Effect of Eye Closures and Openings on Photostasis in Albino Rats

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PURPOSE. To determine the effects of eye closure and opening on photostasis, the regulation of light absorption by retinal rods in the albino rat.

METHODS. The approach was to measure the effect of eye closure and opening on rhodopsin bleaching in situ and to use those results to simulate what happens to rhodopsin when a living rat opens or closes its eyes during daylight exposure. Completely dark-adapted, dead albino rats, each with one eye closed or open, were exposed to a standard lighting situation. The rhodopsin bleaching rate in closed versus open eyes was measured. Rhodopsin bleached at a more reduced rate in closed eyes than in open eyes. This measured reduction of rate in closed eyes was applied to a simulation of rhodopsin bleaching in open and closed eyes. The simulation used idealized conditions to verify the simulation itself, and then it was applied to previously published photostasis results.

RESULTS. Rhodopsin in closed eyes bleaches at half the rate found in open eyes. The absorption spectrum of rat red blood cells was compared with the rat rhodopsin absorption spectrum, and the comparison showed that blood does not absorb the main-band wavelengths of rhodopsin. Simulating rhodopsin bleaching with eyes closed (half intensity) and open (full intensity) during daylight hours showed a slight effect on the total number of photons absorbed in an entire day. The simulation set limits to the maximal effect of eyes open all day versus eyes closed all day. At a habitat intensity of 200 lux, for example, this maximal effect (eyes always open versus always closed) was calculated to be ±9%. At the lowest intensity, 3 lux, this maximal effect was ±28%, but it is only 1% at the highest intensity, 400 lux.

CONCLUSIONS. Eye closures and openings have a slight effect on photostasis in albino rats. There are two reasons for this: The eyelids reduce the effective bleaching intensity by half. Moreover, during the “dim-out” of closure, rhodopsin continues to regenerate and approaches a new, higher value. This accumulation of rhodopsin enhances the rate of photon absorption because the rate is proportional to the product (rhodopsin \times intensity). Thus, the increased rhodopsin concentration in the rods partially compensates for the reduced intensity of lid closure, and the photon absorption rates, with eyes closed, do not decrease by the full factor of 2 implied by the intensity reduction. In addition, when the eyes are subsequently opened after such a dim-out, the retina is suddenly exposed again to the full intensity of the environment. At this time, photon absorption rate, rhodopsin \times intensity, is transiently higher than just before eye opening. Thus, the compensatory interplay between bleaching and regeneration in closed and open eyes results in the near compensation of light absorption and maintenance of the stasis close to \(10^{16}\) photons per eye per day. (Invest Ophtalmol Vis Sci. 1998;39:603–609)

Penn and Williams\(^1\) raised groups of albino rats from birth until 12 weeks of age, with each group exposed to a different intensity of cyclic light, and found that all rats absorbed the same number of photons per eye per day regardless of habitat intensity. The rats living in dim light had long rod outer segments, fast rhodopsin regeneration rates, high rhodopsin concentration in rod outer segment discs, and many rod cells. The reverse was true of rats living in bright-light habitats. These retinal adaptations resulted in the regulation of photons absorbed per eye per day at \(1.0 \times 10^{16}\) (approximately ±20%), the “photostasis number”\(^2\) for rats. Since then, it has been shown that rat retinas are plastic and adapt to new lighting by upregulating or downregulating the retinal properties enumerated above.\(^3\)–\(^4\) Renewal of the rod outer segments is the mechanism by which the majority of the light-absorption properties of the retina are established, and Schremser and Williams\(^5\)\(^6\) have shown that renewal of the rod outer segments is an adaptive phenomenon, permitting long-term adaptation to changes in environmental lighting. Previous work by others\(^7\)–\(^8\) anticipated such a finding, and studies by Chamberlain and coworkers\(^9\)–\(^10\) support this conclusion for invertebrates.

Photostasis calculations, as performed by Penn and Williams,\(^1\) require that rhodopsin in the eyes of living rats come to steady state levels during the daylight hours. Ordinarily, the solution of rate equations by the steady state approximation relies on the assumption of steady state, but, in the case of
Penn and Williams, no such assumption was needed. Rapp
and Williams had earlier shown that, within experimental
error, such steady state levels of rhodopsin exist. These levels
are established after approximately an hour into the lights-on
period and persist throughout the daytime hours. However,
even though the Penn and Williams calculations were not
dependent on an assumption of steady state, they were per-
formed without concern for whether the eyes were open or
closed and, if closed, what fraction of the day they were closed.
Rats frequently close their eyes or sleep during daylight hours.

