*Journal of Physiology* (1993), **462**, *pp.* 465–481
*With 10 figures*
*Printed in Great Britain*



# TEMPERATURE DEPENDENCE OF THE LIGHT RESPONSE IN RAT RODS

BY D. W. ROBINSON*, G. M. RATTO†, L. LAGNADO‡ AND P. A. McNAUGHTON§

*From the §Physiology Department, Biomedical Sciences, King's College London, Strand, London WC2R 2LS and the Physiological Laboratory, University of Cambridge, Downing Street, Cambridge, CB2 3EG*

(*Received 18 March 1992*)

SUMMARY

1. The effects of temperature on the light responses of rat rods have been investigated over the range 17–40 °C.

2. The amplitude of the light-sensitive current increased with temperature with a mean temperature coefficient ($Q_{10}$) of 2·47.

3. The amplitude of the $Na^+$–$Ca^{2+}$, $K^+$ exchange current decreased with temperature when expressed as a fraction of the light-sensitive current, showing that the light-sensitive channel becomes less permeable to calcium as the temperature is raised. The time constant of relaxation of the exchange current was little affected by temperature.

4. The flash intensity required to give a half-saturating response increased with temperature with a mean $Q_{10}$ of 1·68.

5. The responses to single photoisomerizations were determined from amplitude histograms of the responses to dim-flash trains. The amplitude of the response to a single photoisomerization decreased with temperature when expressed as a fraction of the light-sensitive current, but the change was not sufficient to account for the overall decrease in sensitivity.

6. The fraction of dim flashes that produced a photoisomerization decreased with temperature. This decrease in photon capture efficiency together with the decrease in the relative size of the single photon event fully accounts for the observed change in sensitivity.

7. The speed of the falling phase of the dim-flash response was accelerated more by warming than the rising phase, and it was therefore not possible to superimpose light responses at different temperatures by a simple change in time scale.

* Present address: Centre for Neurobiology, University of California Davis, Davis, CA 95616, USA.

† Present address: Istituto di Neurofisiologia del CNR, 56127 Pisa, Italy.

‡ Present address: Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA.

§ To whom correspondence should be addressed.

MS 1218

## INTRODUCTION

The effects of temperature on the light responses of amphibian photoreceptors have been studied in a range of $\pm 10$ °C centred around room temperature (Baylor, Matthews & Yau, 1983; Lamb, 1984). These studies established that the size of the light-sensitive current increases and the time course of the light response is accelerated at higher temperatures. A similar study has never been carried out on mammalian photoreceptors where the working point for the enzymes of the transduction pathway is at least 20 °C higher than in cold-blooded animals. We find that the effects of temperature on mammalian rods are broadly in line with those previously reported for amphibian rods, but a surprising finding which has emerged from the present study is that the quantal efficiency with which photons activate the light-sensitive pathway decreases with temperature.

## METHODS

### *Isolation procedure and suction electrode recording*

Albino (CHFB strain) and pigmented (Lister Hooded strain) rats were reared in a 12 h light–dark cycle (mean intensity in light 7 lm $m^{-2}$) and were fed on rat and mouse diet No. 3 (SDS, Witham, Essex). Rats were dark adapted for two or more hours before being killed, under dim red illumination, by $CO_2$ anaesthesia followed by cervical dislocation. The methods for isolating small fragments of retina and for viewing them under infrared illumination were similar to those described by Hodgkin, McNaughton & Nunn (1985). Retinae were stored at 5 °C for up to 8 h before use. In early experiments all procedures were carried out in modified Hanks solution of composition (mM): NaCl, 130; KCl, 5·4; $MgCl_2$, 0·5; $MgSO_4$, 0·2; $CaCl_2$, 1·0; ascorbic acid, 2·0; glucose, 5·0; Hepes, 10·0, final pH 7·5. In later experiments (the $Na^+$–$Ca^{2+}$, $K^+$ exchange and dim-flash experiments reported in Figs 4 and 8) the viability of rods was found to be considerably improved by using a Hepes-buffered minimal essential medium (Eagle's MEM, Sigma M2645); 26·2 mM $NaHCO_3$, 100 $\mu$M taurine and 0·01 % bovine serum albumin (fraction V, Sigma A9543) were added to the MEM. The medium was filtered before storage, and the pH was adjusted to 7·6 at room temperature before each experiment.

Electrodes were pulled from Pyrex capillary glass of 1·2 mm o.d. in two stages on a horizontal puller (Mecanex, BB-CH, Geneva, Switzerland) in patch-pulling mode. The internal diameter of the tip was typically 1·2–1·8 $\mu$m, measured using a calibrated graticule. The electrodes were coated with tri-*n*-butylchlorosilane (Lamb, McNaughton & Yau, 1981).

### *Recording chamber*

The recording chamber was made from Perspex, with the top consisting of a small piece of microscope glass and the bottom of a coverslip in order to allow the use of high-power objectives on an IM35 inverted microscope (Zeiss). The front of the chamber was left open to allow entry of the suction electrode and the waste pipe, the solution being retained in the chamber by surface tension.

Solution passed through a water-cooled Peltier device controlled by a negative feedback circuit before entering the recording chamber. A small antechamber, open to the atmosphere, was incorporated into the inlet pipe in order to trap bubbles liberated as a result of the rapid heating of the perfusing solution by the Peltier device. The capacity of the recording chamber was about 250 $\mu$l. Perfusion introduces some variation in the electrical baseline, probably because of small fluctuations in temperature around the mouth of the pipette, and for the dim-flash analysis, where a very stable baseline was necessary, electrical stability was improved by dispensing with perfusion. In these experiments the chamber was warmed by a water jacket through which water heated by the Peltier device continuously flowed.

### *Optical stimuli*

The system for optical stimulation and observation under infrared illumination was similar to that described by Baylor & Hodgkin (1973), with the modifications described by Hodgkin *et al.* (1985). Light stimuli were calibrated daily by placing a photometric diode (United Detector

Technology 111A, Santa Monica, CA, USA) immediately below the recording chamber. The Phenol Red in the MEM attenuated the flash by 0·11 log units.

In some experiments the intensity of the stimulating light transmitted through the recording chamber was monitored throughout the experiment by imaging the spot on the cathode of a photomultiplier placed in the binocular port of the inverted microscope. The resulting counts were recorded and analysed to evaluate the stability of the stimulating light flux.

*Signal acquisition and processing*

Signals were low-pass filtered with a six-pole Butterworth filter (cut-off frequency set at either 40 or 20 Hz) and digitized on-line (400 Hz sampling frequency) with an IBM PC-AT, using a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK). The only effect of the filtering on the relatively slowly rising responses in the present study was to impose a delay of *ca* 25 ms (for the 40 Hz filter). Occasionally the data were further digitally smoothed by an averaging window (width specified in figure legends).

*Dim-flash analysis*

The size of each response in a long train of dim flashes was obtained by least-squares fitting the ensemble average to each individual response, after correction for baseline drift and for decline in the light-sensitive current (see Baylor, Lamb & Yau, 1979; Baylor, Nunn & Schnapf, 1984; Ratto, Robinson, Yan & McNaughton, 1991). A similar analysis was carried out on an equal number of interleaved sweeps in which no flash was given. Amplitude histograms of the resulting response sizes were constructed. The amplitude histograms from trials with no flash were fitted with a single Gaussian function using a fitting routine based on the Levenberg–Marquardt minimization algorithm. Histograms from trials with a dim flash were fitted with a linear combination of three Gaussian functions using the methods developed by Baylor and colleagues (1979, 1984 – see eqn (10) of Baylor *et al.* 1979), with the exception that the position of the peak corresponding to two photoisomerizations was found to be best described by the use of a saturating exponential function (eqn (7), see p. 15). Good fits to the observed histograms were obtained and in most experiments peaks corresponding to two or more photoisomerizations were clearly resolved (see Figs 7 and 8).

