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Light adaptation and temperature effects in rat PIII retinal response: Analysis with a two-state model

(visual adaptation/temperature effects/operating functions/stimulus–response/retinal potentials)

JOHN G. GALE* AND THEODORE P. WILLIAMS†

†Institute of Molecular Biophysics and Department of Biological Science, The Florida State University, Tallahassee, Florida 32306; and *Department of Pediatrics, Health Center, University of Florida, Gainesville, Florida 32601

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ABSTRACT **Aspartate-isolated PIII responses from excised perfused retinas of albino rats were studied, with emphasis on background adaptation and temperature effects. When responses are measured at their peaks, it is seen that the proportion of response elicited by the background is equal to the proportion by which a test-flash response is suppressed. This supports the notion that rat PIII, although a complex response, seems to approximate a simple two-state ("compression") system and that it is therefore subject to analysis according to our recently proposed model. This model predicts that (*i*) responses from two-state systems should increase with decreasing temperature within limits; (*ii*) σ, the semisaturation constant of voltage–log intensity functions should shift to lower intensities with decreasing temperature; and (*iii*) the exact magnitude of the σ shift should follow the temperature dependence of the rate of rapid "neural" adaptation of the response. All of these predictions are verified for rat PIII. This suggests that rat PIII, although produced by at least two cell types, is being controlled by only two processes: a light-driven excitation and a rapid first-order "neural" dark adaptation.**

In an earlier paper (1) we presented a simple theoretical model from which the hyperbolic dependence of retinal responses on intensity was derived. Naka and Rushton (2) provided this equation as an empirical best fit for the V–log I function found in their study of goldfish horizontal cells (V, voltage response; I, light intensity). Since then, the hyperbolic equation has proved applicable to many V–log I curves of other retinal cells, including the photoreceptors of many species (3–6) and certain nonneural elements (7, 8).

The postulate of our earlier paper was that a response-generating mechanism, whose output is given by the hyperbolic equation, can be formally modeled as though it were composed of just two states. Photic stimulation serves to drive a responder substrate from its inactive state to an active response-producing state. Return of responding generators to a responsive status is supposed to be a thermal process, namely, "neural" dark adaptation. The model is symbolically represented as

$$A \underset{k_2}{\overset{k_1 \cdot I}{\rightleftharpoons}} B,$$

in which A and B are the two states of the response substrate, k_1 is the photosensitivity of the response generator (some undetermined volume element), and k_2 is the thermal rate constant for dark recovery. A "generator" is some volume element, capable of absorbing photons (it therefore contains one or more photoreceptors) and producing the response in question. This scheme not only results in the hyperbolic function, it also provides a description of a simple "compression" system. That is, the proportion of the maximum response being "used" by a given background light is exactly equal to the proportion *unavailable* to further stimulation by a saturating flash.

The rate of response production is

$$\frac{dB}{dt} = k_1 I A - k_2 B.$$

At the maximum of the response waveform $dB/dt = 0$. Under this constraint,

$$k_1 I A = k_2 B$$

and, if the total number of generators is A_o, then $A_o = A + B$. Whence,

$$k_1 I(A_o - B) = k_2 B$$

and

$$\frac{B}{A_o} = \frac{I}{I + (k_2/k_1)}.$$

This is identical to the Naka–Rushton formulation for V–log I functions because B is proportional to V, A_o to V_{max}, and (k_2/k_1) can be identified with σ, the semisaturation constant.

Naka and Rushton presented this equation *ad hoc* and called it a hyperbolic tangent. (That it is not equivalent to a hyperbolic tangent is shown in the *Appendix*.) However, the function is one limb of a rectangular hyperbola and in the parlance of biochemistry and physiology, it is the Michaelis–Menten equation. Those familiar with the Michaelis–Menten equation of enzyme kinetics recognize that K_m of that equation is the analog of σ. K_m has units of concentration and σ has units of intensity in analogous fashion. From the above two-state model, it is not immediately obvious, but it is possible to show that (k_2/k_1) has units of intensity: photons·generator^{-1}·sec^{-1} (cf. *Appendix*). In this paper we present evidence that the aspartate-isolated PIII response in excised perfused albino rat retinas conforms very well to this two-state formulation.