How does this eye closure affect the finding that a steady
state exists? Furthermore, what effect does eye closure (and
opening) have on the regulation called photostasis?

In a preliminary attempt to answer these questions, we
monitored the behavior of rats in brightly lit cages with closed-
circuit television and found that they neither sleep the entire
day nor always close their eyes even when they appear to be
sleeping. Thus, it was clear that the eyes are in fact open
during a large fraction of the daylight hours; at such times,
there is no reduction in the intensity within the eyes. However,
television monitoring of behavior only qualitatively an-
swered the main question. We now report a more definitive
study on the effect of eye opening and closing.

The present study was conducted in two parts. The first
was an experiment to measure the amount of rhodopsin
bleaching in intact rat eyes, open and closed. The second was
a simulation, during 12 hours of daylight, of moment-by-mo-
ment photon absorption in rods with eyes open and closed.
The simulation was at first based on easily understood model
systems to test the correctness of the calculations. Once cor-
correctness was verified, the simulation was applied to previously
published experimental results. From these studies, we con-
cluded that the net effect of eye closure and opening had only
a slight effect on photostasis in the albino rat. Indeed, the
dynamic nature of rhodopsin bleaching and regeneration with
eyes open and closed may be intrinsic to the photostasis
process in albino rats.

MATERIALS AND METHODS

Bleaching In Situ. The purpose of bleaching in situ was
to determine the rate of bleaching of rhodopsin in albino rat
eyes that were either open or closed. To determine how eye
closure affects photon absorption by rhodopsin, it was appro-
priate to use rhodopsin in situ to report that effectiveness.
Rhodopsin in intact rods acts as its own actinometer and
automatically takes into account not only photon flux at the
retina but the bleaching effectiveness of the transmitted wave-
lengths. Therefore, we chose to use intact rat eyes in their
orbits, sutured closed or held open, as actinometers. Other
studies on (human) eye closure and eyelid transmissivity have
not always used the retina itself as a detector of the
effective intensity at the retina.

In addition to the “actinometer” properties of rhodopsin,
this study makes use of another feature of the intact rat eye: It
is known that regeneration of rhodopsin does not occur in the
dead, intact rat eye. The object of the intact bleaching
study was to determine the bleaching rate uncontaminated by
regeneration because such a determination reports the effec-
tive intensity alone.

All work on these animals conformed to the tenets of the
ARVO Statement for the Use of Animals in Ophthalmic and
Vision Research and the National Institutes of Health guidelines
for the care and use of animals. Rats were dark adapted over-
night and killed by CO2 asphyxiation the following morning in
dim red light, and one retina was removed and sequestered to
act as the dark-control retina. The other eye was either sutured
closed to give the appearance of a normally closed eye or held
open with tape to give the appearance of a normally opened
eye. The rat was positioned in a standard way beside a bank of
fluorescent light bulbs; the intensity had been pre-set to 200
lux at the position of the eye. We chose 200 lux because it lies
in the middle of the range of intensities studied by Penn and
Williams, whose data were used in the simulations.

Once the rat was in position, the lamps were turned on for
a predetermined length of time. The retina in the exposed eye
was quickly removed (in dim red light) for rhodopsin determi-
nation, and the dark-control retina from the same animal was
used for the subsequent procedures.

Retinas were immersed in distilled water and macerated for
5 to 10 minutes to burst cells and release soluble proteins.
After centrifugation at 10,000g for 10 minutes, the supernatant
was removed and 1.0 ml of 1% polyoxyethylene 10 tridecyl
ether (Emulphogene; Sigma Chemical, St. Louis, MO) in Tris
buffer (pH 6.5) was added to the pellets, which were thor-
oughly disrupted for 2 to 3 minutes with a microspatula. These
tubes were set aside to undergo gentle agitation at 4°C for 1
hour. Then they were centrifuged at 10,000g for 10 minutes.
The supernatants were drawn off, the total volumes of each
were measured accurately, and the volumes were scanned
using a diode-array spectrophotometer (Hewlett-Packard, Palo
Alto, CA). Spectra of all samples were obtained before and after
complete bleaching in white light, and difference spectra were
generated. From these, the change in absorbance of the rho-
dopsin at 500 nm was determined. This value was used to
calculate, using the Beer-Lambert law (molar absorbance co-
efficient = 42,000/M per cm), the concentration of rhodopsin
in the extract and, from that, to calculate the number of
molecules per eye. These results were used to calculate the
rate of bleaching (percentage of bleach per minute) in sutured
(closed) or unsutured (open) eyes.

