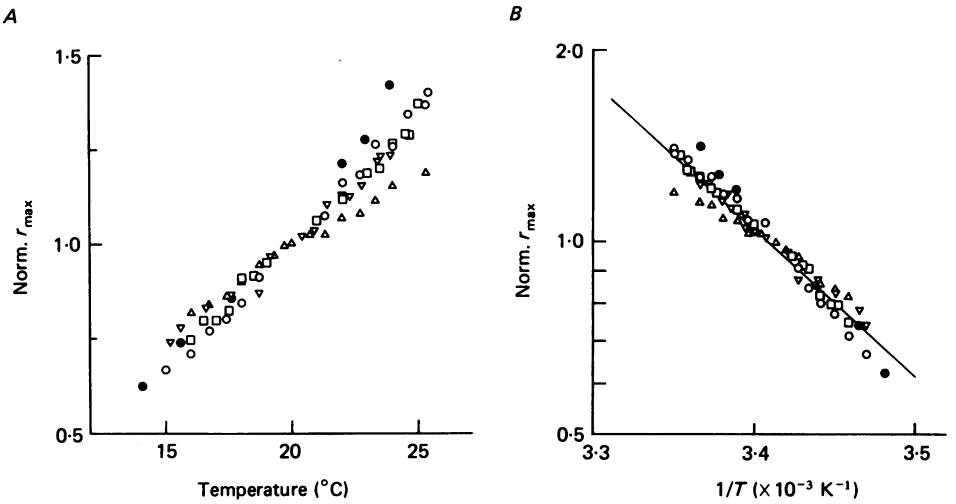


Fig. 2. Dependence of saturating response, $r_{max}$, on temperature. Collected results from five rods. Responses from each cell have been scaled by interpolation to a value of unity at 20 °C. *A*, linear plot of $r_{max}$ *vs.* temperature. *B*, Arrhenius plot of same results. The slope of the linear regression line in *B* corresponds to an activation energy of 10·5 kcal mole$^{-1}$. Saturating amplitudes were determined by intermittent bright flashes as temperature was changed continuously at a rate of 0·5–1·5 °C min$^{-1}$.

*Flash sensitivity and half-saturating flash intensity*

Table 1 and Fig. 3 collect results from six experiments to examine the effect of temperature on the sensitivity $S_F^D$ to dim flashes, estimated from the ratio of the peak amplitude of the average response to the flash photon density. Results were selected from experiments in which the sensitivity returned to at least roughly the initial value when the temperature was returned to its initial value. In about half the experiments this did not occur, making interpretation difficult. As can be seen in Fig. 3, warming usually reduced sensitivity. The $Q_{10}$ of the average effect was 1·61, as calculated from the fractional changes in $S_F^D$. Because warming reduced $S_F^D$ while increasing $r_{max}$, it appears that at a higher temperature each incident photon on average gave a smaller fractional reduction in dark current. This would imply that the half-saturating photon density $i_0$ should increase on warming. The response–intensity relations in Fig. 4 show this effect in the records of Fig. 1. The half-saturating flash intensity $i_0$ was estimated by fitting the Michaelis relation