*Computation of the exchange current during the response to a bright flash*

Assuming that the intracellular free calcium is in rapid equilibrium with its buffering system the total intracellular calcium, $[Ca^{2+}]_i$, can be expressed as:

$$\frac{d[Ca^{2+}]_i}{dt} = \frac{1}{2VF} P J(t) - \frac{J_{ex}(t)}{VF}, \tag{1}$$

where $V$ is the outer segment volume, $F$ is the Faraday constant, $P$ is the fraction of light-sensitive current carried by calcium, $J(t)$ is the light-sensitive current and $J_{ex}(t)$ is the current carried by the $Na^+$–$Ca^{2+}$, $K^+$ exchange. Equation (1) assumes that the exchange extrudes one $Ca^{2+}$ ion for each charge flowing in (Lagnado & McNaughton, 1991). If binding equilibrium between $[Ca^{2+}]_i$ and the exchanger is rapid, and $[Ca^{2+}]_i$ is small relative to the dissociation constant, $K_{Ca}$, of the binding then we can write:

$$J_{ex}(t) = kVF[Ca^{2+}]_i(t), \tag{2}$$

where $k$ is a constant. Then eqn (1) can be rewritten:

$$\frac{dJ_{ex}(t)}{dt} = k\frac{P}{2} J_{tot}(t) - k\left(\frac{P+2}{2}\right) J_{ex}(t) \tag{3}$$

where:

$$J_{tot}(t) = J(t) + J_{ex}(t) \tag{4}$$

is the total membrane current recorded by the suction pipette.

Initially the system expressed by eqns (1)–(4) is at steady state. Thus an initial constraint, obtained by setting $dJ_{ex}/dt$ equal to zero in (3) is:

$$J_{ex}(0) = \frac{P}{2+P} J_{tot}(0). \tag{5}$$

Equation (3) can be integrated by standard procedures to give:

$$J_{ex}(t) = \frac{P}{2+P} J_{tot}(0)\, e^{-\alpha t} + \alpha \frac{P}{2+P} J_{tot}(0)\, e^{-\alpha t} \int_0^t e^{\alpha\tau} J_{tot}(\tau)\, d\tau, \tag{6}$$

where $\alpha = k\ (P+2)/2$ is the rate constant of exponential decay of the final phase of the exchange transient. The rate constant $\alpha$ is determined by fitting $J_{tot}$ with an exponential function after all light-sensitive current has been suppressed by a bright flash. $P$ is computed by an iterative method which minimizes the difference between $J(t)$ (computed as $J_{tot}(t) - J_{ex}(t)$) and a theoretical model for the light-sensitive current (eqn (6.10) of Lamb & Pugh, 1992). This model, which was derived for the case of a voltage-clamped rod stimulated by a very brief light flash, was found to provide an excellent fit to the rising phase of the light response in rat rods, although since our rods were not voltage clamped and were stimulated by a flash of finite duration the fit must be regarded as empirical. The free parameters in eqn (6.10) of Lamb & Pugh (1992) were determined by fitting the rising phase of the light response up to half-suppression of the light-sensitive current; in this range the $Na^+$–$Ca^{2+}$, $K^+$ exchange current forms a small proportion of the total current and has little effect on the fitting procedure.

## RESULTS

### *Basic observations*

The effects of a change in temperature on the bright-flash response are summarized in Fig. 1. The responses labelled *a* to *f* show that the light-sensitive current increases as the temperature is raised from 17 to 40 °C. The light-sensitive current in this cell was 1·1 pA at room temperature (20–22 °C) and 6·5 pA at body temperature (37–39 °C), an increase of 6 times. The effects of raising the temperature were well reversed on return to room temperature (responses *g* to *j*), though a full recovery of the light-sensitive current to its initial level was not in general observed. The decline was of the order usually seen in a long experiment such as that shown in Fig. 1 even without a change in temperature.

The responses to saturating and dim flashes of light delivered at 20·0 °C and 38 °C are shown in Fig. 2. As the temperature is increased both the time to peak and the duration of the light response decrease. The absolute amplitude of the dim-flash response (flash intensity 2·7 $\Phi\ \mu m^{-2}$ at both temperatures) increases with temperature, but the amplitude relative to the size of the light-sensitive current decreases by a factor of 1·4. The sensitivity therefore decreases with temperature when the changes in light-sensitive current are taken into account.

### *The effect of temperature on the light-sensitive current*

In six cells the average light-sensitive currents were $1{\cdot}5 \pm 0{\cdot}24$ and $5{\cdot}9 \pm 1{\cdot}13$ pA (means ± S.D.) at room and body temperature respectively, an average increase of approximately 4 times. This effect of temperature is plotted in Fig. 3 on both linear (*A*) and Arrhenius (*B*) co-ordinates. In the Arrhenius plot the current of each cell at different temperatures has been normalized by the value of the light-sensitive current at 30 °C to compensate for differences in current between cells. The average temperature coefficient ($Q_{10}$) for the change in light-sensitive current was $2{\cdot}47 \pm 0{\cdot}35$ (mean ± S.D.).

### *The effect of temperature on the $Na^+$–$Ca^{2+}$, $K^+$ exchange*

The relaxation visible on the plateau of the bright-flash response (see Fig. 2) has been demonstrated to be due to the operation of an electrogenic $Na^+$–$Ca^{2+}$, $K^+$ exchange which reduces $[Ca^{2+}]_i$ after a bright flash (Yau & Nakatani, 1985; McNaughton, Cervetto & Nunn, 1986; Hodgkin, McNaughton & Nunn 1987; Tamura, Nakatani & Yau, 1991).

Figure 4 shows the relaxation in $Na^+$–$Ca^{2+}$, $K^+$ exchange current after a bright flash at temperatures of 19·5 and 38·0 °C. At both temperatures a single exponential fits the relaxation of exchange current after all light-sensitive current has been suppressed. Changes in temperature had little effect on the time constant of decay;

