Extreme Responsiveness of the Pupil of the Dark-Adapted Mouse to Steady Retinal Illumination

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PURPOSE. To measure the dependence of the size of the pupils of mice on steady retinal illumination.

METHODS. Anesthetized C57BL/6 mice aged 7 to 8 weeks were placed in a ganzfeld chamber in darkness, and in monochromatic (510 nm) and white light whose intensity was varied more than 6 log units. The pupils of the mice were photographed with an infrared video camera and recorded on videotape and the pupil areas determined by digital image analysis of the video recordings.

RESULTS. Fully dark-adapted murine pupils had an area of 2.29 ± 0.35 mm². The minimum pupil size at saturating intensity was 0.10 ± 0.05 mm². The steady state pupil area declined to half its dark-adapted maximum when ganzfeld luminance was 10⁻⁵ scotopic candela (scot. cd) per meter squared. Pupil area declined to 20% of the dark-adapted magnitude at approximately 10⁻³ scot. cd/m².

CONCLUSIONS. The mouse pupil can regulate retinal illumination by a factor exceeding 20. The neural circuitry that determines steady state murine pupil size is extremely sensitive to retinal illumination and under these experimental conditions is controlled almost exclusively by rod signals. This follows, because the ganzfeld illuminance (10⁻⁵ scot. cd/m²) that causes the pupil to constrict to half its dark-adapted value corresponds to only approximately 0.01 photoisomerization per rod per second, whereas 80% reduction in pupil area occurs at approximately 1 photoisomerization per rod per sec. Based on this extreme responsiveness to steady illumination, the hypothesis is proposed that the murine pupil functions to protect a retinal circuit that can become saturated at extremely low photon capture rates. General principles of dark-adapted retinal circuitry support the identification of the first three neurons in the circuit as the rod, the rod bipolar, and the All-amacrine. The rod and rod bipolar neurons do not approach saturation at the intensities at which the pupil constricts, however, and it seems unlikely that the All-amacrine does. Thus the retinal neurons protected from saturation by the mouse pupil constrictions are probably ganglion cells with large receptive fields that have sustained responses.


The mouse has become increasingly important in the investigation of retinal function and retinal disease. This importance comes from the general use of the mouse as a model for mammalian genetic manipulation and from the specific development of lines of mice that have transgenes for retinally expressed proteins, including rhodopsin transgenes that cause retinal degeneration resembling the human disease retinitis pigmentosa.

It has long been known that exposure to fairly intense light can cause retinal damage in rodents, and the conditions under which such damage occurs have been much investigated. More recently, it has been established that light-rearing conditions that cause no damage per se, nonetheless regulate many components of the albino rat retina, including outer segment length, rhodopsin content, and rate of regeneration of rhodopsin after a substantial bleaching exposure, amounts of the phototransduction cascade proteins and message for transducin and arrestin, and the message levels of interphotoreceptor retinoid binding protein. Recently, it has been shown that the time course and severity of retinal degeneration in mice that have mutant rhodopsin transgenes is light dependent. That is, mice expressing transgenes such as rhodopsin P23H and P347S are more susceptible to light damage than nontransgenic control animals.

The phenomena of light-dependent regulation of retinal protein expression and of increased susceptibility to light damage of mice with transgenes dictate a need to specify in physiologically relevant units the light loads to which the murine retina is subjected in controlled rearing environments. We undertook this investigation to determine the relationship between mouse pupil size and retinal illumination.

METHODS

Animals

Animals were maintained and all experiments were performed in accordance with protocols approved by the University of Pennsylvania Animal Care and Use Committee, whose regulations are strictly governed by National Institutes of Health guidelines, and in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Female
C57BL/6 mice aged 7 to 8 weeks and weighing 15 to 20 g were obtained from a commercial supplier (Charles River, Wilmington, MA) and maintained on a 12-hour dark-12-hour light cycle at 2.5 lux for 1 to 2 weeks before testing. For experimentation, the animals were anesthetized under red light with an intraperitoneal injection of a mixture containing 25 mg/kg ketamine, 10 mg/kg xylazine, and 1000 mg/kg urethane. After the injection of anesthetic, the previously dark-adapted mouse was placed in the completely darkened experimental box (described later) for a half-hour before testing began. Testing typically lasted 60 to 80 minutes.