We used rats of the Sprague-Dawley strain. These are
ture-breeding albinos, and, accordingly, their eyelids have no
significant pigmentation in them other than blood. Thus, be-
sides loss of intensity through scattering, the only potentially
significant loss of intensity on transmission through the lids is
that caused by optical absorption by blood. Therefore, we
measured the absorption spectra of single rat red blood cells
and of single rat rod outer segments. The spectra of hemoglo-
in and of rhodopsin, combined in Figure 1, help explain why
albino eyelids are not effective occluders of bleaching light.
The methods for such single-cell measurements have recently
been published.

Calculation of Effects of Closure and Opening. To
estimate the effects of eye closure and opening on photon
absorption rates, the simultaneous and dynamic processes of
bleaching and regeneration must be taken into account. For
example, bleaching rates are proportional to the product of the
intensity and the rhodopsin concentration (I X R), and the
latter is variable over time in light until a steady state is at-
tained. Furthermore, when a living albino rat is in a lighted
environment with its eyes open, the retina will be exposed to
full intensity (iridial responses are ineffective at blocking light
in this animal\textsuperscript{13}). When the eye closes, the intensity of light falling on the retina decreases immediately, but, because regeneration is occurring, the level of rhodopsin will begin to increase immediately. It is assumed that this regeneration rate will correspond to the rate just before the lids were closed. The validity of this assumption is discussed later.

The rate equation that describes simultaneous bleaching and regeneration in vivo is:

\[
-\frac{dR}{dt} = \Phi I R - k_b (R_o - R).
\] (1)

The first term on the right-hand side is the bleaching term, and the second term is the regeneration term. \(\Phi\) is the photo-sensitivity of rhodopsin for bleaching, a property of rhodopsin itself and assumed to be constant; \(I\) is the intensity of light falling on the rods in the intact eyes; \(R\) is the rhodopsin concentration in the rods at any time, \(t\), during the lights-on period; \(R_o\) is the dark-adapted (that is, maximal) rhodopsin concentration in the rods at time zero; and \(k_b\) is the rate constant for regenerating rhodopsin from the bleached state (units = \(t^{-1}\)).

Equation 1 can be rewritten as follows:

\[
-\frac{dR}{dt} = k_b R - k_b (R_o - R),
\] (2)

where \(k_b\), the pseudo-first-order bleaching rate constant, has been substituted for \(\Phi I\). In other words, \(k_b\) embodies the intensity factor, and, when intensity is to be changed in the calculation from open- to closed-eye intensity, the parameter that is changed is \(k_b\).

Equation 2 was solved by an iterative method using a desktop computer, supplied with spreadsheet software (Excel; Microsoft, Redmond, WA). In this procedure, \(dt\) was set at a small fraction of the entire length of the day (namely, \(1/720\)) and \(dR\) was calculated. Then, a new value for \(R\) was calculated, \(R[\text{inf}mli](t + dt) = R[\text{inf}mli]t + dR; dt\) was increased in small increments, and the whole process was repeated for 720 \(dt\) units = 1 daytime.

The objective was to calculate \(R\) at any time, \(t\), during an exposure to either full intensity (eyes opened) or half intensity (eyes closed). To achieve this intensity change, the calculation was programmed, at the appropriate time, with the new value of \(k_b\) inserted. To achieve the desired durations of opened and closed eyes, the calculation was continued with a given \(k_b\) for the desired interval, at which time the previous value of \(k_b\) was reinserted. We had no information about the durations of eye openings and closings for the rats used in the original photostasis study, so we decided that applying this method to a model system at first would provide confidence that the calculation was correct. After verifying that this model was correct, we proceeded to simulate the data of Penn and Williams.\textsuperscript{1}

### RESULTS

#### In Situ Bleaching

Table 1 presents the results of the in situ bleaching experiments. In this table, \(n\) refers to the number of animals used in a given part. It can be seen that suturing the eyelids closed reduced the average percentage of bleach per minute of exposure by a factor of approximately 2. This means that the eyelids of albino rats occlude approximately half the light effective for bleaching rhodopsin. This reduced factor does not account for the filtering out of other visible wavelengths, some of which are absorbed by blood.