$$\frac{r}{r_{max}} = \frac{i}{i+i_0} \qquad (1)$$

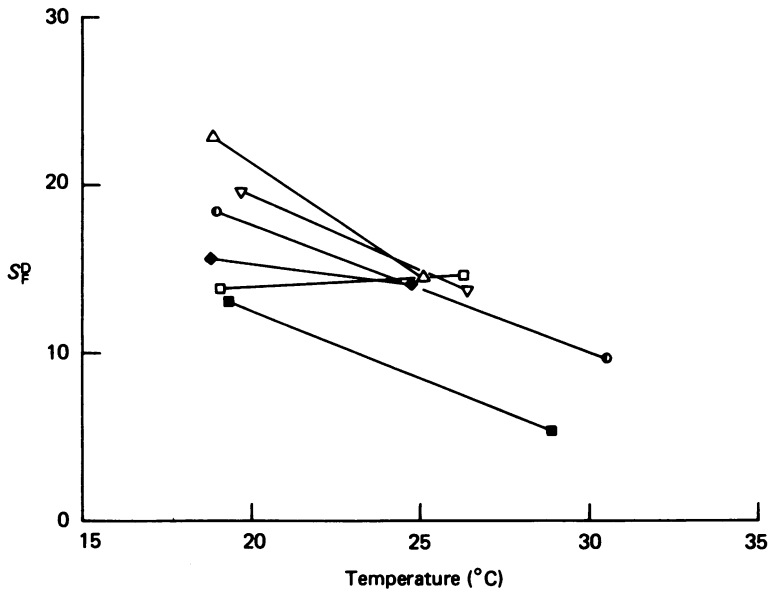


Fig. 3. Temperature dependence of flash sensitivity, $S_F^D$ (pA photon$^{-1}$ $\mu$m$^2$). Collected results from cells 1–6 of Table 1; same cell symbols. Sensitivities at the starting temperature were averaged from the initial and final values.

TABLE 1. Dependence of flash sensitivity on temperature. Cell symbols as in Figs. 3 and 6. $S_F^D$ was measured after temperature change had reached steady state. $r_{max}$ was averaged from values just before and after the dim flash run to determine $S_F^D$. $i_o$ was estimated by interpolation in plots of response amplitude *vs.* log intensity

| Cell | Temperature (°C) | $r_{max}$ (pA) | $S_F^D$ (pA photon$^{-1}$ $\mu$m$^2$) | $i_o$ (photons $\mu$m$^{-2}$) | $r_{max}/S_F^D$ (photon $\mu$m$^{-2}$) | $t_{peak}$ (sec) |
|---|---|---|---|---|---|---|
| 1. ◆ | 25·0 | 15·0 | 16·9 | 0·82 | 0·89 | 1·05 |
| | 18·8 | 11·5 | 15·6 | 0·72 | 0·74 | 1·47 |
| | 24·5 | 13·0 | 11·4 | 0·86 | 1·14 | 1·14 |
| 2. △ | 18·9 | 17·8 | 25·4 | 0·55 | 0·70 | 1·71 |
| | 25·1 | 21·0 | 14·5 | 0·88 | 1·45 | 0·96 |
| | 18·9 | 13·9 | 20·4 | 0·46 | 0·68 | 1·56 |
| 3. ▽ | 20·0 | 16·3 | 22·7 | 0·51 | 0·72 | 1·52 |
| | 26·4 | 17·5 | 13·7 | 1·05 | 1·28 | 0·84 |
| | 19·5 | 13·5 | 18·0 | 0·59 | 0·75 | 1·56 |
| 4. □ | 19·2 | 16·3 | 13·4 | 0·75 | 1·22 | 1·20 |
| | 26·3 | 21·0 | 14·6 | 1·2 | 1·43 | 0·68 |
| | 19·1 | 15·2 | 14·2 | 1·05 | 1·08 | 1·48 |
| 5. ◐ | 18·7 | 18·4 | 21·2 | — | 0·87 | 1·66 |
| | 30·5 | 25·9 | 9·7 | — | 2·63 | 0·53 |
| | 19·2 | 12·5 | 15·6 | — | 0·80 | 1·27 |
| 6. ■ | 20·0 | 16·9 | 15·9 | — | 1·06 | 1·36 |
| | 28·8 | 21·2 | 5·4 | — | 4·00 | 0·50 |
| | 18·7 | 13·6 | 10·3 | — | 1·32 | 1·90 |

to the experimental points at each temperature. In *A*, cooling from 22·5 to 18·8 °C shifted the curve to the left and reduced the value of $i_0$ from 0·62 to 0·41 photons $\mu m^{-2}$. In *B*, warming from 19·2 to 26·3 °C increased $i_0$ from 0·92 to 1·33 photons $\mu m^{-2}$. Similar results are evident in Table 1 for cells 1–3, where the values of $i_0$ were estimated by interpolation in plots of peak flash response amplitude *vs.* intensity.