Experimental Apparatus

Figure 1 is a diagram of the experimental chamber, as it would look from above during an experiment. The chamber is light-tight and made of aluminum. Its interior is accessed through a light-tight door, and contains three ports: A baffled port is the entry point for the visible illumination, which is supplied by a xenon arc lamp (described more fully later); a second port holds a fiber optic light guide that provides infrared (IR) illumination for visualizing the mouse pupil; and a third port holds a macro lens connected by a custom-made tube to a video camera with extended infrared sensitivity (ITC-46, with IR 747-second vidicon; Ikegami Electronics, Maywood, NJ).

The interior of the chamber is painted with a highly reflective, flat white paint (No. 35-3516, Eastman Kodak, Rochester, NY). In the experiments the anesthetized mouse was situated on a rotatable plastic restraint near the chamber's center, so that its eye was 5 cm from a cap that nearly covered the macro lens. The lens cap was painted with the same paint as the chamber interior, except for a centered, 1-cm diameter hole through which light reached the camera. The hole was made as small as possible for compatibility with adequate light collection: Whereas its diameter subtended 20° of visual angle at the mouse's eye, the hole in the lens cap occupied only $\pi(0.5)^2/2\pi(5)^2 = 1/200$ of the total solid angle of the hemisphere into which the mouse's eye was looking.

Illumination was provided by a xenon arc lamp (XBO 150W/1; Osram, Berlin, Germany) situated in a multiport housing and powered by a highly stabilized direct current supply. Monochromatic visible illumination was provided by one port of the arc lamp housing, which held a 510-nm interference filter of 8 nm bandwidth (full width at half-maximum; Ealing Electrooptics, Holliston, MA), and also had place for calibrated neutral density filters (Inconel; Eastman Kodak). Infrared illumination was provided by a fiber optic filament that carried light from a second port of the housing after it was first passed through a filter with its 1% transmittance cutoff at 790 nm (Wratten 87C; Eastman Kodak).

Light Calibrations

Light intensities were measured as described previously. In brief, a supplier-calibrated photodiode (Pin 5DP/SB, United Detector Technology, Hawthorne, CA) with a 5.1-mm2 surface area was placed at the plane of the mouse pupil, and the total 510-nm radiant energy flux ($F_\lambda$, in watts) falling onto its surface was measured with the diode operating in the photovoltaic mode. The luminous flux ($F_{\text{scot}}$, scot. lumens) was obtained from the relation

$$F_{\text{scot}} \text{ (scot. lumens)} = K'_m V'_\lambda = 1700 F_\lambda \text{ (watts)},$$

where $K'_m = 1700$ (scot.) lumens/W. The luminance ($L_\lambda$) was then computed from the relation

$$L_\lambda \text{ (scot. cd/m}^2\text{)} = F_{\text{scot. lumens}} / 2\pi A_{\text{euc}} (m^2)$$
This latter relation follows directly from the definition of luminance and the assumption that the photodiode is collecting flux from one hemisphere of a uniform ganzfeld chamber. To determine whether the luminance in the chamber at the plane of the mouse's pupil was uniform—that is, to ensure that the chamber acted as a proper ganzfeld—the following procedure was performed. The photodiode was covered by an elongated pinhole aperture probe that had an angular acceptance of less than 2° and was placed on a gimbeled mount at the position of the mouse's eye (Fig. 1). With the flux into the chamber set to a convenient level, the diode was then directed toward each of a grid of 25 evenly spaced directions (5 azimuthal, 5 elevational) in the hemisphere into which the mouse's eye projected. Thus, considering the direction of the camera lens as the (0,0) direction, the five azimuthal angles (in degrees of visual angle) were −90°, −45°, 0°, 45°, and 90°, and likewise for the angles of elevation. These measurements were within 20% of the mean value, with the following exceptions: in the (0,0) direction, for which the intensity declined to approximately 25% of the mean and in the lower negative quadrant, where the intensities were approximately 30% lower than the mean. These deviations from uniformity were caused by the presence of the camera lens and the inability to change its position.