The PIII response arises in the distal retina (9, 10). Part of it is closely associated with receptor activity (11–14), and part may be generated by the Müller cells (8, 11). Importantly, the rat PIII also has a V–log I function of the hyperbolic form, and it closely approaches a simple compression system in its response to background light (cf. ref. 15).

Effects of temperature on the response of the preparation are also reported here. The isolated retina is ideal for manipulation of this experimental parameter. The results give additional support to the two-state character of PIII by showing that the semisaturation constant, σ, is temperature-dependent, as the model predicts. Finally, rates of rapid "neural" adaptation from short periods of light exposure were determined at various temperatures. The results show that the responsible quantity in σ-shifts is the rate of "neural" dark adaptation, k_2.

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MATERIALS AND METHODS

The PIII electrical records in this report were obtained from retinas that were removed from albino rat eyes and maintained in an isolated perfusion chamber, constructed after the design of Sickel (16). Choice of this preparation was prompted by the desire to manipulate retinal temperature and to illuminate the retina evenly, without the complication of physiological optics. The albino rat also provided a retina with a nearly homogeneous class of receptors (rods), and this helps to simplify the analysis.

Surgery and Perfusion Chamber. Adult rats were dark-adapted overnight prior to an experiment and the experimental preparation was made under dim red light. After a rat was anesthetized with halothane, its eyeball was removed by snipping the extraocular muscles. The anterior portion was sliced away and the eyecup was immersed in a dish filled with oxygenated perfusate. The retina was carefully peeled from the underlying pigment epithelium and freed by cutting the optic nerve. It was mounted in the perfusion chamber, which had been kept immersed in the dissection dish. This resulted in the retina being held between two supporting pieces of nylon mesh.

Photic Stimulator. Placement in a double-channel photic stimulator and connection of the chamber to the perfusion system and to electrical recording leads completed the preparation. The perfusion system consisted of two separate reservoirs and flowmeters, one each for the anterior and posterior spaces of the retina chamber. Each reservoir included a sintered glass disk through which oxygenation of the perfusate was accomplished. Flow was by gravity and was regulated by ball-in-tube flowmeters; in these experiments flow was 5 ml/min on each retinal side. On its way to the perfusion chamber the perfusate's temperature was regulated by traversal of glass tubing enclosed in a water jacket. The temperature of the circulating water of the jacket was controlled by a Neslab RTE-4 refrigerated bath/circulator; temperature was monitored by a thermistor probe in the perfusion chamber. Except for the bath/circulator, everything was enclosed by a metal cabinet that afforded electrical shielding.

Photic stimulation was provided via two channels, from separate sources. The more intense, used for brief "flash" stimulation, was from a Bausch and Lomb high-intensity monochromator and the xenon light source designed for it. The slit widths of the instrument were adjusted for a 10-nm half-maximal bandwidth. The other source was less intense and was used for steady "background" stimulations; the output of a tungsten source was directed through a Bausch and Lomb "microscope illuminator" monochromator whose bandpass was set at 15 nm. Both monochromators were adjusted and fixed so as to deliver light at 500 nm. Although spectral shaping was primarily due to the monochromators, extra filters were interposed to ensure that no UV or infrared radiation reached the preparation. Independent temporal control of each beam was made possible by two Uniblitz shutters and specially made timing circuitry.

Light intensity at the retina was calibrated by two methods: (*i*) use of a model J-16 Tektronix photometer, and (*ii*) partial bleaches of solutions of rhodopsin. Intensity measurements by the two methods were comparable.

Perfusate. Perfusate of the following composition was found to abolish the PI*i* component (b-wave) of the isolated retina electroretinogram and maintain the PIII component: 95 mM NaCl, 25 mM sodium aspartate, 25 mM $NaHCO_3$, 20 mM $MgSO_4$, 5 mM KCl, 2 mM $CaCl_2$ and 20 mM glucose. Perfusate was made from dry chemicals on the day of each experiment; its pH was adjusted to 7.4 by addition of small amounts of HCl or NaOH in concentrated solutions. It was bubbled with 95% O_2/5% CO_2, "medical oxygen."