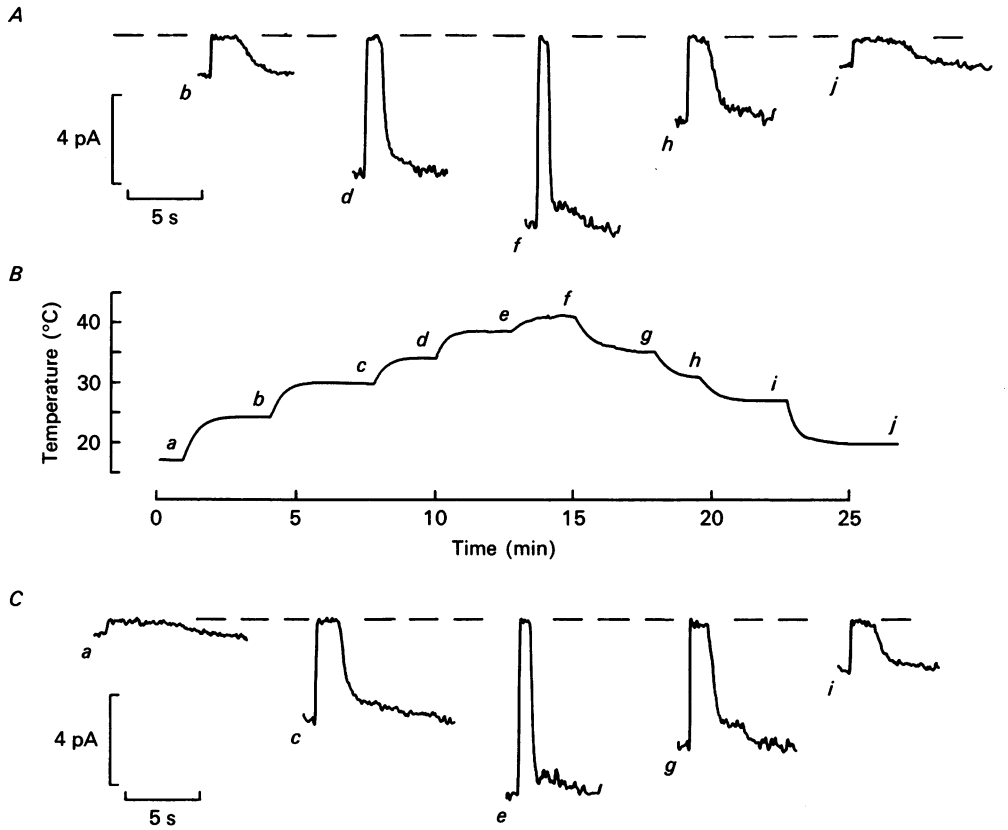


Fig. 1. The effect of temperature change on the light-sensitive current. *B*, the temperature changes during the recording. The traces labelled *a*, *b*, *c*, *d*, *e* and *f* are the average responses to saturating flashes of light at different increasing temperatures, whilst traces *g*, *h*, *i* and *j* are recorded during a decrease in temperature. The light-sensitive current at the various temperatures is plotted, along with that of five other cells in which a similar protocol was adopted, in Fig. 3. The light intensities ($\Phi$ $\mu m^{-2}$ $flash^{-1}$) and number of sweeps employed for each set of recordings are: *a*, 540 ($n = 4$); *b*, 1100 (4); *c*, 4600 (6); *d*, 4600 (4); *e*, 8900 (3); *f*, 8900 (3); *g*, 8900 (4); *h*, 4600 (2); *i*, 1100 (4); *j*, 1100 (4). The traces are all plotted relative to the maximum light-sensitive current with inward current represented as downwards. All traces have been filtered at a bandwidth of 20 Hz.

in five cells the time constant was $111 \pm 31$ ms (mean $\pm$ S.D.) at 20 °C and $102 \pm 29$ ms at 38 °C. The turnover rate of the exchange would be expected to increase with temperature, which would cause a shortening of the time constant at higher temperature. A possible explanation for the lack of an observed effect on the time constant is that the increase in light-sensitive current as temperature is raised causes

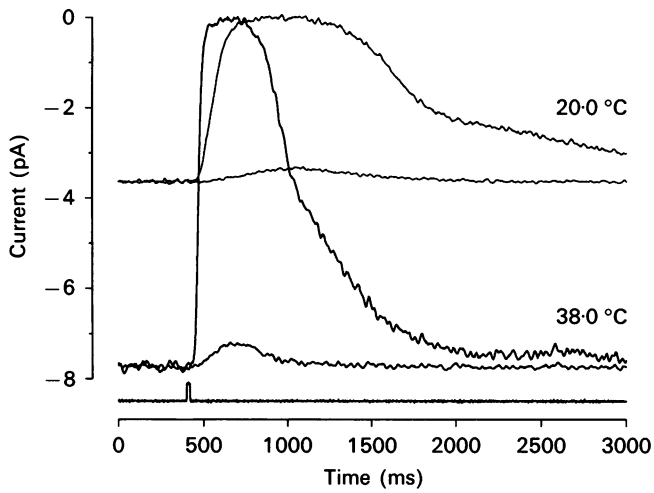


Fig. 2. Average responses to saturating flashes of 214 and 564 $\Phi\ \mu m^{-2}$ ($n = 19, 16$) and to dim flashes of 2·7 $\Phi\ \mu m^{-2}$, delivered as indicated by the lower trace, at 20·0 and 38·0 °C ($n = 78, 80$), respectively. The membrane current of the outer segment is represented relative to the level in darkness with inward current plotted downwards. The light-sensitive current was 3·65 and 7·74 pA and the responses to flashes delivering 2·7 $\Phi\ \mu m^{-2}$ were 0·32 (9%) and 0·52 pA (7%) at 20 and 38 °C, respectively (percentage of the light-sensitive current in parentheses). Recordings were filtered at 30 Hz. This cell is referred to as cell 4 in the following figures and tables.

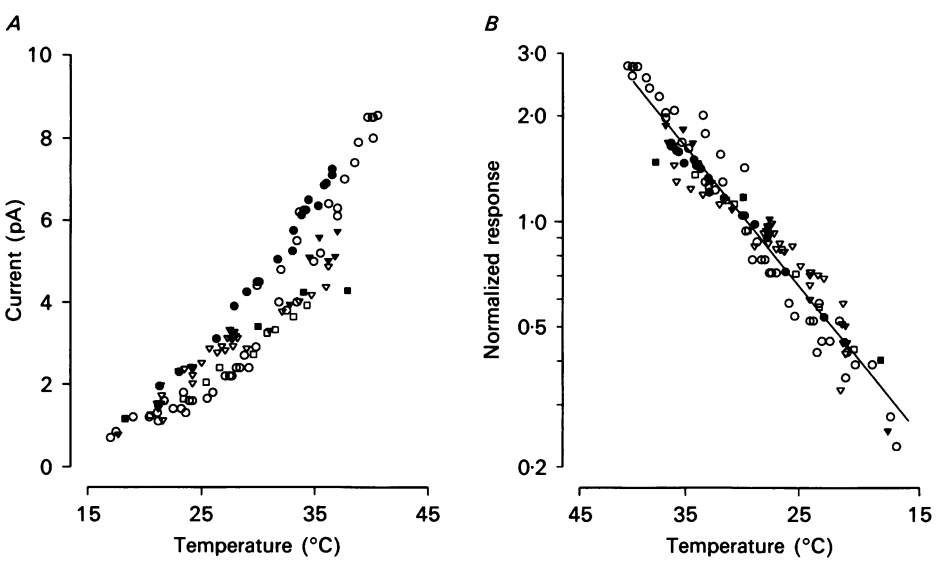


Fig. 3. Effect of a continuous change of temperature on the light-sensitive current for six cells in which a similar protocol to that described in Fig. 1 was used. *A* is a linear plot while *B* is the Arrhenius plot; note that the abscissa in *B* is linear in $1/T$, where $T$ is the absolute temperature, but has for convenience been reconverted to °C. In *B*, the responses of each cell, at the various temperatures, were normalized to the value of the light-sensitive current in that cell at 30 °C. Each cell is represented by the same symbol in both *A* and *B*. Regression line in *B* corresponds to a temperature coefficient ($Q_{10}$) of 2·47.

a rise in $[Na^+]_i$ and an inhibition of the $Na^+$–$Ca^{2+}$, $K^+$ exchange sufficient to cancel out the increase in turnover rate of the exchange. Another possibility is that the properties of the intracellular calcium buffer may be altered by temperature (see eqn (15) of Lagnado, Cervetto & McNaughton, 1992).