To achieve a luminance level in excess of 3 scot. cd/m² the 510-nm interference filter was removed from the entry port, and the xenon white light delivered directly to the chamber. Scotopic visual equivalence between white and 510-nm light was established by the lights' relative effectiveness in eliciting the b-wave of the scotopic electroretinogram; only the 510-nm interference filter was removed from the entry port of the ganzfeld chamber.20

Measurements of Pupil Area

With the aid of deep red light, a dark-adapted, anesthetized mouse was positioned at the distance of precisely 5 cm from the camera lens cap, in such a way that the video image of the dark-adapted pupil appeared circular. As part of the record of each experiment, a millimeter ruler was positioned as close as possible to the mouse's cornea, imaged with the infrared video camera lens cap, in such a way that the video image of the dark-adapted pupil appeared circular. As part of the record of each experiment, a millimeter ruler was positioned as close as possible to the mouse's cornea, imaged with the infrared video camera and stored on videocassette. The mouse was then left in the restraint in the experimental chamber to adapt to darkness for an additional 30 minutes before experimentation began.

Ninety-second video recordings were recorded with a high-quality videorecorder (model 6500; Sony, Tokyo, Japan) at each illumination level tested. In each mouse an initial recording was made in total darkness (save for the infrared illumination), and then recordings were made of the pupil in the presence of each of a series of ascending intensity steps of 0.5 to 1.0 log units (depending on the illumination level), beginning at a luminance of approximately 10⁻⁶ scot. cd/m² and terminating at approximately 100 scot. cd/m². In some mice at the end of the experiment the pupil was measured again in complete darkness and after instilling a drop of 1% tropicamide solution (Mydriacil, Alconox, NY, NY) into the eye.

Our goal was to determine the steady state pupil size as a function of luminance. Inspection by slow-motion playback of the recorded pupil responses to the stepwise increments in luminance used showed the steady state pupil size to be reached in less than 30 seconds. To estimate the steady state pupil size for each illumination level, three individual frames were "grabbed" at random with an image analysis system (model 150; Itex, Bedford, MA) from the video record beginning 30 seconds after the particular illumination commenced. These images were stored as TIF files for subsequent analysis.

Pupil areas were computed with a software program written in code (MatLab; Mathworks, Natick, MA). A standard target with a precision-drilled 1-mm diameter hole was placed 5 cm from the camera lens at the plane normally occupied by the mouse. The image of this target hole provided an absolute scaling factor for the images. To measure the area of the pupil, a 20- to 30-point polygon that circumscribed the pupil was first drawn with the computer on each image. The area enclosed in pixel units was then computed with the "region of interest polygon" image analysis function of the software. This procedure was performed for each of the three frames, and the average of the three pupil images in pixels, scaled according to the area of a single pixel, was accepted as the absolute area of the pupil of a given mouse at a given intensity.

RESULTS

Four images of the pupil of an individual mouse, obtained at four luminances spanning the intensity range across which the pupil responded, are shown in Figure 2. The images in each of the four rows are duplicates: that in the left column shows the raw image, and that in the right column shows the same image with a white ring drawn on it to identify the region measured as the pupil. Each image is from an individual video frame taken at least 30 seconds after the light intensity was stepped to the particular level.

The pupil areas of five female mice aged 7 to 8 weeks, as a function of the chamber luminance, are plotted in Figure 3. Each point on the graph (Fig. 3A) represents the average of three measurements of the pupil of an animal exposed to the luminance shown by the abscissa, with each measurement having been made from an individual image (Fig. 2). The inset (Fig. 3A) shows the SDs of the individual measurements. For pupil areas smaller than approximately 1 mm² the SDs are roughly proportional to the mean, with proportionality constant of approximately 5%. For such pupil areas, the variability most likely reflects the reliability of the measurement method per se is not the cause of the higher variability seen in the middle range of the U-shaped curve. Finally, in the largest pupil areas, which represent the fully dark-adapted state, the SDs are small again, typically less than 5% of the total area. The low variance of the pupil areas in the dark provides evidence that the measurement method per se is not the cause of the higher variability seen in the middle range of the U-shaped curve.