Electrical Recording. Electrical recording was via shielded leads, which connected to silver ring electrodes located in the perfusion chamber on each side of the retina near its edge. These electrodes were polished and chlorided just before each experiment. The leads exited from the cabinet and connected to a Grass P-16 preamplifier. Direct coupling was used for all experiments; the amplifier was set in differential mode. Records were made either oscillographically or by using a Brush pen-writer. All amplitudes reported here were measured from the baseline to the response peak (see *Discussion* for an elaboration of this). The peak-time is a function of temperature; hence it is not possible to specify the point in time at which it was measured, but it was always later than 1 sec after the flash.

RESULTS

Response Elicitation and Suppression by Backgrounds. Changes in bright-flash responsivity effected by long-duration "background" lights indicate a two-state constraining mechanism at some point in albino rat PIII production. Figs. 1 and 2, from the same preparation, typify the experimental evidence for this. In Fig. 1 the left column is composed of tracings of responses elicited by various background flashes. These, together, amount to an intensity–response series for this retina at 35°C. Values for V/V_{max} for these responses appear under the tracings, and they have been plotted vs. log I as the open circles of the 35°C data of Fig. 2. The line through them is a hyperbolic function.

The column on the right in Fig. 1 is made up of responses to a constant-intensity bright test flash. The uppermost tracing is with no background illumination; the others are responses to the flash with the various intensities of backgrounds present. Increasing background illumination is seen to reduce the size of response to the superimposed flash; the proportion of response *suppressed* (compared to dark) by each background appears below the traces.

Comparison of the effect of background light in eliciting responses *per se* and in suppressing flash responses may now

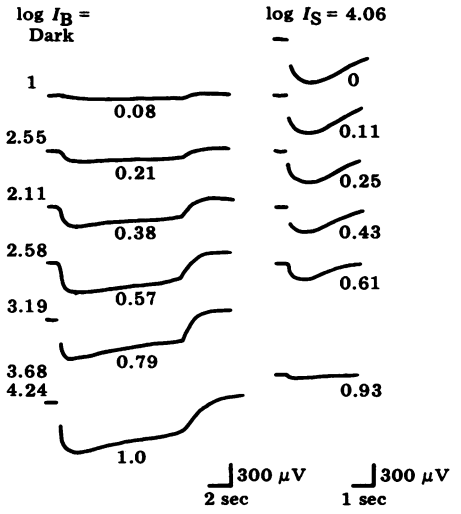

FIG. 1. Tracings of aspartate-isolated rat PIII responses to 10-sec background stimuli (left column) and 10-msec flash stimuli (right column). The intensities of background stimuli (log q incident/rod-sec, in which q = number of photons) appear on the left. All flash responses were elicited by stimuli of log I_s = 4.06 q incident/rod-flash; the top response on the right was with no background, the responses beneath it were in the presence of the backgrounds on their left. The numerical quantities appearing underneath all the responses are explained in the text. Temperature = 35°C.

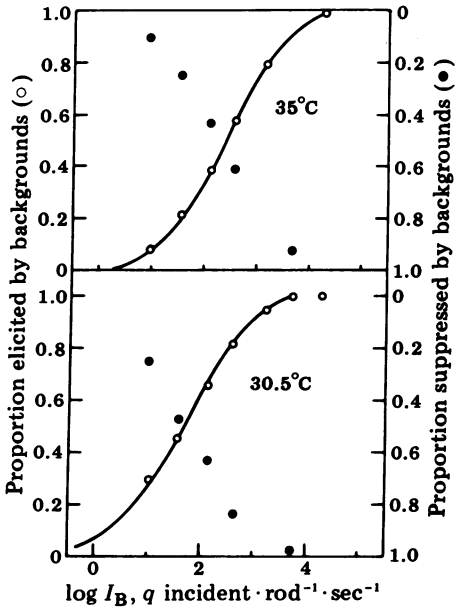

FIG. 2. PIII responses elicited by 10-sec background stimuli plotted as proportion of maximum response (left ordinates, O). Proportion of flash response suppressed by backgrounds (right ordinates, ●). Data are from same preparation at two temperatures, 35°C and 30.5°C. Data from 35°C are from responses of Fig. 1. Lines fitted to intensity–response data are hyperbolic functions.