Figure 1 is a comparison of the absorption spectra of rat red blood cells and rhodopsin. In red blood cells, the prominent Soret band appeared at approximately 418 nm and weaker absorption peaks at approximately 546 nm and 582 nm. The absorbance scale is correct for the blood cells, but the rhodopsin ordinates were multiplied by 15 to give a realistic value for the axial absorption by rods in albino rat that have been raised in low-to-moderate intensity.\textsuperscript{18} However, the main absorption band of rhodopsin resides to a large extent between the red blood cell absorption peaks. This means that blood, the main pigment in albino rat eyelids, transmits fairly well the wavelengths that rhodopsin absorbs. In turn, this explains why bleaching rates are only reduced by a factor of 2 when rats close their eyes. The main intensity reducer in the albino eyelids appears to be scattering caused by the tissue itself.

#### Calculations of Photon Absorptions: Applied to a Model

We chose model conditions that would give easily understood results, namely, that the rate constants for bleaching (\(k_b\)) and regeneration (\(k_r\)) were equal and \(R_o\) was unity. With such parameters, the photo-steady state would result in a 50% steady state bleach when the rat eyes are open and intensity is full for an entire day (Fig. 2A). Note that these conditions produce a steady state after approximately 1 arbitrary time unit. This was intentional because we know from Rapp and Williams\textsuperscript{13} that it takes approximately 1 hour to establish a steady state in the albino rat retina. The dashed line in Figure 2A is the expected rhodopsin bleaching function when the intensity on the pigment is reduced by a factor of 2 (eyes are closed all day). In this case, the predicted steady state level of rhodopsin is 67\%, not 50\% as it was when the intensity was full (eyes open). Thus, this model gives the predictable, correct values of these rhodopsin levels for open and closed eyes. What happens to rhodopsin when the model begins with full intensity and suddenly the intensity is reduced by a factor of 2 (that is, when the eyes suddenly close)?

Figure 2B shows what happens when eyes are open and then closed for 2 time units. Intensity is reduced immediately,
FIGURE 1. Comparison of absorption spectra: rat rhodopsin (thin line) and rat red blood cell (heavy line). The former is the average of two spectra obtained from single rod outer segments and fitted with a high-order polynomial. The red cell spectrum is the average of spectra from 20 single cells. Note that the main absorption bands of blood and of rhodopsin do not overlap extensively. This indicates that blood in the eyelids is not an effective blocking filter for bleaching wavelengths.

but some rhodopsin begins to accumulate at the ongoing regeneration rate that, with eyes open, matches the bleaching rate constant. Now, however, regeneration transiently exceeds bleaching and the rhodopsin level increases to 67%, the correct level for closed eyes. Beyond this point, the simulation shows a new steady state level for rhodopsin that is achieved when the 67% level is attained and that persists as long as the eyes are closed. Then the eyes open for the remainder of the daylight hours, and the 0.5 level of rhodopsin is reestablished. Thus, the model correctly describes the "open-closed-open" conditions. As far as photostasis is concerned, the important questions are: What is the integral of photons absorbed over the daylight hours of this model? How do these integrations depend on whether the eyes are open or closed? What is the maximal effect obtainable if eyes are always open or always closed?