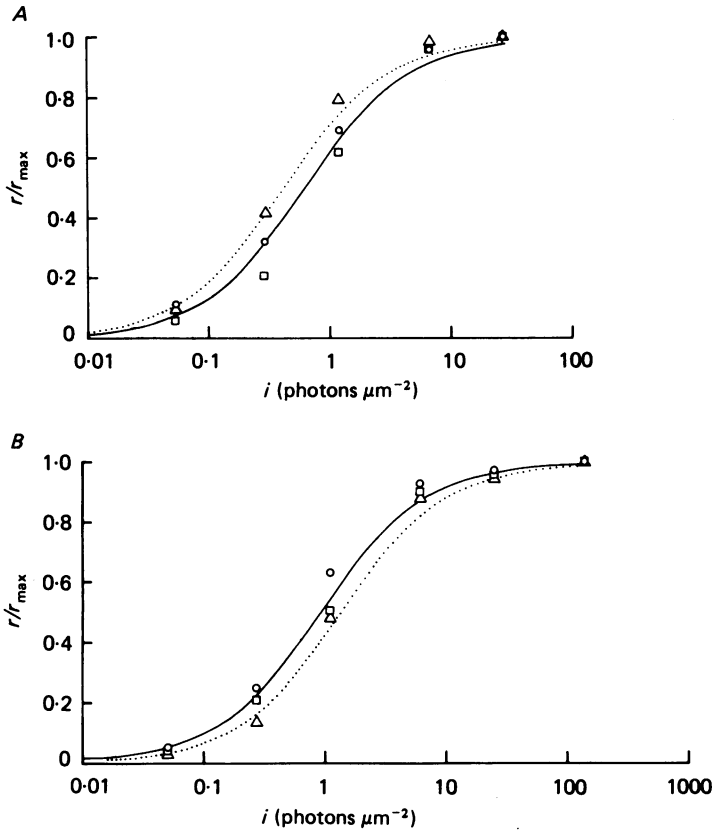


Fig. 4. Response-intensity relations at different temperatures. Results from the two rods of Fig. 1. Peak response amplitude divided by saturating amplitude plotted against flash photon density (logarithmic scale). *A*, results from Fig. 1*A*, determined in the sequence: □, 22·5 °C; △, 18·8 °C; ○, 22·5 °C. Continuous curve drawn from eqn. (1) with $i_0$ = 0·62 photon $\mu m^{-2}$, fitted by eye to points at 22·5 °C. Dotted curve drawn from eqn. (1) with $i_0$ = 0·41 photon $\mu m^{-2}$. *B*, results from Fig. 1*B*, determined in the sequence: □, 19·2 °C; △, 26·3 °C; ○, 19·1 °C. Continuous curve drawn from eqn. (1) with $i_0$ = 0·92 photon $\mu m^{-2}$ fitted to data at 19·2 and 19·1 °C; dotted curve drawn from eqn. (1) with $i_0$ = 1·33 photon $\mu m^{-2}$.

The average effect on four cells, including that of Fig. 4*B*, had a $Q_{10}$ of 1·89. The effect on $i_0$ in cells where a range of flash intensities was not used can be estimated from the ratio $r_{max}/S_F^D$, which, for a Michaelis relation, is identical to $i_0$. The average effect on this parameter from all six cells of Table 1 had a $Q_{10}$ of 2·29.