The results averaged over the five animals are shown in Figure 3B. Mean pupil areas (±SD) ranged from an average of 2.29 ± 0.35 mm² in the dark to a minimum area at saturating intensities of 0.10 ± 0.05 mm². The smooth gray curve through the data was generated with the formula:
Figure 2. Images of an individual mouse's pupil, taken at four luminances: $10^{-6.1}$, $10^{-5.1}$, $10^{-4.1}$, and $10^{-3.5}$ scotopic candela/m². The images are repeated in two columns: in the column at left they are shown in their raw form; in the right column, each image is shown with a white ring drawn around the pupil. The ring was drawn by connecting 15 to 30 points with straight lines placed on the pupil limbus at approximately equal angles. This illustrates the process used to determine the pupil area from an enclosed polygon (see Methods section). Three images were analyzed in this manner at each luminance to which the mouse was exposed, and the pupil area was considered to be the average of the three measurements. The pupil areas thus estimated, from top to bottom, were 2.17, 1.15, 0.43, and 0.12 mm². Scale bar, 1 mm.
Figure 3. Pupil measurements of five mice, as a function of luminance. (A) Each point represents the average of three measurements of steady state pupil area, in a mouse exposed to the luminance provided by the abscissa value. Different symbols represent data of individual mice. (●) Data of the mouse with pupil images shown in Figure 2. Inset: The SDs of the repeated pupil area measurements are plotted as a function of the mean pupil area, with the same symbols used in (A) representing the data of each mouse. (B) The group average pupil areas at each luminance are plotted; the error bars are the SDs of the individual means from the group mean values. The thickened gray curve drawn through the data was generated with equation 1. The parameters of optimum fit were found with the nonlinear regression “wizard” of SigmaPlot 4.0 (SPSS, Chicago, IL).

\[ A_p(L) = A_{p,\text{dark}} \left( \frac{\alpha}{\left( 1 + \frac{L}{L_1} \right)^{n_1}} + \frac{1 - \alpha}{\left( 1 + \frac{L}{L_2} \right)^{n_2}} \right) \]  

where \( A_p(L) \) is the pupil area at ganzfeld luminance \( L \), \( A_{p,\text{dark}} \) the area in the dark, \( \alpha \) a fraction of the pupil area that declines more rapidly (component 1), and \( 1-\alpha \) the complementary fraction (component 2) that declines more slowly with intensity. The parameters \( L_1 \) and \( L_2 \) characterize the sensitivity of each component to light, and \( n_1 \) and \( n_2 \) determine the rates of pupil area change per unit increase in light. For the curve fit to the data (Fig. 3B) the parameter values are \( A_{p,\text{dark}} = 2.3 \text{ mm}^2 \), \( \alpha = \)
FIGURE 4. Normalized pupil areas of each mouse, plotted against the computed rate of rod photoisomerization in each mouse. The data of each mouse were normalized by the pupil area measured in the dark. The rate of photoisomerization was computed as described in the Discussion section. (●) Data of the mouse whose pupil images are shown in Figure 2. The remaining symbols represent data of individual mice.

As in humans, the size of the fully dark-adapted mouse pupil is smaller than the pupil dilated with mydriatic agents. In two of the five mice, after exposure to the most intense chamber luminance was completed, the light was extinguished and a drop of tropicamide was instilled in the eye. The pupil of one mouse in which the initial dark-adapted area was 2.5 mm² dilated to 2.9 mm², and that of the second, in which the initial value was 1.8 mm², dilated to 2.75 mm². We had previously estimated the area of the pharmacologically dilated pupil of the mouse to be 3 mm² (ref 19). Taking 2.9 mm² as the area of the maximally dilated pupil and 2.3 mm² (the average of all five mice) as the area of the fully dark-adapted but not pharmacologically dilated pupil, it then followed that the dark-adapted pupil had reached approximately 90% of its maximum possible diameter.

Insight into the mechanism controlling pupil size can be obtained by replotting the pupil area data of Figure 3 as a function of the rate of rod photoisomerization (Fig. 4). The graph reveals the extraordinary facts that the pupils of these mice contract to 50% of their dark-adapted area at a ganzfeld luminance that produces, on average, less than 1 photoisomerization per 100 rods per second, and that the pupils contract to 20% of their dark-adapted area at luminances producing less than 1 photoisomerization per rod per second.

In closing this section, we feel obligated to report an additional finding. In the preliminary stages of the investigation in which we were determining the proper range of intensities to use in the experiments, we measured the pupil areas of several mice of the same strain that were older than those whose data are reported in Figures 2, 3, and 4. We found several animals aged more than 4 months to have little or no pupil response, even though these animals had been reared throughout their lives in the same strict light regimen (12-hour dark-12-hour light cycle at 2.5 lux) as the animals for which data are reported here. In parallel electroretinographic experiments, the maximum a-wave and scotopic b-wave amplitudes of these older animals were also found to be reduced. These preliminary findings are reported as a cautionary note, so that
the data reported here are not considered to be representative of mice of all ages.