be made. It is seen that the proportion of flash response suppressed by a given background is the same as V/V_{max} for that background. This finding is emphasized graphically in Fig. 2. The open circles are V/V_{max} vs. log I_B; data are fit with hyperbolic functions (already mentioned in reference to 35°C data). The proportions of test flash response suppressed appear as filled circles plotted with regard to the right-hand ordinate. Fig. 2 makes clear that, as various intensity backgrounds elicit greater proportions of the slow PIII response, they also suppress superimposed flash responsivity in equal measure. It might be argued that this mirror-image relationship is an artifact owing to our inability to completely saturate the response with our backgrounds. However, data from the same preparation at 30.5°C are included in Fig. 2 to counter this argument. At this temperature the responses to background stimuli are clearly saturated, and yet the same equality of proportion of response elicited and proportion of flash response suppressed is observed.

Our interpretation of this result (to be reiterated more fully in *Discussion*) is that PIII response generators always exist in one of two states within the time scale of the observed PIII potential. In terms of the two-state model, the proportion of all response generators "used" by a background light to produce a response is exactly that deprived an ensuing bright flash.

Effects of Temperature on Rat PIII. The ability to easily alter and control retinal temperatures in the isolated preparation was exploited to investigate its effect on PIII responses. A characteristic finding is shown in Fig. 3, in which V/V_{max} is plotted vs. log I_B for the same preparation at two temperatures. Altering the temperature is seen to shift the normalized operating characteristic along the abscissa. A change from 35°C to 21.5°C moved the curve 1.1 log units to the left. Because there was no change in the curve's shape, this result may be viewed simply as a temperature effect on $V_{max}/2$, or σ, of the hyperbolic functions fitted to the data. In terms of the two-state model, this shift of the V–log I curves is understandable in terms of the ratio, k_2/k_1. If k_2 is the rate constant for a "dark"—i.e., "thermal"—reaction, it should be temperature dependent. On

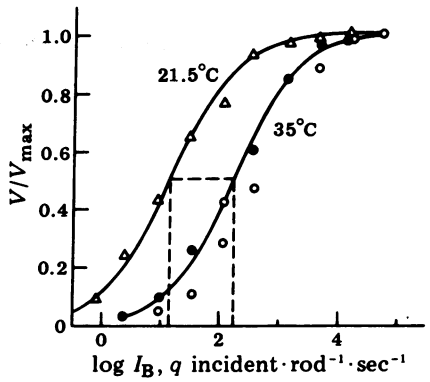

FIG. 3. V/V_{max} vs. I_B (q incident/rod·sec, 60-sec duration) of aspartate-isolated rat PIII at two temperatures. ●, Initial data at 35°C; △, data at 21.5°C; O, final data at 35°C. Curves fitted to data are hyperbolic functions. Note: σ changes by 1.1 log unit.

the other hand, if k_1 is, as already mentioned, the photic rate constant, it should be independent of temperature. The ratio of these—i.e., σ—should change with temperature in the same way and to the same extent as k_2 (see below).

It should be emphasized that the curves of Fig. 3 are normalized to maximum response. Even though these temperature effects were reversible (as seen in Fig. 3), deterioration characteristically did occur in the responses themselves. This steady process resembles one already reported for the isolated rat retina by Ernst and Kemp (15). Normalization of the responses to V_{max}, of course, mathematically eliminates it. Other experiments we have performed show these shifts to be independent of the order of temperature alteration—i.e., from low to high and from high to low.

An expectation concerning PIII's thermal behavior may be made from our suggestion that the temperature shift of the V–log I curve is through its influence on k_2. The two-state model predicts (see *Appendix*) that a shift in equilibrium *toward* the response-producing state should occur at lower temperatures, and that, therefore, the response to a given stimulus should *increase* with decreasing temperature.

