Are Albino Rats Night Blind?

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Based on single-unit recordings from the superior colliculus and optic nerve, albino rats recently were reported to have dark-adapted thresholds that are 2 log units higher than those of pigmented rats. To confirm this result, electroretinograms (ERG) were recorded with pupillary light reflex thresholds from the same strains of albino (CD) and pigmented (Long-Evans hooded) rats. Neither ERG nor pupil measurements showed higher dark-adapted thresholds for albino relative to pigmented animals. Both groups had dark-adapted thresholds close to the thresholds found for hooded animals in the reported study. These experiments measuring ERGs and pupillary light reflexes do not verify the report of night blindness in albino rats. Invest Ophthalmol Vis Sci 32:2366-2371, 1991

Dark-adapted thresholds in albino rats and mice have been reported to be elevated relative to those of normally pigmented animals.1,2 To account for the loss in sensitivity associated with albinism, one suggestion was that a reduction in melanin in the retinal pigment epithelium led to abnormalities in ionic environment of the subretinal space.2 Earlier studies on the retina of the hypopigmented pearl mouse showed that visual sensitivity in the pearl mouse returns to normal when the retina is isolated from the pigment epithelium.3,4 This finding was particularly fascinating in view of the report that the retinal pigment epithelium of albinos shows reduced calcium binding.5 Taken together, these two results fit in well with the calcium hypothesis of visual sensitivity regulation6-9 and suggest that Ca++ levels in the subretinal space can have powerful influences on dark-adapted sensitivity. Thus, finding "night blindness" in a number of different albino animals is potentially important in understanding the processes that regulate the sensitivity of rod photoreceptors.

There is a problem, however. Although the elevated absolute thresholds of the pearl mouse are well documented3,10,11 the report of elevated dark-adapted thresholds in albino rats is surprising, given the extensive literature on light sensitivity in rats12-20 None of the previous literature suggested that albino rats were "night blind." Albino rats were reported to have the same or slightly lower dark thresholds than normally pigmented animals.13,19 This was our impression, based on experiments on both albino and pigmented rats17-18,21,22 but we had not made direct, detailed comparisons of the increment thresholds of albino and pigmented animals under identical experimental conditions.

At the point we began these studies, there seemed to be several possibilities. It was conceivable that we and others had missed the differences between albino and pigmented rats. However, and perhaps more likely, the reported sensitivity loss might have been present in the animals used by Balkema2 but not in the animals we used. In the previous work from this laboratory, we consistently used Sprague-Dawley animals dark reared from birth in our own colony. In contrast, Balkema used the CD strain of albino animals obtained from Charles River Laboratories, a commercial animal supplier that rears animals in well-lighted rooms. Thus, it seemed possible that either strain differences or light exposure differences might underlie the Balkema finding. We compared the visual thresholds of the two strains of rats from the Charles River supplier. As reported here, we were unable to find any substantial differences in the visual sensitivity of these albino and pigmented rats.

Materials and Methods

We measured electroretinograms (ERGs) and pupillary light reflexes on dark-adapted pigmented and albino rats. All procedures were in compliance with the ARVO Resolution on the Use of Animals in Research.

Animals

Twenty-two rats, 12 hooded Long-Evans and 10 albino CD, weighing between 300-500 g, were obtained from Charles River Laboratories (Wilmington,
MA). On arrival in our facility, the animals were housed in cyclic lighting on a 12-hr/12-hr light–dark cycle.

**ERGs**

Before an experimental session, the animal was dark adapted for 12 hr or more and then anesthetized with intraperitoneal injections of pentobarbital (50 mg/kg). The surgery and setup were done under dim red dark-room lights. Supplementary doses of anesthetic were given when needed. The pupils were dilated with atropine (1% atropine sulfate), and the dilated pupils were about 4.0 mm in diameter. A topical anesthetic (tetracaine) was applied to the corneas. The eyelids were retracted with surgical silk thread. Body temperature was kept constant at 37°C by placing the animal on a thermostatically controlled heating pad. The rat was laid on its side with its head fixed in place with surgical tape. The ERGs were recorded with two silver-silver chloride cotton-wick electrodes. The active electrode was placed on the cornea under a half-shell of a Ping-Pong ball, and the reference electrode was placed on a small cut at the nose. Tetracaine was applied to the cut and to the lid sutures. After setup, the animals were dark adapted for at least 10 min before the experiment began. Electrical potentials were recorded with an alternating-current coupled system similar to the one described previously23 controlled by placing calibrated neutral-density filters in the beams. The density filters could be changed in 0.5-log unit steps. The test flash duration (50 msec or 0.5 sec) was controlled with an electromagnetic shutter (Uniblitz, Vincent Associates, Rochester, NY). The unattenuated test light produced a luminance of about 3.0 log cd/m² (ERG experiments) and the unattenuated background light produced 2.4 log cd/m² on the inside of the Ping-Pong ball. Intensity–response functions were measured against backgrounds of various intensities using a 50-msec duration test and a steady background (appearing 2 sec before the test). The amplitude of a response and the associated time-to-peak were quantified as follows. The response was digitized (500 samples/sec), and the baseline was determined by averaging 50 prestimulus samples. A least-squares second-order polynomial was fit to a 30-msec segment of record containing the peak, and then the maximum response amplitude was obtained from the parameters of the parabolic fit. Thresholds (50 μV) were determined by fitting a Michaelis-Menton curve in a least-squares sense to the lower position of the intensity–response curves.