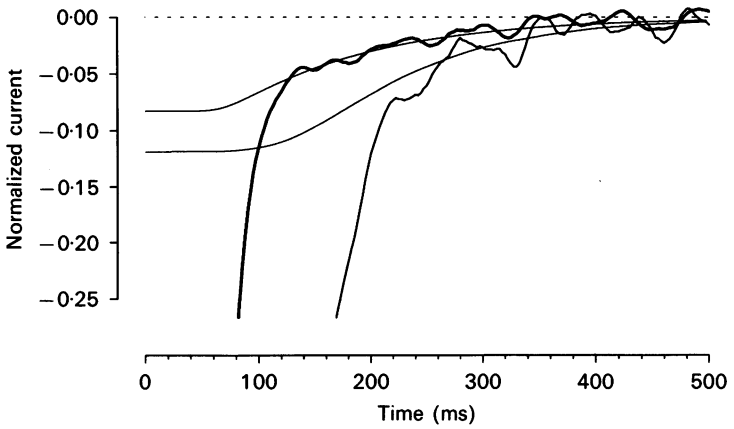


Fig. 4. Effect of temperature on the $Na^+$–$Ca^{2+}$, $K^+$ exchange current. Experimental traces are the responses to saturating flashes of 203 and 527 $\Phi$ $\mu m^{-2}$ recorded at 19·5 °C (thinner traces, $n = 41$) and 38·0 °C (thicker traces, $n = 28$). At body temperature the flash was followed by a weaker (700 ms, 2500 $\Phi$ $\mu m^{-2}$ $s^{-2}$) steady background to keep the light-sensitive conductance closed. The membrane current of the outer segment is represented relative to the level in darkness, with inward current plotted downwards. Light flash (20 ms) was delivered at $t = 0$. Smooth traces represent the exchange currents $J_{ex}(t)$ calculated as described in Methods.

The entire time course of decay of the $Na^+$–$Ca^{2+}$, $K^+$ exchange current after a flash, $J_{ex}(t)$, can be obtained by fitting eqn (6) to the response to a bright flash (see Methods). The exchange currents obtained from eqn (6) are shown in Fig. 4 for responses recorded at both room and body temperature. The initial amplitude of the exchange current in this cell was 11·9% of the dark current at room temperature and 8·3% at body temperature. In five cells the initial amplitude of the exchange current was $9·4 \pm 2·3$ and $5·2 \pm 2·4$% at room and body temperature, respectively (means $\pm$ S.D.). The difference between the two set of values is highly significant ($P = 0·027$, Student's paired $t$ test). Since one charge enters the cell for every $Ca^{2+}$ ion extruded by the $Na^+$–$Ca^{2+}$, $K^+$ exchange, the magnitude of the exchange current in the steady state is equal to half the inward current carried by calcium through the light-sensitive conductance. Thus the observation that the amplitude of the exchange current declines as a fraction of the light-sensitive current at higher temperatures shows that a smaller fraction of the light-sensitive current is carried by $Ca^{2+}$ as the temperature rises.

The above calculation ignores the effect on the exchange current of the hyperpolarization caused by the suppression of the light-sensitive current. Hyperpolarization increases the initial amplitude of the exchange current, causing an overestimate of the contribution of calcium current to the light-sensitive current

(Lagnado *et al.* 1992). This effect will be larger at higher temperatures where the light-sensitive current is greater. When the effect of hyperpolarization is taken into account, therefore, the difference between the fraction of light-sensitive current carried by calcium at room and body temperatures will be even more pronounced.

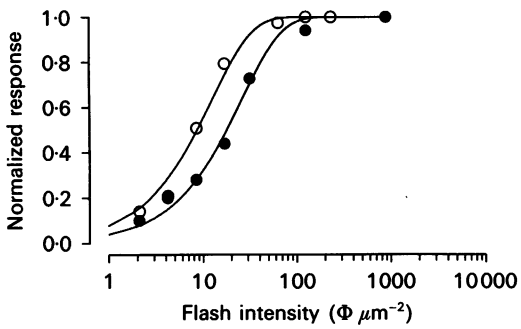


Fig. 5. Intensity–response relationships for a cell at two different temperatures. Abscissa gives flash intensities ($\Phi\ \mu m^{-2}$) on a logarithmic scale. Continuous curves show eqn (7) fitted to the data by a minimization routine. Half-saturating intensities are: 9·4 $\Phi\ \mu m^{-2}$ (20·0 °C, ○), 18·2 $\Phi\ \mu m^{-2}$ (30·0 °C, ●).

*The effect of temperature on the flash sensitivity*

Flash response amplitudes as a function of flash intensity, $I$ (see Fig. 5) were found to be well fitted at all temperatures by a saturating exponential function of the form (see Lamb *et al.* 1981):

$$\frac{R}{R_{max}} = 1 - e^{-\beta I}. \tag{7}$$

The constant $\beta$ is related to the light intensity required to obtain a half-saturating response:

$$i_0 = \frac{\ln(2{\cdot}0)}{\beta}, \tag{8}$$

where the $i_0$, the half-saturating flash intensity, is a useful measure of sensitivity in the present study as it is unaffected by changes in the amplitude of the light-sensitive current.

In all experiments a decrease in sensitivity with temperature was observed, but the magnitude of this effect varied from cell to cell. Collected results from eleven cells are shown in Fig. 6. The mean value of $i_0$ from eight cells recorded in the temperature range 19–23 °C was $11{\cdot}4 \pm 6{\cdot}1\ \Phi\ \mu m^{-2}$ (mean ± S.D.), and in the same eight cells recorded at over 36 °C the $i_0$ was $30{\cdot}0 \pm 15{\cdot}6\ \Phi\ \mu m^{-2}$. The average $Q_{10}$ of $i_0$ in all the cells shown in Fig. 6 was 1·94 ± 0·89. In one rod an abnormally high $Q_{10}$ of 4·6 was recorded; if this value is discarded the $Q_{10}$ of $i_0$ in the remaining rods is 1·68 ± 0·28.

*Responses to single isomerizations*

A series of responses to dim flashes of light delivered at room temperature is shown in Fig. 7*A*. The responses clearly exhibit the quantal nature described by Baylor and colleagues (1979, 1984).