The responses in Fig. 4 lend support to this prediction. In the figure, a stimulus of 1.0 log (q incident/rod·sec) of 10-sec duration is seen to elicit responses that increase in magnitude as preparation temperature is decreased. The simple interpretation of this as reflecting a shift in the two-state equilibrium is not without limits, though. For one thing, this phenomenon is observed only if low-intensity flashes are used. Also, at temperatures lower than about 26°C, the response begins to decrease with decreasing temperatures, and it abruptly disappears at about 16–18°C. We attribute this low temperature effect to the onset of some other process—e.g., failure of membrane

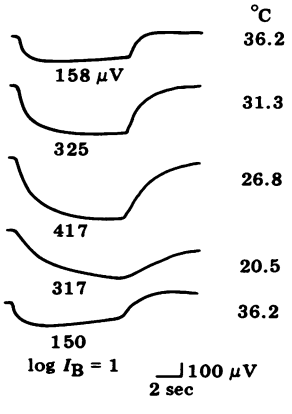

FIG. 4. Aspartate-isolated rat PIII responses to 10-sec background stimuli of log I_B = 1.0 q incident/rod·sec at different temperatures. The responses increased in magnitude as temperature was decreased except at the lowest temperature. The responses were obtained in the order top to bottom.

pumps to sustain high dark currents. Or, perhaps, for part of the temperature range, we simply did not use a flash sufficiently long to observe the full amplitude. (Note in Fig. 4 that the response measured at 20°C has not reached its maximum. Nevertheless, we chose not to use longer flashes because they increase the bleaching beyond acceptable limits.)

The last experimental result we now present was aimed at determination of the temperature dependence of one of the rate constants, k_2, alone. The idea was to see if k_2's temperature dependence predicts the direction and magnitude of σ shift with temperature. The approach was to adapt a retina, repeatedly, with 10-sec background lights and then determine recovery of responsivity as a function of time in the dark after the background was shut off. Full recovery was allowed to take place between each adaptation. In essence we have followed the course of rapid ("neural") dark adaptation and have assumed that its rate is k_2.

Repeating this procedure at different temperatures, we determined the temperature dependence of the recovery process. Fig. 5 is composed of recovery curves from one retina at several temperatures plotted in a semilogarithmic manner. The linear functions resulting from this treatment signify that the process is kinetically first order; this permits computation of rate constants, k_2, from the slopes of the lines. Rapid "neural" dark adaptation in the rat is temperature-dependent as evidenced by the fact that lower temperatures result in shallower slopes.

In Fig. 6 are shown, in the Arrhenius manner, the rate constants, k_2, at various temperatures. The slope of this function gives the activation energy for "neural" dark adaptation in the excised perfused rat retina; it is 35.6 kcal/mol (149 kJ/mol). The fact that k_2 decreases with decreasing temperature shows that it changes in the correct manner to explain the decrease of σ with decreasing temperature (Fig. 3). But, more importantly, the *extent* of change in k_2 is exactly the same as that of σ. That is, Fig. 3 showed that σ varied by 1.1 log unit between 21.5°C and 35°C. Fig. 6 shows that the same change occurs in k_2 over these temperatures.

DISCUSSION

The two-state system, described in the Introduction, requires that the response to a background plus the response to a saturating test flash superimposed on it must always sum to the same value, namely the V_{max} of the dark-adapted state. This is the hallmark of a *simple compression system*. (The model, as presently formulated, is not intended to deal with σ shifts with background.) Fortunately, rat PIII conforms well to these constraints (Figs. 1 and 2) and can be treated with the two-state model. It is important that although the waveform is not maintained (Fig. 1), measuring at the peak of the response fulfills the mathematical requirement that $dB/dt = 0$ that was used in the derivation of the hyperbolic function. Furthermore, despite the waveform sag, the PIII "generators" behave, in their responsivity, as though they were producing a steady output. We can only conclude that recording this mass response is not adequate for determining the true waveform of the generator output. This conclusion is similar to the one proposed by Boynton and Whitten (3) in their study of monkey cones.