Figure 3 shows the integrals of photons absorbed during the whole 12-hour day of the model. These functions, given in this separate figure for the sake of clarity, correspond to the same conditions modeled in Figure 2. Here, the top function is the integral of photons absorbed when the eyes are open all day. The value of this integral at the end of the day is 6.23, which is slightly more (as it should be) than the steady state approximation value of 6.0. (The steady state value of this integral is the product of \( R_o \) = 0.5, \( k_b = 1.0 \), and total time = 12 units, that is, 6.0.) The bottom function is the integral representing eyes closed all day. The total number of photons absorbed at the end of the day in this condition is 4.07. (Steady state approximation gives 4.02.) Finally, the middle function (thin line) is the integrated number of photons representing eyes open at first, then closed for 2 time units, and then open again for the remainder of the day. The integral here lies, as it should, between the other two functions, and the number of photons absorbed for the entire day is 5.56. However, note the slopes that comprise this last integral. At first the eyes are open and the integral begins as usual. When the eyes close at time = 4 units, the slope becomes shallower but does not become flat; photon absorptions are still accumulating because the eyelids transmit bleaching light. When the eyes open after this transient closure, the rate of photon absorption is transiently higher than it would have been if the eyes had not closed at all. This is because the rate of absorption is proportional to the product, \( R \times I \), and rhodopsin has been elevated by regeneration in the dimmer light of eye closure, but the intensity is full because the eyes are open. Thus, eye closure does not reduce the photon absorption rate by half, even though the intensity is lower by half (compare the top integral with the bottom integral in Fig. 3).

Eye opening after such closure produces an additional increase in absorbed photons because full intensity falls now on an enhanced rhodopsin level (compare slope segments among the integrals in Fig. 3).

Calculations of Photon Absorptions Applied to Experimental Results

Penn and Williams\(^1\) presented a table of experimental results that can be used to simulate open and closed eyes. They measured the \( R_o \), \( R_d \), and \( k_b \) values for groups of rats raised in various intensities of cyclic light—12-hours of light and 12-hours of darkness. From their values of these parameters at a habitat intensity of 200 lux, we calculated \( k_b = \Phi \times I \). This value, in units of minutes\(^{-1}\), is \( 7.8 \times 10^{-2} \) for rats raised in 200-lux light. Therefore, in the following calculations, we used
FIGURE 2. (A) Model system simulation of the full-intensity (eyes open) effect on rhodopsin bleaching-regeneration (solid line) and simulation when the intensity is half-full (eyes closed; dotted line). The number of rhodopsin molecules in the darkadapted eye is set to unity. When lights came on and eyes were opened (full intensity), the rhodopsin bleached to a steady state of 50% present. If the intensity was half-full (eyes are closed), the rhodopsin level at steady state was 67%. Both these steady state levels are correct for the relative values of bleaching and regeneration rates used in this model. (B) Simulation of the rhodopsin levels when the day began with eyes open until \( t = 4 \) units, closed for 2 units, and reopened for the remainder of the day. Note that rhodopsin levels in full intensity and in half-full intensity are correctly calculated by the simulation. Also note that the rhodopsin level began to increase immediately on closure and that, at the end of eye closure, it was greater than it would have been if the eyes stayed open the entire time.

this value for full intensity (eyes open) and for half that value \((3.9 \times 10^{-3})\) when eyes were closed.

Figure 4A shows that the simulation returns the correct

\[ R_{\text{ss}} \] value, namely, \(0.17 \times 10^{15}\) molecules per eye. This means that the calculation, when supplied with the experimentally determined values of \( k_b \) and \( k_r \), properly returns the correct \( R_{\text{ss}} \) level. However, having demonstrated this correctness, we return to the main objective of examining the maximum effect expected from eye opening or closing.

Figure 4B shows the integrated photon function if eyes are open the whole day \((k_b = 7.8 \times 10^{-3}/\text{minute})\). The value of this integral at the end of the day is \(1.0 \times 10^{16}\) photons absorbed per eye per day. Penn and Williams reported \(0.97 \times 10^{16}\) at this habitat intensity. Also shown is the integral if the eyes are closed all day \((k_b = 39 \times 10^{-2}/\text{minute})\) (Fig. 4B; dashed line), and the number absorbed per eye per day by the end of the day is \(0.82 \times 10^{16}\). It is less than the open-all-day value by 18%. Thus, the maximum expected effect is \(\pm 9\%\) for eyes open half the day and closed the other half. We calculated (but do not show here) the integrals for eyes open for half the day and closed the other half, but the openings and closings last 1 hour each and follow each other in succession. In this case the value of photons absorbed at the end of the day is \(0.92 \times 10^{16}\), which lies, as it should, halfway between the values for always open and always closed eyes.
Thus, when data from rats born and raised in 200 lux are used, these calculations indicate that eye closing for the whole day causes, at most, an 18% reduction in the photostasis number. But because we have previously observed that rats do not keep their eyes closed all day,12 we surmise that the expected effect of eye openings and closings on the total number of photons absorbed per eye per day is, more realistically, approximately 10% less than the condition in which eyes are open continually.