**DISCUSSION**

**The Extreme Responsiveness of the Dark-Adapted Mouse Pupil**

Similar to that in other mammals including humans, the steady state area of the murine pupil is a monotonically declining function of retinal illuminance (Fig. 3). The pupils of young mice are exceptionally responsive, constricting to 50% of their dark-adapted area at a luminance that generates only 1 photoisomerization per 100 rods per second (Fig. 4). A previous study of rodent pupils provides evidence consistent with this extreme responsiveness. Trejo and Cicerone\(^ \text{22} \) reported that the pupil of a 1-year-old albino rat, with an area of 7.1 mm\(^2 \) when fully dark adapted, constricts to 50% of this area in response to a 500-msec, 500-nm ganzfeld stimulus producing an irradiance at the cornea of 5620 photons/mm\(^2 \) per second. With a half-maximum pupil area of 3.5 mm\(^2 \), a media transmissivity of 0.8, and a retinal surface area of 55 mm\(^2 \),\(^ \text{12} \) the ganzfeld stimulus that Trejo and Cicerone\(^ \text{22} \) found to cause 50% pupil constriction caused a retinal irradiance of approximately 3.6 \times 10 \(^ {-14} \) photons/(\( \mu \text{m}^2 \) \cdot s). Thus, assuming an end-on collecting area for the rod of 1.3 \( \mu \text{m}^2 \) (ref. 19), the stimulus produced a rate of photoisomerization of approximately 1 per second per 2100 rods. To compare this latter number with the value, 1 per second per 100 rods from our steady state pupil data, the rod integration time should be considered (approximately 0.2 seconds), as should the greater sensitivity of the transient phase of the pupillary response compared with that of its steady state phase. Reasonable adjustments for these factors predict a steady state albino rat pupil responsiveness close to that of the young mouse.

Although the transient responses of human and rodent pupils may have comparable thresholds (expressed in retinal illuminance) in the fully dark-adapted state,\(^ \text{22,23} \) the steady state response functions are markedly different. Moon and Spencer,\(^ \text{24} \) summarizing results of several investigations, report that 50% reduction of the dark-adapted human pupil area is reached at a steady retinal illumination of approximately 0.5 photopic troland (td). Spring and Stiles,\(^ \text{25} \) reporting the size of the pupils of 12 human subjects to a uniform steady field of 52° diameter, show the subjects’ pupils to diminish to 50% of the dark-adapted area at retinal illuminances ranging from 1 to nearly 100 photopic td. Assuming that for the light sources used by these investigators, 1 photopic td was equivalent to approximately 1.5 scot. td, and that 1 scot. td produces approximately 5 photoisomerizations per rod per second, it follows that when the most responsive human pupils diminish to 50% of their dark-adapted area, rods are undergoing at least 2 photoisomerizations per second. Thus, those human pupils that are most responsive to steady illumination seem to be at least two orders of magnitude less responsive than rodents. Therefore, it may be concluded that the extreme responsiveness of the rodent pupil is not a general feature of mammalian visual systems or even of mammals with strongly duplex retina.

**A Puzzle of Function**

It is puzzling that the mouse pupil remained constricted at such extremely low steady intensities, because on general grounds it is expected that at such intensities the signal-to-noise ratio of any retinal cell is improved by an increase of the rate of photoisomerization, as follows: The ability of any retinal neuron to signal reliably the presence of steady illumination under conditions when rods are capturing fewer than 1 photon per integration time (approximately 200 msec) is necessarily set by photon capture fluctuations, by dark photolink spontaneous events in the rods, and by other intrinsic noise.\(^ \text{26–29} \) Ignoring the other intrinsic noise, the signal-to-noise (s/n) ratio of a retinal cell counting quantal inputs from \( N_\text{rods} \) rods under such low light conditions should vary with intensity according to the formula

\[
\frac{s}{n} = \frac{N_\text{rods}I}{\sqrt{N_\text{rods}(I + I_\text{dark})}} \tag{2}
\]

where \( I \) is the mean photoisomerization rate per rod produced by the steady light, and \( I_\text{dark} \) is the mean rate of spontaneous (dark) photolink events, estimated to be 0.006 seconds in monkey rods.\(^ \text{27} \) Equation 2 is predicated on the insights that under the dimmest light conditions vision must operate in a single-photon counting mode, and that Poisson statistics govern the signal-to-noise ratio of such an event-counting process. The numerator is the input signal to a cell at which single-photon signals are pooled, and the denominator is the SD of the combination of photon-triggered events and spontaneous events. From equation 2 it can be seen that \( s/n \) is a two-limbed function of \( I \), increasing linearly in \( I \) when \( I < I_\text{dark} \) and as \( \sqrt{I} \) when \( I > I_\text{dark} \). On either limb \( s/n \) grows steadily with \( I \), in agreement with the general notion that near the limits of vision more \( I \) is better.