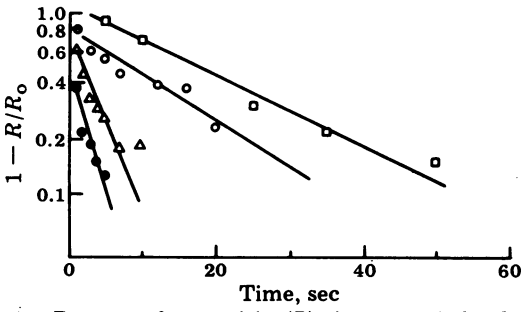

FIG. 5. Recovery of responsivity (R) of aspartate-isolated rat PIII to 10-msec flashes of log I_T = 4.06 q incident/rod·flash from 10-sec background stimuli of log I_B = 3.68 q incident/rod·sec. Recovery plotted as fraction of dark-adapted flash responsivity remaining to recover, determined at four temperatures. □, 20.0°C; ○, 24.0°C; △, 28.5°C; ●, 31.5°C. Slopes are equal to $k_2/2.3\ I_B$.

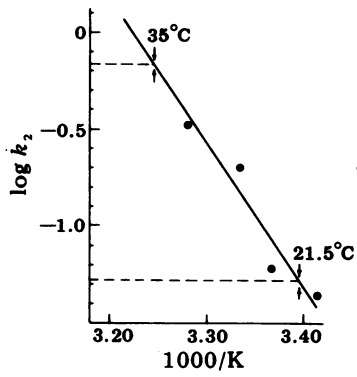

FIG. 6. Arrhenius plot of rate of rapid ("neutral") dark adaptation of rat PIII. Values of k_2 were obtained from results given in Fig. 5. Recovery at 35°C was too fast to measure accurately; therefore the line was extrapolated to ascertain the value of k_2 at 35°C. This plot shows that k_2 varies by 1.1 log units between 21.5°C and 35°C and corresponds exactly to the change in σ shown in Fig. 3.

The equilibrium constant, K_{eq}, appropriate between states A and B of the model, when expressed as rate constants, and when I is constant, is k_1/k_2; the inverse of this is k_2/k_1, or σ, of the derived hyperbolic function. When k_2/k_1 is decreased, as by lowering temperature, the effect amounts to an *increase* in K_{eq}; that is, it favors the responding state, B. Whether this will be manifest in a given system as a larger response or not depends on the complexity of the PIII response-generating system and the position of the two-state section in it. One manifestation of this in our experiments is the greater ease with which we are able to saturate the response at low temperatures (Fig. 2). The most conspicuous effect of this is that larger responses *do* occur with decreasing temperature under restricted conditions (see Fig. 4); those conditions, it would seem, are when the two-state mechanism temperature dependence dominates that of the system *in toto*. For example, responses to *low*-intensity flashes get larger when temperature is decreased, but responses to higher intensities show complex behavior. This is probably because, at low I, the rate-limiting step *forward* is the rate at which photons are absorbed, k_1, but, at higher I, the rate limitation could be the reaction of the activated pigment with substrate. The latter probably has a temperature dependence of its own and thus σ would take on a complex temperature dependence.

Rat PIII is a complex response. A fast component is generated across the receptor outer segments, a slow component is probably produced by glial cells, and there may even be a third process that contributes (ref. 11; see also ref. 8). Would the agreement between the response and the model be as good if, in fact, any one of the components were analyzed separately? We made an attempt to answer this question by trying to sort out fast from slow-PIII as did Witkovsky *et al.* (8), but we conclude this is not possible in our case because changing temperature also changes the time courses of the fast and slow components. Thus, it would be improper to set a single criterion time and always measure the fast component at that point. Each temperature would require a different criterion time. The difficulty is that obvious discontinuities between the fast and slow components were not evident at all temperatures (see Figs. 1 and 4). We conclude that, in order to analyze only one of the PIII components in the way we have reported here, it will be necessary to record with a pair of electrodes as Arden (11) has done.

In any case, why should such a complex response conform to such a simple model? Perhaps because it is being *controlled* by processes that can be reduced to the simple formal input–output model. In this case "controlled" refers to "rate-limited." And, while it is clear that visual transduction and PIII production are both multistage processes, it may not be unreasonable to expect that the rate of a *single* process controls the excitation and another *single* process controls recovery. This is the underlying assumption of the simple formal model we proposed earlier, and it appears that rat PIII corresponds closely to its tenets.