*Quantum efficiency*

The results just described showed that warming decreased $S_F^D$ and increased $i_0$. These changes might result from a reduction in the size of the single photon effect or from a decrease in the probability that a photon incident on the rod will trigger a response. The probability of response generation can be assessed by determining the probability that a very dim flash of fixed applied intensity generates no response

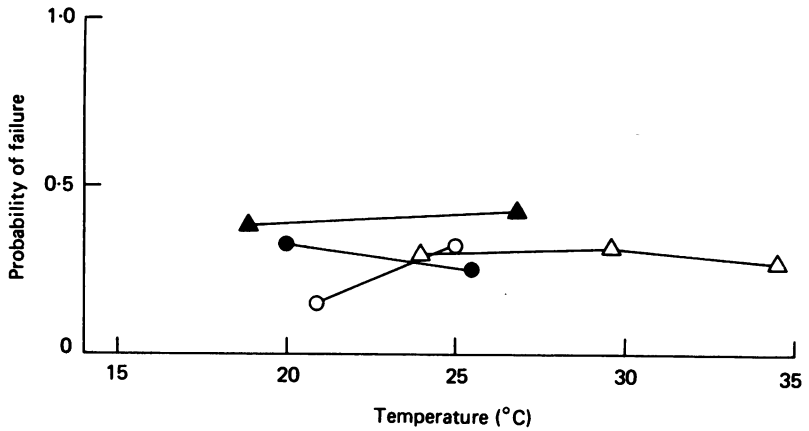


Fig. 5. Probability of failure in dim flash runs at different temperatures. Collected results from four rods, with each symbol representing a different cell. Each flash run was at fixed intensity and consisted of thirty-one to sixty trials. Intensities were adjusted so that the probability of failure exceeded 0·25. The proportion of failures was estimated from histograms of response amplitude as described by Baylor *et al.* (1979*b*).

at all. Such 'failures' are an inevitable consequence of the Poisson statistics governing the probability that dim lights will evoke photoisomerizations. Fig. 5 collects results from four experiments in which the probability of failure was determined at different temperatures. The strength of the 500 nm flash was adjusted so that failures occurred in about one third of the trials. The probability of failure was estimated from response amplitude histograms as described previously (Baylor *et al.* 1979*b*). There is no sign that the probability of failure varied systematically with temperature in the range explored. Given the scatter in the results and the difficulty in performing the experiment satisfactorily, a small temperature effect cannot be ruled out. Nevertheless, the temperature dependence of $S_F^D$ and $i_0$ observed here probably cannot be explained by a changed likelihood of quantal response generation. Instead, warming appeared to lower $S_F^D$ by reducing the size of the quantal photocurrent. The concomitant rise in $i_0$ implies that upon warming a smaller fraction of the light-sensitive conductance was blocked during the successful quantal response.

*Kinetics of light response*

Warming strongly accelerated the flash response. In the experiment of Fig. 1*A*, for example, the time-to-peak, $t_{peak}$, of the dim flash response shortened from 2·4 sec at 18·6 °C to 1·4 sec at 22·5 °C. In the experiment of Fig. 1*B*, $t_{peak}$ shortened from