The amplitude histogram for the complete experiment, obtained as described in Methods, is shown in Fig. 7*B*, and clearly exhibits three peaks, associated with failures and with one and two photoisomerizations. The smooth curve has been obtained as the sum of three Gaussian functions, fitted to the histogram as described

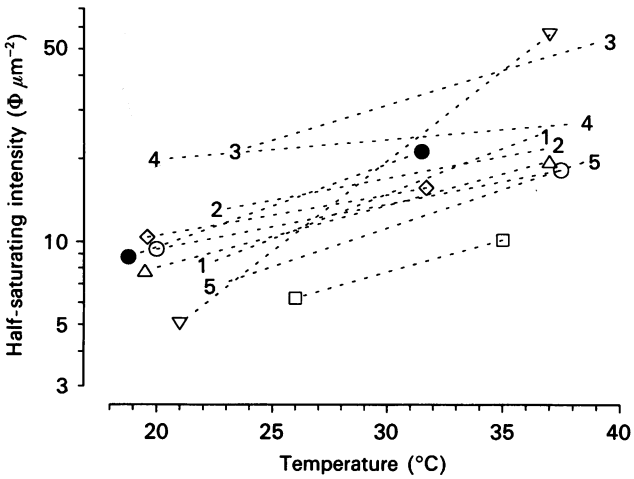


Fig. 6. The effect of temperature on the light intensity required to give a half-saturating response in eleven cells. On the ordinate is plotted, on a logarithmic scale, the half-saturating intensity calculated from the intensity–response relation as described in the legend to Fig. 5. The cells represented by symbols (excepting △) along with the one labelled 5 are all from adult albino rats whereas the remainder are from adult pigmented rats. Data from the cells labelled from 1 to 5 in this and following figures are shown in Table 1. The dotted lines connect the sensitivities observed at different temperatures in the same cell and are not intended to represent any particular function. The measurements of sensitivity from the cells represented by the open symbols and those labelled 1, 2 and 5 have been obtained first at room and then at body temperature. The sensitivity of the cell represented by ● was measured at room temperature, 31·5 °C and room temperature again. The final sensitivity at room temperature was within the symbol diameter of the initial value.

by Baylor and colleagues (1979, 1984; see Methods). The mean and variance of the Gaussian describing failures is obtained from interleaved blank trials (see inset). The amplitude of the single-photon response in this experiment, obtained from the mean of the second peak, was 0·71 pA. The amplitude of the response to two photoisomerizations was 1·28 pA, less than twice the amplitude of the single-photon response, as expected from the exponential compression function relating response to flash strength (eqn (7)).

The variance of the single-photon response, $\sigma_1^2$, is equal to the difference between the variances of the first and second peaks. The mean $\sigma_1$ from the four best experiments at room temperature (see Fig. 8 below) was 0·06 pA, or 11% of the single-photon response amplitude, and at body temperature was 0·12 pA, or 17% of the single-photon response. Corresponding figures for toad at room temperature and monkey at body temperature are 20 and 28% respectively (Baylor *et al.* 1979, 1984).

From the amplitude histogram the sweeps corresponding to zero, one and two

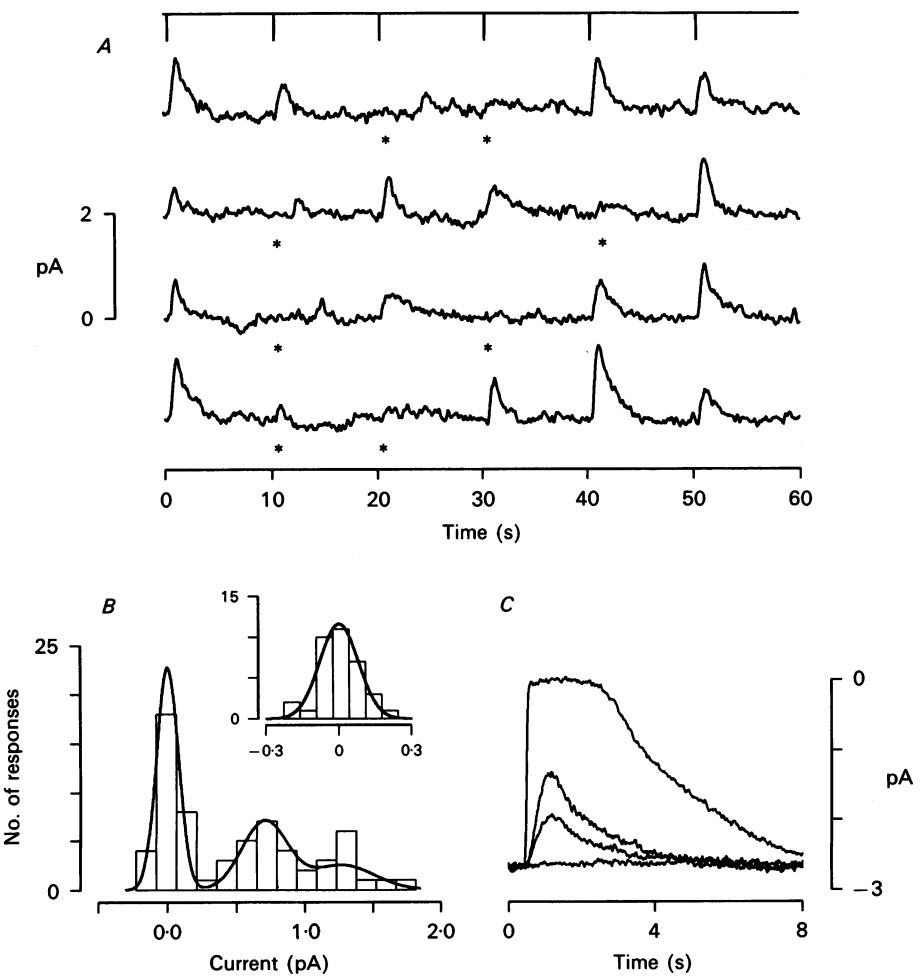


Fig. 7. *A*, responses of an albino rat rod, at 21·6 °C, to twenty-four dim flashes (3·5 $\Phi$ $\mu m^{-2}$), delivered as indicated by the flash train at the top of the figure, illustrating the quantal nature of the light response. The responses have been filtered at 30 Hz and digitally smoothed with 75 ms averaging window. From the amplitude histogram for this rod a criterion level of 0·29 pA was set to distinguish failures from responses to one or more photoisomerizations (see *B*); flashes that have failed to elicit a response are marked with an asterisk. *B*, amplitude histogram of all responses from rod shown in *A*. Response amplitudes determined as described in Methods. Inset shows histogram of interleaved trials in which no flash was delivered, used to determine standard deviation of 'failures' peak in main histogram. Continuous curve in main histogram is the sum of three Gaussian functions with amplitudes, means and standard deviations calculated as described in Methods. *C*, mean responses to zero, one and two photoisomerizations shown with response to a saturating flash. Traces computed by averaging all responses falling between the following criterion levels, determined from the amplitude histogram: failures, < 0·29 pA; one photoisomerization, from 0·29 to 1·01 pA; two photoisomerizations, from 1·01 to 2·0 pA.