**Results**

**Dark-Adapted Responses**

**ERG b-waves:** Figure 1 shows sample ERGs of individual pigmented and albino animals (Fig. 1A, albino; Fig. 1B, pigmented) measured in the dark using 50-msec flashes of white light. The flash intensity was increased in 0.5-log unit steps from −2.9 log cd/m² to −0.9 log cd/m². For both animals the first sign of a response was a positive-going deflection (b-wave) rising out of the baseline noise of the recording at a flash intensity of about −2.9 log cd/m². We saw no sign of a low-threshold, negative-going scotopic response described to occur in some other species.24,25 The low-level responses in these two animals were virtually identical.

Figure 2 summarizes the results for all ten animals in this series by showing the dark-adapted intensity–response functions for the b-wave. Each point plots the mean amplitude of the b-wave (bars represent the standard errors of the measurements, n = 5). The smooth curve drawn through both sets of data is the Michaelis-Menton function

\[
V = V_{\text{max}} \frac{I}{1 + \frac{I}{I_0}}
\]

where I is the intensity of the flash and σ is the semi-saturation constant. The responses from the two groups
Pupil Light Reflex

Pupillary light reflex thresholds were determined on four albino and four pigmented animals. The intensity required to produce a just discernible pupillary contraction was determined by monitoring the infrared image of the pupil on a television monitor. The average pupillary threshold for the albino was $-3.09 \log \text{cd/m}^2$ (SD = 0.28) and for the pigmented animals was $-2.96 \log \text{cd/m}^2$ (SD = 0.30). The pupils in both pigmented and albino animals had resting diameters of 1.5–2.5 mm. We did not correct for the difference in retinal illumination resulting from the differences in pupil size in the pupil and ERG experiments.

Increment Thresholds

Figure 4 shows increment threshold functions measured on albino and pigmented rats (four in each group). As previously, the threshold criterion was a 50-μV b-wave response. The threshold intensity was determined by fitting a curve to the data obtained against each background (Fig. 3C). Again, both albino and pigmented animals had essentially the same sensitivity, and whatever differences were evident were always in the direction of albinos being more sensitive.

Two methodologic issues are of possible interest. These are the effects of flash duration and the time course of light adaptation. Figure 5A shows the effect of stimulus duration on low-level ERG responses. The flash duration varied from 50–800 msec. The responses increased with flash duration only up to about 100 msec duration. Thus the temporal integrating time of the b-wave was about 100 msec. The results did not differ significantly. If there was any difference, it was that the albino animals were slightly more responsive than the pigmented animals.

Thresholds

B-waves: The absolute dark-adapted threshold can be estimated by determining the stimulus intensity that produces a response significantly above the baseline noise of the recordings in complete darkness. To do this, we chose a 50-μV response to be our threshold. The intensity required to produce a threshold response was determined by fitting a Michaelis-Menton curve to the dark-adapted responses (Fig. 3B). This procedure yielded an average dark-adapted b-wave threshold of $-2.60 \log \text{cd/m}^2$ (standard deviation [SD] = 0.16, n = 7) for hooded and of $-2.75 \log \text{cd/m}^2$ (SD = 0.35, n = 7) for albino. Again, albino and pigmented animals had essentially the same sensitivity, with albinos being slightly more sensitive.
Fig. 3. The peak of an ERG response recorded from a dark adapted albino rat to a flash of intensity $-2.6 \, \log \text{cd}/\text{m}^2$ has been fit by computer with a parabola. A response amplitude of 208 \, nV was obtained from the parameters of the best fitting parabola, shown as a solid line (A). A Michaelis-Menton curve has been fit by computer to the b-wave intensity-response data obtained from another dark-adapted albino rat. Each point is a single measurement obtained by fitting a parabola to the peak of the response. The threshold intensity (50 \, \mu V criterion), obtained from the Michaelis-Menton curve, was $-2.7 \, \log \text{cd}/\text{m}^2$ (B). Intensity response data obtained from an albino rat light adapted with a background intensity of 0.9 \, \log \text{cd}/\text{m}^2 (same animal in “B”). The smooth curve is the best fitting Michaelis-Menton curve and the threshold obtained from this curve is $-0.47 \, \log \text{cd}/\text{m}^2$ (C).