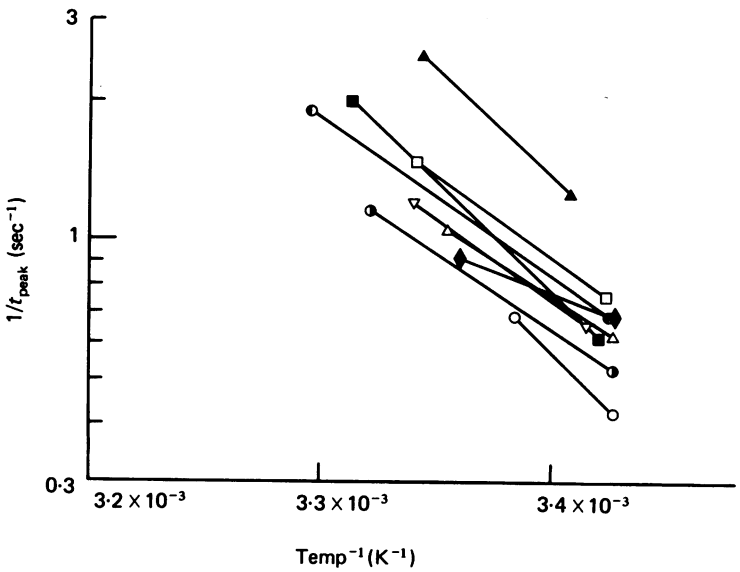


Fig. 6. Arrhenius plots of reciprocal time-to-peak ($1/t_{peak}$) of dim flash response against reciprocal absolute temperature. Collected results from nine rods, each symbol representing a different cell. The values of $t_{peak}$ before and after the temperature change have been averaged, as well as the control temperatures, which were within 1·3 °C of each other. The mean slope of the straight lines fitted to individual experiments is 8427 ± 2106 K (S.D.), giving an activation energy of 16·8 kcal mole$^{-1}$.

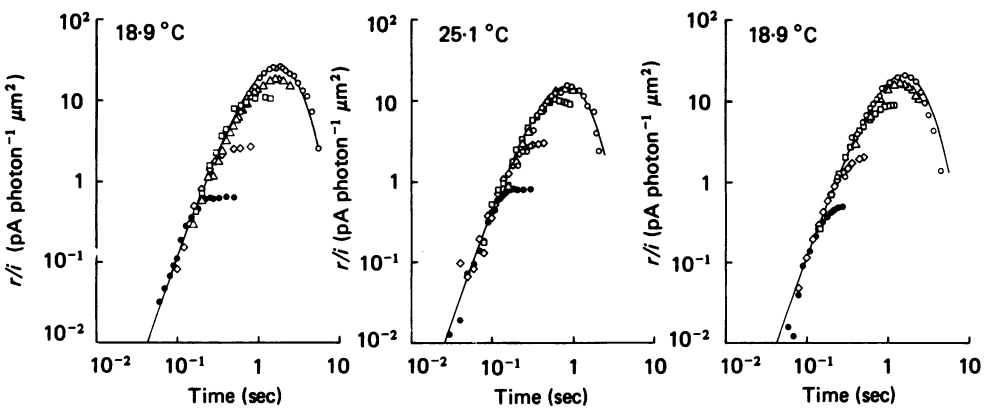


Fig. 7. Effect of temperature on kinetics of flash responses. Response divided by flash photon density plotted against time after the flash on double logarithmic coordinates. Temperature was changed from 18·9 to 25·1 °C and returned. At each temperature, families like those of Fig. 1 were obtained, with flash intensities varying by factors of about 4·7 from 0·051 to 25 photons μm$^{-2}$. 20 msec flashes at 500 nm. Responses to the three brightest flashes not low-pass filtered (□, ◇, ●); responses to lower intensities (○, △) processed with a 6-pole low-pass filter of 20 Hz cut-off. Continuous curves drawn according to eqn. (3) with $\alpha = 0{\cdot}82$ sec$^{-1}$ (first run at 18·9 °C), 1·54 sec$^{-1}$ (25·1 °C), and 0·87 sec$^{-1}$ (final run at 18·9 °C). Cell 2 of Table 1.

1·2 sec at 19·2 °C to 0·68 sec at 26·3 °C. Fig. 6 shows Arrhenius plots of $1/t_{peak}$ against reciprocal absolute temperature collected from nine cells. The mean slope of the straight lines fitted to the points from individual experiments corresponds to an activation energy of 16·8 kcal mole$^{-1}$ and a $Q_{10}$ of about 2·7 between 15 and 25 °C. The activation energy estimated here is close to the value of 15·8 kcal mole$^{-1}$ reported by Penn & Hagins (1972) for rat rods.