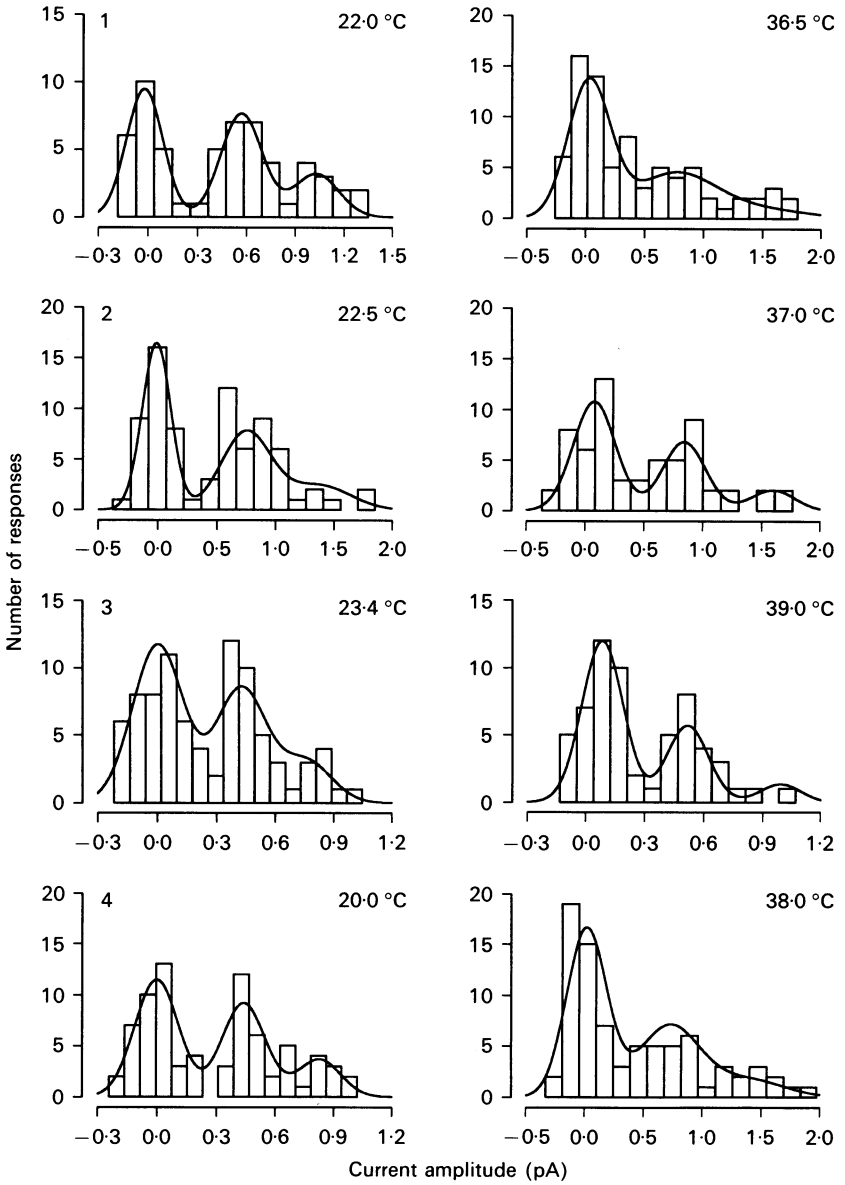


Fig. 8. Amplitude histograms of dim-flash responses from four cells, recorded at the indicated temperatures, and fitted with a sum of three Gaussians (see Methods). The means of the Gaussians give the peak current change produced by zero, one and two photoisomerizations, and the values are reported in Table 1. Each pair of histograms has been obtained from the same cell. Cells 1 and 2 were recorded at room temperature and then at body temperature, while cells 3 and 4 were recorded first at body temperature then at room temperature. Note that in each case the proportion of failures is higher at body temperature.

photoisomerizations can be extracted. The averages of these sweeps are shown in Fig. 7*C*.

*Effect of temperature on the response to a single photoisomerization*

In five experiments dim-flash responses have been successfully recorded from the same cell at room and body temperature. The amplitude histograms from four of these cells are shown in Fig. 8.

TABLE 1. Relationship between dim flash response statistics and sensitivity

| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell | $T^R$ (°C) | $J_\Phi^R$ (pA (%)) | $P_0^R$ | $i_0^R$ ($\Phi\ \mu m^{-2}$) | $T^B$ (°C) | $J_\Phi^B$ (pA (%)) | $P_0^B$ | $i_0^B$ ($\Phi\ \mu m^{-2}$) | $\frac{J_\Phi^B}{J_\Phi^R}$ | $\frac{J_\Phi^B}{J_\Phi^R}\frac{m^B}{m^R}$ | $\frac{i_0^R}{i_0^B}$ |
| 1 | 22·0 | 0·57 (19·5) | 0·40 | 8·15 | 36·5 | 0·75 (10·3) | 0·55 | 24·5 | 0·53 | 0·34 | 0·33 |
| 2 | 22·5 | 0·75 (17·2) | 0·44 | 12·9 | 37·0 | 0·84 (10·6) | 0·53 | 21·9 | 0·62 | 0·48 | 0·59 |
| 3 | 23·4 | 0·43 (24·2) | 0·48 | 17·2 | 39·0 | 0·52 (8·53) | 0·62 | 58·3 | 0·35 | 0·24 | 0·30 |
| 4 | 20·0 | 0·44 (12·1) | 0·45 | 19·8 | 38·0 | 0·72 (9·30) | 0·51 | 27·0 | 0·77 | 0·58 | 0·73 |
| 5 | 22·6 | 0·48 (22·6) | 0·45 | 6·90 | 38·5 | 0·56 (11·2) | 0·49 | 19·5 | 0·50 | 0·45 | 0·35 |
| Mean | 22·1 | 0·53 (19·1) | | | 37·8 | 0·68 (10·0) | | | 0·55 | 0·42 | 0·46 |

$J_\Phi$, absolute size of the single photon response (pA), percentage of the saturating response given in parentheses; $P_0$, failure probability; $i_0$, half-saturating flash intensity. The superscripts 'R' and 'B' refer to room and body temperature, respectively. Column 10 gives the ratio between the relative sizes of the single-photon response. Column 11 gives the expected change in sensitivity taking into account the change in single-photon response and the change in capture efficiency ($m$ is the mean number of photoisomerizations per flash). Column 12 gives the observed change in sensitivity (ratio between columns 5 and 9). Cells numbered 1, 2 and 5 were recorded at room temperature first followed by body temperature and the cells numbered 3 and 4 were recorded at body temperature first.

The average single-photon response size calculated from the five cells, at room temperature, was $0{\cdot}53 \pm 0{\cdot}13$ pA (mean $\pm$ S.D.) or $19{\cdot}1 \pm 4{\cdot}8$% of the light-sensitive current. The single-photon response size calculated in the same cells at body temperature was $0{\cdot}68 \pm 0{\cdot}13$ pA (mean $\pm$ S.D.) or $10{\cdot}0 \pm 1{\cdot}1$% of the light-sensitive current. The mean relative size of the single-photon response therefore changed by a factor of 0·55 over this temperature range, while the sensitivity changed by a factor of 0·46 in the same rods (see columns 10 and 12 in Table 1). The difference could be accounted for by a fall in the efficiency with which incident photons are converted into quantal events. This possibility has been investigated further by counting the proportion of failures in response to a train of dim flashes. The effect of temperature on the failure probability is shown in Fig. 9 for the five cells. The change in failure probability was highly significant ($P = 0{\cdot}011$, Student's paired $t$ test). The change in failure probability together with the change in single event size fully accounts for the change in sensitivity (last two columns in Table 1).