Table 2 summarizes these results and displays the calculated integrals for the other intensity groups of Penn and Williams. The column marked *(calculated) open halftime* gives the results for eyes that were open half the day, 1 hour at a time, for a total of 6 hours open and 6 hours closed. Note that eye closures and openings have their greatest effect on the lowest intensity group, 3 lux, and the least effect on the highest intensity group, 400 lux.

**DISCUSSION**

The results of this work show that eye opening and closing, coupled with the dynamic nature of the rhodopsin changes, work together to partially sustain the rate of photon absorption and thus help maintain the stasis. Albino rat eyelids reduce the bleaching rate of rhodopsin by a factor of 2. Rat eyelids are thin and should not scatter light as much as the thicker eyelids of humans do, for example.13,14 Another reason the reduction is slight is that the only significant pigment of albino rat eyelids, blood, does not strongly absorb many of those wavelengths that effectively bleach rhodopsin.

When the measured reduction in bleaching effectiveness was incorporated into a simulation of eye opening and closing, it was found that the effects are far less than a factor of 2 on the number of photons absorbed per eye per day. The dynamic interplay of intensity with rhodopsin bleaching and regeneration helps compensate for the intensity lowering. During the dim-out of eye closure, the ongoing regeneration raises the rhodopsin concentration, which in turn raises the rate of absorption. The increases in rhodopsin thus partially compensate for the lower intensity. Similarly, when the eyes open after such a dim-out, the retina is again exposed to full intensity and, because the rhodopsin level has been enhanced, the rate of photon absorptions is enhanced above that which prevailed just before eye opening.

These views of bleaching in dim versus bright light are not new. They involve standard photochemical analysis to measure bleaching. The novelty here is that they are applied to considerations of eye closure and opening, and these, in turn, are applied to photostasis. Regarding regeneration, however, there is an assumption that has been implicit in the development of the computer simulations. It is that regeneration continues at the rate that prevailed just before eye closure.

There are no data in the literature that address this point. It is known that regeneration rates vary inversely with the extent of bleach and with habitat intensity.1,10,20 The studies that show this were conducted so that the intensity was not lowered from one level to another but was instead completely shut off. Regeneration rates were then measured as functions of time in the dark. The results of these studies are all in agreement: Lower intensities during bleaching are associated with faster regeneration rates in the dark. Thus, when we assume that the prevailing rate of regeneration persists into the dim-out of eye closure, perhaps we underestimate the accumulation of rhodopsin during lid closure. If this is the case, we have underestimated the compensatory effects that occur, and we have, thereby, underestimated the stability of the photostasis. What is needed to solve this problem is information about the rapidity with which regeneration rates change when intensity is abruptly lowered. In lieu of such studies, we can only report that our results probably underestimate the compensation.

In this regard, a question arises about lid closure in pigmented species. Their eyelids contain melanin, which would seriously absorb bleaching-effective wavelengths. In large animals or humans, the eyelids are thick and scatter more light than do albino rat lids.13,14 Because thick, nonalbino eyelids block more light, do eyes with such lids also undergo the kinds of compensatory processes we have shown here? If so, are the compensations even more exaggerated than we have shown them to be for the albino? This is possible if an adaptation, known to exist in humans, generalizes to other species: When human eyelids are closed for longer than 0.3 second, the pupils beneath those eyelids dilate.21 The effect of this dilation, in the present context, would be to admit more light into the closed eyes than would be expected given the optical density of the eyelids and the pupil diameter extant just before lid closure. If this occurs, the density of the eyelids would be offset (partially, at least) by the widening of the pupillary aperture. Although unimportant for albino rats, this possibility deserves detailed study in persons with differing concentrations of melanin in their skin and eyelids. Two recent studies14,15 have examined part of this question and have found no differences in lid transmission with degree of melanization. Of these, only one15 has used the retina to report the intensity transmitted to the retina. That study was conducted on humans and reported no differences between the transmissivity of eyelids of white and Asian persons. However, the study was not designed to show
the possible compensatory effects of pupil dilation when eyes are closed. This is left to future work.

References