Despite substantial shortening in the response at higher temperatures, the wave form was preserved, as illustrated in Fig. 7. In this experiment response families were obtained from a rod at two temperatures, as in Fig. 1, and the response amplitude divided by flash intensity is plotted against time on double logarithmic co-ordinates, allowing comparison of wave forms in the presence of changes in scale (see Baylor, Hodgkin & Lamb, 1974; Baylor *et al.* 1979*a*). The continuous curve which fits the dim flash response at both temperatures is a plot of

$$r(t) = ike^{-\alpha t}(1-e^{-\alpha t})^3, \tag{2}$$

where $k$ is a sensitivity constant, $\alpha$ is a rate constant, $i$ is flash intensity and $t$ is time after the flash. This is the impulse response of a sequence of four delay stages with time constants $1/\alpha$, $1/2\alpha$, $1/3\alpha$ and $1/4\alpha$ arranged in any order; this expression, or the alternative one described below, has been shown to describe the rod's dim flash response near 20 °C (Baylor *et al.* 1979*a*). In Fig. 7, $\alpha$ has values of 0·82 sec$^{-1}$ (left) and 0·87 sec$^{-1}$ (right) at 18·9 °C and 1·54 sec$^{-1}$ at 25·1 °C. Thus the shape of the response was preserved apart from a change in time scale, corresponding to a shift along the time axis in Fig. 7. On the filter model this would be expected if all four time constants had approximately the same temperature dependence.

Broadly similar results were obtained from nine other rods. In five cells the dim flash response near 20 °C was fitted better by the expression

$$r(t) = ik(\alpha t)^3 e^{-\alpha t}, \tag{3}$$

which represents the impulse response of four delay stages each having the same time constant $1/\alpha$ (see Fuortes & Hodgkin, 1964; Baylor *et al.* 1974; Baylor *et al.* 1979*a*). In these cells also the main effect of temperature was to shift the responses on the logarithmic time axis. At 20 °C or lower such double log plots invariably showed the initial rise of the flash response to have a slope of 3, consistent with the four-stage models of eqn. (2) and (3). At temperatures above 25 °C, however, three of the ten rods required five or six stages to fit their flash responses; this might be explained by the unmasking of additional shorter delays less sensitive to temperature.

*Continuous component of dark noise*

The membrane current of toad rods is noisy in darkness but becomes quiet when the light-sensitive conductance is lowered by a bright light (Baylor *et al.* 1980). The dark noise has been separated into a discrete component, thought to represent thermal isomerization of rhodopsin, and a continuously present component of smaller amplitude. Little is known about the continuous component, but it has been suggested to arise within the cascade of processes that controls the internal transmitter concentration of the outer segment. Further support for this notion is provided by the similar dependence of the noise spectrum and flash response kinetics

on temperature. In the experiment of Fig. 8, the shape of a rod's dim flash response and its continuous noise spectrum were determined at three temperatures, 15 °C (*A*), 19·8 °C (*B*) and 25·5 °C (*C*).

The difference spectra of the continuous noise were obtained by subtracting bright-light spectra from spectra of stretches of dark record not containing obvious