Careful precautions have been taken to exclude possible artifacts which might account for the decrease in photon capture efficiency at higher temperatures. The possibility that the flash intensity may be reduced by an elevation in temperature, perhaps because of a change in the transparency of the medium or condensation on the lenses, has been investigated by measuring the intensity of each flash during the

course of an experiment with a photomultiplier mounted in place of the binocular head on the inverted microscope (see details in Methods). No change in light intensity related to a change in temperature was observed over the temperature range 18–40 °C. A small and progressive reduction in light intensity was observed during

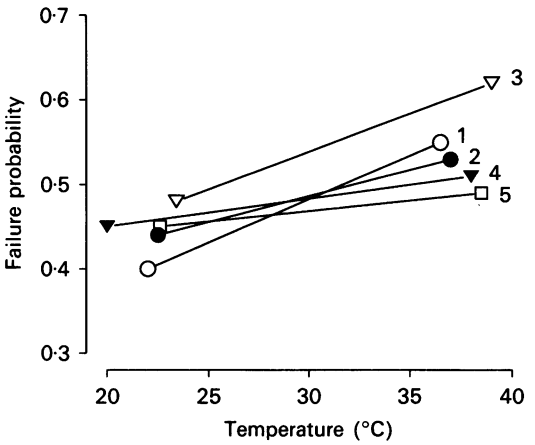


Fig. 9. The effect of temperature on the probability of failure of response to a dim flash. The numbers refer to the cells in Table 1 and Figs 6 and 8. The symbols have been joined arbitrarily by straight lines.

the course of the experiment, irrespective of changes in temperature, but did not exceed 5% over a period of 2 h. The possibility that this change in light intensity might account for the apparent decrease in capture efficiency, or that the results may be affected by a progressive change in cell condition, was investigated by comparing experiments in which the temperature was lowered with others in which it was raised. In all experiments (see Table 1 and Figs 8 and 9) an increase in capture probability was observed at the lower temperature. The magnitude of the change was similar in all cells, irrespective of the direction of change of temperature (see legend to Table 1).

The collecting area of the rod outer segment, $A_c$ ($\mu m^2$), can be calculated from the amplitude histograms by dividing the mean number of photoisomerizations per flash by the photon density of the flash. In five cells the average effective collecting areas at room and body temperature were $0{\cdot}30 \pm 0{\cdot}09$ and $0{\cdot}22 \pm 0{\cdot}07$ $\mu m^2$ respectively (means $\pm$ S.D.). Note that these values were obtained with unpolarized light incident normal to the long axis of the outer segment, and that values approximately two times larger would be obtained either with light polarized at right angles to the outer segment or with axial stimulation. An estimate for the axial pigment density, $\alpha$, can be obtained from the following approximate relation (Baylor *et al.* 1979, eqn (14)), valid when self-screening is negligible (i.e. $2{\cdot}303\,\alpha d \ll 1$):

$$A_c = \frac{\pi d^2 l}{4} Q f 2{\cdot}303\,\alpha, \tag{9}$$

where $A_c$ is the effective collecting area, $l$ is the length of the outer segment, $d$ is the diameter of the outer segment and $Q$ is the quantum efficiency for isomerization of

rhodopsin. For unpolarized light incident normal to the axis of the outer segment the factor $f$ is 0·5 to allow for the failure of rhodopsin to absorb light polarized parallel to the long axis of the outer segment. Using values of $l = 25{\cdot}3\ \mu m$, $d = 1{\cdot}52\ \mu m$, $Q = 0{\cdot}67$ (Dartnall, 1972) and $A_c = 0{\cdot}3\ \mu m^2$ (see above), the axial pigment density for the

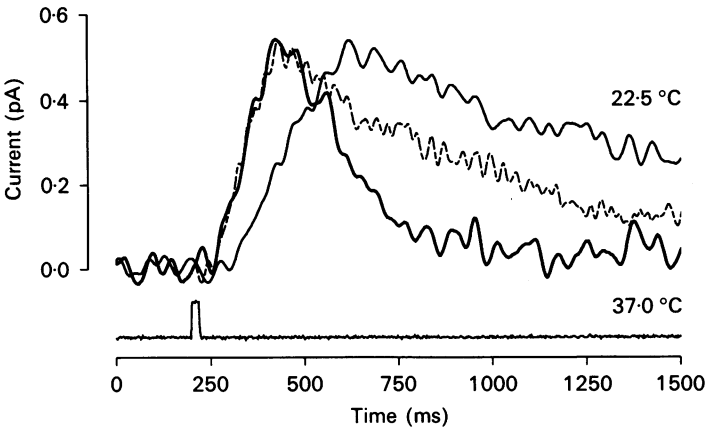


Fig. 10. The effect of temperature on the kinetics of the dim-flash response. Continuous lines show two average responses of similar amplitude (0·50 pA), to a flash which delivered 2·26 $\Phi\ \mu m^{-2}$ at 22·5 °C and 37 °C. The dashed line represents the response at 22 °C scaled in time to have the same time to half-peak as the body temperature response. The scaling factor used was 1·85. The traces are averages of seventy-three and sixty individual responses recorded at 22·5 °C and 37 °C, respectively, and digitally smoothed with a 12·5 ms averaging window. The responses are obtained from cell 2 of Table 1.

rat rod at room temperature was 0·0085 $\mu m^{-1}$. This value is comparable to, but somewhat smaller than, the values of 0·0106–0·0188 $\mu m^{-1}$ obtained in amphibian rods by spectroscopic analysis (Harosi, 1975).

*Time course of the flash response*

Lamb (1984) found that in the salamander the light responses at different temperatures could be superimposed by a simple scaling of the time axis. Such simple behaviour was not observed in rat rods, as shown in Fig. 10.

A temperature increase accelerates the rising phase of the light response less markedly than the falling phase, and it is therefore not in general possible to superimpose a response at room temperature on a response obtained at body temperature by simply contracting the time scale. The activation energies of the elements of the light-sensitive cascade contributing to the falling phase must therefore be greater than those contributing to the rising phase.

## DISCUSSION

*Amplitude of the light-sensitive current*

Over the temperature range 17–40 °C we found that the $Q_{10}$ of the light-sensitive current was 2·47 ± 0·35 (mean ± S.D.), compared with values in the toad rod of 1·8 reported by Baylor *et al.* (1983) and 2·1 by Lamb (1984) over a temperature range

15–25 °C. The $Q_{10}$ for the $Na^+$ channel is 1·6 (Chandler & Meves, 1970), so our value of 2·47 suggests that the increase in light-sensitive current is due to a recruitment of more channels as temperature rises. The absolute size of the single photon response changes by only about 20% over the temperature range 18–38 °C (see p. 14), corresponding to a $Q_{10}$ of 1·1. These observations suggest that the gain (the number of channels shut by a single photoisomerization) changes relatively little with temperature, but that more channels are open in darkness at higher temperatures.

*Dependence of $Na^+$–$Ca^{2+}$, $K^+$ exchange on temperature*

Raising the temperature from 18 to 38 °C had surprisingly little effect on the time constant of the $Na^+$–$Ca^{2+}$, $K^+$ exchange current relaxation after a bright flash, in spite of the 4·5-fold increase of the light-sensitive current. The initial amplitude of the exchange current and the amount of charge transferred during the relaxation were also little affected by temperature. Since the calcium current in darkness is approximately equal to twice the initial amplitude of the exchange current it seems clear that the *relative* contribution of calcium to the total current is lower at body temperature, implying that the permeability to $Ca^{2+}$ of the light-sensitive channel decreases with temperature.

The fall in free $[Ca^{2+}]_i$ after a flash is proportional to the charge transferred by the exchange, provided that the properties of calcium buffering are unaffected by changes in temperature. From the observation that the charge transferred remains constant it seems likely that free $[Ca^{2+}]_i$ is little affected by temperature in spite of the substantial increase of light-sensitive current. The maintenance of a constant free $[Ca^{2+}]_i$ may be important in amphibian rods, which are subjected to a wide range of temperature changes.