An overriding exception to the signal-to-noise rule that more \( I \) is better is provided by the requirement for retinal neurons to operate below their saturation limits. Could it be then, that the mouse pupil serves to protect from saturation a neuron with very high amplification between photon capture and its input, a neuron that is therefore subject to saturation at extremely low light levels? If so, what might the cell type be that is protected from saturation by the murine pupil?

**The Neuronal Circuit Protected from Saturation by the Mouse Pupil Contraction**

The dark-adapted murine pupil size is controlled exclusively by signals originating in rods. Across most of the operating range of the pupil, no rod in the retina undergoes more than 1 photoisomerization per second (Fig. 4). Because mammalian rods are linear in their responses to steady lights producing as many as 10 or more photoisomerizations per second\(^ \text{27,29} \)—a level 1000 times greater than that which causes the pupil to constrict 50%—it is certain that the pupil contraction does not serve to protect rods from saturation.

It is now generally accepted that the second-order neuron that processes the single-photon responses of rods is the rod bipolar,\(^ \text{28,30,31} \) and thus the second neuron in the circuit controlling the dark-adapted mouse pupil is the rod bipolar. To evaluate the hypothesis that the rod bipolar neuron is protected from saturation by the pupil, we consider first the rod — rod bipolar convergence. In the cat the convergence is approximately 20 \( \rightarrow \) 1,\(^ \text{30} \) and it may be as high as 100 \( \rightarrow \) 1 in the rabbit.\(^ \text{32} \) The convergence in the mouse is not known, but even if the highest convergence ratio, 100 \( \rightarrow \) 1, applies for the
Table 1. Saturation Parameters of the Rodent Scotopic b-Wave

<table>
<thead>
<tr>
<th>Species</th>
<th>$b_{\text{max}}$ (μV)</th>
<th>$\Phi_{1/2}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>520</td>
<td>0.7</td>
<td>39</td>
</tr>
<tr>
<td>Rat</td>
<td>700</td>
<td>~2</td>
<td>40</td>
</tr>
<tr>
<td>Mouse ($n = 8$)</td>
<td>392 ± 110</td>
<td>2.5 ± 3.2</td>
<td>41</td>
</tr>
<tr>
<td>Mouse ($n = 4$)</td>
<td>292 ± 120</td>
<td>0.9</td>
<td>42</td>
</tr>
<tr>
<td>Mouse ($n = 4$)</td>
<td>493 ± 105</td>
<td>0.7 ± 0.2</td>
<td>21</td>
</tr>
</tbody>
</table>

The corneally recorded scotopic b-wave peak amplitude versus intensity function was fitted in each instance with a hyperbolic (Naka-Rushton) saturation function, with the form $b_{\text{peak}}\Phi_{\text{max}} = \Phi(\Phi+\Phi_{1/2})$, where $b_{\text{peak}}$ is the peak amplitude for a flash producing $\Phi$ photoisomerizations/rod, $\Phi_{\text{max}}$ is the saturating amplitude of the scotopic b-wave, and $\Phi_{1/2}$ the flash intensity that produces a half-saturating amplitude.

* The mice were of the 129SV genetic strain (mean age, 4 months) and were reared under unspecified light conditions. The saturating b-wave amplitude (790 ± 380 μV) reported in reference 42 (Table 1) was erroneously reported as the scotopic b-wave, but in fact was the full rod- and cone-driven b-wave of the dark-adapted animal. The value shown here is the exclusively scotopic b-wave.

† The mice were C57BL/6 aged 2 to 3 months and had been reared exclusively on a 12-hour dark-12-hour light cycle at 2.5 lux.

The mouse, the intensity producing 50% constriction of the pupil would generate only 1 photoisomerization per rod bipolar per second.