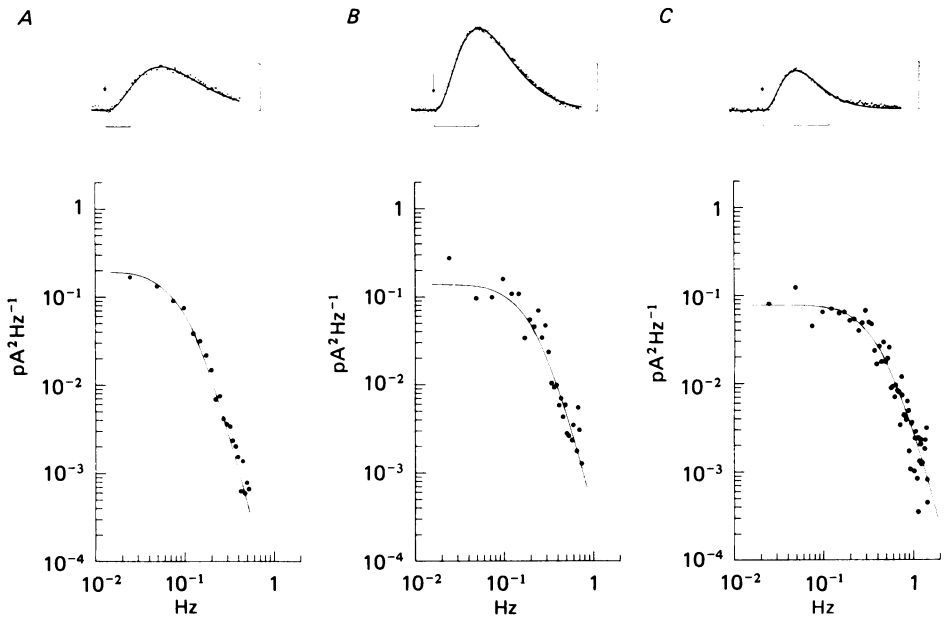


Fig. 8. Power spectra of continuous dark noise at three temperature *A*, 15 °C; *B*, 19·8 °C; *C*, 25·5 °C. Noise spectra (bottom) obtained by subtracting spectrum in bright light from spectrum of selected stretches of dark current containing no discrete events. Continuous curves through the power spectra were calculated from $S(f) = S(0)\,[1+(2\pi f/\alpha)^2]^{-2}$, where $S(0)$ is an arbitrary constant (zero-frequency asymptote), $f$ is frequency and $\alpha$ is a constant with values of 0·71 $sec^{-1}$, 1·50 $sec^{-1}$ and 3·00 $sec^{-1}$ in *A*, *B* and *C* respectively. Top insets show averaged responses to dim flashes at each temperature. 20 msec flash delivered at time indicated by arrow. Continuous curves in the insets drawn according to eqn. (3) with same $\alpha$ value as in the corresponding noise spectrum. Inset calibration bars 2 sec and 1 pA.

discrete noise events. At all three temperatures the form of the noise spectrum could be predicted on the assumption that the underlying event was shaped by two of the four slow processes in the flash response (Baylor *et al.* 1980). For example, at 15 °C (*A*) the flash response was fitted by eqn. (3) with $\alpha = 0{\cdot}71$ $sec^{-1}$. The curve fitted to the noise spectrum at this temperature is a product of two Lorentzians, each of cut-off frequency $0{\cdot}71/2\pi$ Hz. The same equations were fitted at higher temperature, with values for $\alpha$ of 1·50 and 3·00 $sec^{-1}$ respectively at 19·8 and 25·5 °C. Thus despite the changes in time scale the flash response and noise remained in the same relation to one another. Similar results were observed in each of four other experiments.

## DISCUSSION

*Dark current.* It is the light-sensitive conductance that limits the dark current (Bader, MacLeish & Schwartz, 1979), so that the increased current observed with warming would be simply explained by an increase in outer segment conductance. Although we cannot exclude an increase in driving force on the current, due to accelerated sodium pumping, the form of the current–voltage relations observed by Bader *et al.* (1979) suggests that very large changes in reversal potential would be required to give the observed changes in current. Unfortunately our experiments lacked sufficient time resolution to indicate whether warming increased the dark current instantaneously, as expected for a direct effect on the rate of ion permeation. Recent experiments by Lamb (1982), with a rapid flow system, show that the current change follows the temperature change with a delay less than about 5 sec. The ionic conductance of bulk aqueous solution has a $Q_{10}$ of 1·23 (see Moore, 1964), while conduction through the Na channel of squid axon has a $Q_{10}$ of 1·6 (Chandler & Meves, 1970). The dark current of rods had a $Q_{10}$ of 1·8 in our experiments and 2·1 in Lamb's (1982) experiments with faster temperature changes. The temperature sensitivity of the rod current might imply that the mechanism of ion permeation is different from that in conventional aqueous channels. Alternatively, however, temperature might exert its large effects by an indirect mechanism involving changes in the concentration of internal transmitter.