The relaxation of the $Na^+$–$Ca^{2+}$, $K^+$ exchange is much more rapid in rat rods than in amphibian rods, which may be due to the difference in size. From eqn (15) of Lagnado *et al.* (1992) it can be shown that if the properties of intracellular calcium buffering and both the activity of $Na^+$–$Ca^{2+}$, $K^+$ exchange molecules and their density per unit membrane area are constant then the time constant of relaxation of $[Ca^{2+}]_i$ after a bright flash is proportional to the outer segment diameter. In salamander rods the time constant of relaxation of the exchange current is 700 ms (Lagnado *et al.* 1992) and the outer segment diameter of 11 $\mu$m is 7 times larger than the value of 1·5 $\mu$m in rats, where the time constant is about 100 ms (see p. 469). It appears therefore that the different time constants in different species may be largely accounted for by differences in outer segment diameter.

*Sensitivity*

The value of the axial pigment density of 0·0085 $\mu m^{-1}$ at room temperature obtained in the present study is about 2 times smaller than the value of 0·016 $\mu m^{-1}$ found for red rods of the toad, *Bufo marinus*, but even within amphibian species the pigment density varies by as much as 70% (Harosi, 1975). The collecting area determined for rat rods using unpolarized illumination was 0·22 $\mu m^2$ at body temperature, or approximately 0·44 $\mu m^2$ for polarized illumination (see p. 477), compared with the value of 1·4 $\mu m^2$ obtained by similar methods using polarized illumination in the rods of the Macaque monkey (Baylor *et al.* 1984, p. 589).

The light intensity required to give a half-saturating response increased from a value of 11·4 $\Phi$ $\mu m^{-2}$ at room temperature to 30·0 $\Phi$ $\mu m^{-2}$ at body temperature (see Fig. 6). The decrease in sensitivity can only arise from two mechanisms: a decrease either in the size of the single photon response as a fraction of the light-sensitive current, or in the efficiency with which photons are captured and converted to a signal by the rhodopsin molecule (the capture efficiency). Both mechanisms in fact contribute to some extent. In five rods in which a full analysis was carried out the relative size of the single photon event changed by a factor of 0·55 between room and body temperature, which is insufficient to account fully for the sensitivity change of 0·46-fold. The capture efficiency was evaluated by counting the number of failures in response to a flash of constant intensity at the two temperatures. The resulting probability that a flash will fail to elicit a response, $P_0$, is plotted in Fig. 9 and shows that in all the cells the capture efficiency decreases at body temperature. The overall sensitivity change can be fully accounted for by the combined effect of the change in size of the single photon response and quantum efficiency (Table 1).

Baylor *et al.* (1983) have also investigated the possibility of a change in capture efficiency with temperature. They found no effect over a more limited temperature range and with a smaller number of flash trials than in the present study, although they do note that an effect could not be ruled out. The present study shows that capture efficiency clearly does decrease with elevations in temperature.

The change in capture efficiency may be due either to a decrease in the efficiency of absorption of photons or in the probability of an isomerization occurring after a photon has been absorbed (the quantum efficiency). The normal quantum efficiency of 0·67 (Dartnall, 1972) may be reduced as the temperature is elevated because the forward reaction rate from photoactivated state to the production of a single-photon event and the reverse reaction rate from photoactivated to dark state have different temperature dependencies. Raising the temperature may affect these two rates independently in such a way as to increase the fraction of rhodopsin molecules that return to the unisomerized state after a flash. An alternative explanation could be that the quantum efficiency of rhodopsin is regulated by a cytosolic factor and that changing the temperature modifies the quantum efficiency via this factor. Recent results (Barlow, Birge & Kaplan, 1991) indicate that pH regulates the rhodopsin stability in the *Limulus* ventral photoreceptor. Temperature-dependent changes in intracellular pH could be responsible for the observed changes in quantum efficiency which we have observed in rat rods.

We would like to thank Luigi Cervetto and Mike Lunn for their participation in early experiments and Richard Perry and Trevor Lamb for helpful discussions. This work was sponsored by the MRC, the EEC and NATO.

## REFERENCES

BARLOW, R. B., BIRGE, R. R. & KAPLAN, E. (1991). Photoreceptor noise: where does it come from? *Journal of General Physiology* **98**, 15a.

BAYLOR, D. A. & HODGKIN, A. L. (1973). Detection and resolution of visual stimuli by turtle photoreceptors. *Journal of Physiology* **234**, 163–198.

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

BAYLOR, D. A., MATTHEWS, G. & YAU, K.-W. (1983). Temperature effects on the membrane current of retinal rods of the toad. *Journal of Physiology* **337**, 723–734.

BAYLOR, D. A., NUNN, B. J. & SCHNAPF, J. L. (1984). The photocurrent, noise and spectral sensitivity of rods of the monkey *Macaca fascicularis*. *Journal of Physiology* **357**, 575–607.

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

DARTNALL, H. J. A. (1972). Photosensitivity. In *Photochemistry of Vision*, ed. DARTNALL, H. J. A., pp. 122–145. Springer, New York.

HAROSI, F. I. (1975). Absorption spectra and linear dichroism of some amphibian photoreceptors. *Journal of General Physiology* **66**, 357–382.

HODGKIN, A. L., MCNAUGHTON, P. A. & NUNN, B. J. (1985). The ionic selectivity and calcium dependence of the light-sensitive pathway in toad rods. *Journal of Physiology* **358**, 447–468.

HODGKIN, A. L., MCNAUGHTON, P. A. & NUNN, B. J. (1987). Measurement of sodium–calcium exchange in salamander rods. *Journal of Physiology* **391**, 347–370.

LAGNADO, L., CERVETTO, L. & MCNAUGHTON, P. A. (1992). Calcium homeostasis in the outer segments of retinal rods from the tiger salamander. *Journal of Physiology* **455**, 111–142.

LAGNADO, L. & MCNAUGHTON, P. A. (1991). Net charge transport during sodium dependent calcium extrusion in isolated salamander rod outer segments. *Journal of General Physiology* **98**, 479–495.

LAMB, T. D. (1984). Effects of temperature changes on toad rod photocurrents. *Journal of Physiology* **346**, 557–578.

LAMB, T. D., MCNAUGHTON, P. A. & YAU, K.-W. (1981). Longitudinal spread of activation and background desensitization in toad rod outer segments. *Journal of Physiology* **319**, 463–496.

LAMB, T. D. & PUGH, E. N. JR (1992). A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. *Journal of Physiology* **449**, 719–758.

MCNAUGHTON, P. A., CERVETTO, L. & NUNN, B. J. (1986). Measurement of the intracellular free calcium concentration in salamander rods. *Nature* **322**, 261–263.

RATTO, G. M., ROBINSON, D. W., YAN, B. & MCNAUGHTON, P. A. (1991). Development of the light response in neonatal mammalian rods. *Nature* **351**, 654–657.

TAMURA, T., NAKATANI, K. & YAU, K.-W. (1991). Calcium feedback and sensitivity regulation in primate rods. *Journal of General Physiology* **98**, 95–130.

YAU, K.-W. & NAKATANI, K. (1985). Light induced reduction of cytoplasmic free calcium in retinal rod outer segment. *Nature* **313**, 352–354.