Physiological data provide confirmation of the point that rod bipolar neurons are operating well below saturation at the intensity levels at which the mouse pupil constricts to half its dark-adapted area. Recent evidence has supported the identification of the scotopic b-wave of the electroretinogram as a field potential generated primarily by rod bipolar neurons (reviewed in reference 21). As summarized in Table 1, from investigations of the scotopic b-wave in rodents, it can be concluded that flashes producing approximately 1 photoisomerizations per rod cause the rod bipolar neuron to reach half-saturation. Based on a rod integration time of approximately 200 msec, in the absence of adaptation half saturation of the scotopic b-wave response can be predicted to occur in response to a steady light producing approximately 5 photoisomerizations per rod per second, an intensity 500 times greater than that at which the pupil constricts to half its dark-adapted area. If adaptation of the rod bipolar neurons occurs at these extremely low intensities, the steady intensity producing half saturation will be higher and even more discrepant from the pupil response function. Thus, from anatomic and physiological considerations we conclude the rodent pupil’s steady state response does not serve to protect rod bipolar neurons from saturation, except possibly above 0.1 scotopic cd/m², an intensity at which the pupil has already constricted to less than 10% of its fully dark-adapted area.

The third neuron in the circuit of the dark-adapted pupil is the AII-amacrine neuron, which in the cat collects signals from five rod bipolar neurons. Given a possibly higher convergence number at rod → rod bipolar and rod bipolar → AII-amacrine in the mouse than in the cat, it seems possible but still somewhat unlikely that the pupil’s constriction protects the AII-amacrine neuron from saturation. Nonetheless, the most likely retinal neuron that might be protected from saturation by the mouse’s pupil response is a specific ganglion cell type. Several properties of this cell type can be derived by combining our results with evidence in the literature to arrive at the following: First, the ganglion cell receives its input from AII-amacrine neurons and has a sustained response. Second, the ganglion cell collects from at least 900 rods and begins to saturate (decline from linear response) at or below the intensity $I = 0.01$ photoisomerization per second per rod. This property follows from application of equation 2 with the specific value of $I_{\text{dark}} = 0.006$ per second per rod. In equation 2 must be 900 or more to achieve $\Phi/n$ of unity at the intensity 0.03 photoisomerization per rod per second, at which rate the mouse pupil constricts 50%. Third, the ganglion cell has a small fiber axon and projects to the pretectal olivary nucleus, wherein lie the constricter cells that drive the pupillary response.

Mouse Trolands

The conversion from the luminance of the ganzfeld chamber to the rate of photoisomerizations per rod used in Figure 4 is related to the troland unit of retinal illumination. A troland is defined as the retinal illumination resulting from viewing a stimulus patch whose $L$ is expressed in candelas per square meter in a pupil having a 1 mm² area. Alternatively, the retinal illumination produced by any extended source is the product of the source $L$ in candelas per square meter multiplied by $A$, the pupil area in square millimeters. For the human eye, a retinal illumination of 1 scot.td has been estimated to produce from 5 to 8.6 photoisomerizations per rod per second.

A mouse troland has the same definition as a human troland, but the retinal illumination (at the same luminance and for a pupil of the same area) will be far higher in the mouse, because of the much smaller posterior nodal difference of the mouse eye. Taking the human posterior nodal difference to be 16.7 mm and that of the mouse to be 1.75 mm, then for the same pupil area, the retinal illumination would be (16.7/1.75)² = 91 times higher in the mouse. Thus, a mouse troland corresponds to approximately 450 to 780 photoisomerizations per rod per second, if the end-on collecting area of the mouse rod is assumed to be the same as that of the human rod.

The dark-adapted pupil size scales roughly with the eye size, however, so a fully dark-adapted human eye having approximately 40 mm² of pupil area will capture 16 times as many photons as the mouse eye with 2.5 mm² area. The result is that the fully dark-adapted (or dilated) mouse retina receives only approximately 6 times as much irradiance as the human retina from an extended source of the same scotopic luminance.

Unresolved Issues

We are puzzled by the apparent loss of pupillary function in some mice of age 4 months or more, an effect that could be caused by any of a number of factors, including hereditary factors in the strain of mice (C57BL/6 from Charles River). A consequence of this loss of function, if general, would be that in any specific light-rearing condition, animals of such ages would be exposed to considerably higher light loads than younger animals.

Another issue, raised by a reviewer but not addressed in this study, is the possibility that the pupil may be contracting in response to light impinging directly on the iris itself, as for example has been demonstrated in many nonmammalian species. This possibility has been investigated in several studies.
that have been reviewed by Lowenfeld in her extensive book on the pupil. Based on the absence of any pupil responsiveness in the absence of intact optic nerves in seven mammalian species, including man, Lowenfeld concludes that the mammalian iris is not directly responsive to light.

References