*Quantum efficiency.* A photon incident on a rod can cause an electrical response only if it is absorbed in a rhodopsin molecule, isomerizes it, and changes the internal transmitter concentration near the light-sensitive channels. In our experiments the joint probability of all three events showed no obvious temperature dependence. Although several lines of evidence indicate that warming increases the molecular extinction coefficient of rhodopsin at long wave-lengths (St. George, 1952; Denton & Pirenne, 1954; Srebro, 1966) this effect is small and confined to wave-lengths longer than about 600 nm. For the 500 nm light used here the probability of absorption in rhodopsin should have been constant. The constant probability of response thus indicates that absorbed photons continued to trigger electrical changes with the same efficiency at different temperatures. Previous work indicated this to be about 0·5 near 20 °C (Baylor *et al.* 1979*b*).

*Kinetics.* The temperature-induced changes in the time-to-peak of the dim flash response were consistent with an activation energy of 16·8 kcal mole$^{-1}$. This is much larger than that of 9·8 kcal mole$^{-1}$ found in turtle cones (Baylor *et al.* 1974), suggesting an important difference in the transduction mechanism.

In the four-stage filter models of eqns. (2) and (3) the time-to-peak is proportional to the time constant $1/\alpha$. Since in most experiments the temperature fixed the value of $\alpha$ without changing the number of stages or the symmetry of the flash response, the apparent activation energy of 17 kcal mole$^{-1}$ may be regarded as characteristic of each of the four processes, or perhaps an average over the four processes.

This work was supported by grant EY 01543 from the National Eye Institute, USPHS and by the Alfred P. Sloan Foundation.

## REFERENCES


BADER, C. R., MACLEISH, P. R. & SCHWARTZ, E. A. (1979). A voltage-clamp study of the light response in solitary rods of the tiger salamander. *J. Physiol.* **296**, 1–26.

BAYLOR, D. A., HODGKIN, A. L. & LAMB, T. D. (1974). The electrical response of turtle cones to flashes and steps of light. *J. Physiol.* **242**, 685–727.

BAYLOR, D. A., LAMB, T. D. & YAU, K.-W. (1979*a*). The membrane current of single rod outer segments. *J. Physiol.* **288**, 589–611.

BAYLOR, D. A., LAMB, T. D. & YAU, K.-W. (1979*b*). Responses of retinal rods to single photons. *J. Physiol.* **288**, 613–634.

BAYLOR, D. A., MATTHEWS, G. & YAU, K.-W. (1980). Two components of electrical dark noise in retinal rod outer segments. *J. Physiol.* **309**, 591–621.

CHANDLER, W. K. & MEVES, H. (1970). Rate constants associated with changes in sodium conductance in axons perfused with sodium fluoride. *J. Physiol.* **211**, 679–705.

DENTON, E. J. & PIRENNE, H. M. (1954). The visual sensitivity of the toad, *Xenopus laevis*. *J. Physiol.* **125**, 181–207.

FOURTES, M. G. F. & HODGKIN, A. L. (1964). Changes in time scale and sensitivity in the ommatidia of *Limulus*. *J. Physiol.* **172**, 239–263.

GOOD, N. E., WINGET, G. D., WINTER, W., CONNOLLY, T. N., IYAWA, S. & SINGH, R. M. M. (1966). Hydrogen ion buffers for biological research. *Biochemistry* **5**, 467–477.

LAMB, T. D. (1982). Effect of rapid temperature changes on the photocurrent of toad rods. *J. Physiol.* **332**, 15*P*.

MOORE, W. J. (1964). *Physical Chemistry*. Englewood Cliffs, N.J.: Prentice Hall.

PENN, R. D. & HAGINS, W. A. (1972). Kinetics of the photocurrent of retinal rods. *Biophys. J.* **12**, 1073–1094.

ST. GEORGE, R. C. C. (1952). The interplay of light and heat in bleaching rhodopsin. *J. gen. Physiol.* **35**, 495–517.

SREBRO, R. (1966). A thermal component of excitation in the lateral eye of *Limulus*. *J. Physiol.* **187**, 417–425.

YAU, K.-W., MCNAUGHTON, P. A. & HODGKIN, A. L. (1981). Effect of ions on the light-sensitive current in retinal rods. *Nature, Lond.* **292**, 502–505.