It is interesting that Sillman *et al.* (17) have also shown a temperature dependence of rapid adaptation in bullfrog late receptor potential—the rate being slower at low temperature. Although they studied only two temperatures, their Fig. 3 indicates an activation energy of *ca.* 40 kcal/mol, and this compares favorably with our value of 35.6. They point out, however, that more than one process goes on in rod dark adaptation and that both (or all) of these are temperature sensitive. Thus, it appears that rapid dark adaptation in bullfrog probably does not obey our simple two-state model.

Finally, Hofert and Ubels (18) have shown that the a-wave (leading edge of the PIII) in trout is temperature sensitive: it is larger at lower temperatures, within limits. This agrees with our results with the rat.

While this paper was in the process of being reviewed, an article by Oakley *et al.* (19) appeared. They have studied (intracellularly) the receptor potential in *Bufo* rods as a function of temperature. Their recordings show that the amplitude of the response transient *increases* by nearly 2-fold when temperature is *lowered* from 27.5°C to 13.5°C. This is the direction predicted in our paper, and their results represent the third instance of this kind of temperature dependence that we know of. It is important to point out that the three cases span three classes of animals: amphibia, fish, and mammals. Although the two-stage model is not supported by some preliminary results on grasshoppers, it may have sufficient generality to be useful in studies of vertebrates.

APPENDIX

The Rectangular Hyperbola. Some confusion exists among vision researchers regarding the function that describes visual responses as a function of intensity (cf. ref. 20). The equation usually employed is

$$V/V_{max} = I^n/(I^n + \sigma^n). \quad [1]$$

In its early usage, the curve given by this equation was often called a "hyperbolic tangent" but in fact it is not; the hyperbolic tangent is given by

$$\tanh x = (e^{ax} - e^{-ax})/(e^{ax} + e^{-ax}). \quad [2]$$

On a linear scale of x, this gives an S-shaped curve that looks similar to the operating curves of retinal responses when the latter are plotted on a log X (i.e., log I) scale. When the tanh is plotted on a log scale, it is very steep and will not fit visual response data.

In addition to this failure to fit the shape of visual data, the tanh has asymptotic limits of ± 1 at $x = \pm\infty$. Visual responses, on the other hand have limits of 0 and V_{max}, or, if normalized, 0 and 1.0 at $\log I = \pm\infty$.

Eq. 1 is, in fact, *part* of a rectangular hyperbola. In order to show this, one starts with the equation for a rectangular hyperbola whose center is at (0,0) and whose vertices lie at $\pm X$:

$$x^2 - y^2 = a^2. \quad [3]$$

This is rotated $-45°$ and then translated until the center is at $(-1,1)$. Then, the equation describing it is

$$(x + a)(y - a) = -a^2, \quad [4]$$

in which $a = 1.0$. Rearranging and collecting terms, one obtains a form of the Michaelis–Menten equation:

$$y = \frac{x}{1 + x}. \quad [5]$$

When y (analog of the visual response) is plotted against $\log x$ (analog of $\log I$), the standard sigmoid curve obtains and the slope of the function agrees well with visual response data.

Units of k_2/k_1. The model proposes that "generators" of a response exist and that these occupy "volume elements" within the retina. The volumes present cross-sectional areas to the incoming light and hence the intensity can be expressed as "photons·sec^{-1}·generator^{-1}," in which it is understood that a generator represents an (average) area. With intensity in these units, then, the units of k_1 become: generator·photon^{-1}. Now, because the units of k_2 must be sec^{-1},

$$k_2/k_1 = \text{photons·sec}^{-1}\text{·generator}^{-1},$$

that is, intensity.

K_{eq} and Temperature. The equilibrium constant, K_{eq}, for $A \rightleftharpoons B$ is k_1/k_2. This is, according to the model, σ^{-1}. That K_{eq} for chemical reactions is temperature dependent is to be expected, but what was surprising about these results on rat PIII was that they became *larger* with decreasing temperature. This is now easily understood in terms of the model, because lower temperatures should shift the $A \rightleftharpoons B$ equilibrium toward the B state—i.e., toward the response-producing state—because k_2 is reduced by lowering the temperature.

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