Fig. 4. Increment threshold functions for albino and pigmented animals. Each point plots the mean threshold intensity for evoking a 50 \, \mu V b-wave response on a steady background (four animals in each group). Error bars indicate the standard error of the mean.

Fig. 5B illustrates how light adaptation develops over time. Response amplitude is plotted against the time that elapsed after the onset of the background. These measurements confirm our earlier finding that albino rats\textsuperscript{18} light adapt rapidly and reach steady state in seconds.

**Discussion**

Given the recent report by Balkema\textsuperscript{2} of depressed sensitivity in hypopigmented rats, we wanted to compare his results with ours. Although a direct comparison of ERG and single-unit thresholds may not be strictly valid, the comparison of our ERG thresholds with Balkema’s single-unit results was interesting. Figure 6A, for albinos, showed that in the dark, our b-wave thresholds were 2.0 \, \log units lower than what Balkema found. His increment threshold curve had a different form than ours. A background of $-2.5 \, \log \text{cd}/\text{m}^2$ significantly depressed the dark-adapted sensitivity of our animals, but Balkema’s animals required a background of $-0.5 \, \log \text{cd}/\text{m}^2$ to produce an equivalent effect. With bright backgrounds, the two sets of data converged. For backgrounds brighter than 1.0 \, \log \text{cd}/\text{m}^2 (log of luminance = 0), his albino animals and ours had about the same thresholds.

Figure 6B shows the same comparison for pigmented rats. By contrast with the albino animals, our pigmented animals had thresholds more similar to Balkema’s. Against dimmer backgrounds, there was a fixed 0.4 \, \log difference that might be explained by the different response measures used to determine thresh-
Fig. 5. Effect of stimulus duration on the amplitude of the dark-adapted b-wave response measured from an albino rat. The averaged (n = 5) responses shown were measured with a flash intensity of -2.6 log cd/m² and stimulus durations of 50, 100, 330, and 800 msec (same animal as in fig. 3a) (A). Time-course of light adaptation. A -2.1 log cd/m² flash was presented in the dark and at various times after the onset of a -1.6 log cd/m² background. The amplitudes of the b-wave response are plotted as a function of the duration of light adaptation. Results from both a pigmented (dashed line) and an albino animal (solid line) are illustrated. The two points at zero time indicate the amplitude of the dark-adapted control responses (B).

With brighter backgrounds, our pigmented rats and his had the same thresholds.

Table 1 summarizes the information on visual sensitivity we were able to extract from the literature. Threshold is expressed in terms of the energy in log cd/m²-sec required to elicit the threshold response.

Our experiments provided no support for the idea that albino rats have elevated dark-adapted thresholds. We used two measures of retinal sensitivity (b-wave and pupillary light reflex), and with both, we found that albino rats are as sensitive as pigmented rats. We attempted to compare Balkema’s single-cell thresholds with our b-wave and pupillary thresholds.

The problem was that we were comparing responses that were very different in character. The approach we took was to compare thresholds, defined as the stimulus intensity that elicits a just-detectable signal. In the pigmented animals, our pupillary thresholds and Balkema’s ganglion cell thresholds were identical. In the albino animals, they differed by two orders of magnitude.

Comparing b-waves and cellular responses are a bit more problematic. B-wave thresholds are to a large extent determined by the noise of the recordings (about 25 µV peak to peak). We arbitrarily took a 50-µV response to be “threshold.” Thus the just recordable b-wave was not a just-detectable signal in the same sense that a ganglion cell response is. Support for the validity of doing this comes from Green and Powers who compared b-wave thresholds with gan-
Table 1. Summary of literature on dark-adapted thresholds of albino and pigmented rats

<table>
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<tr>
<th>Reference</th>
<th>Albino</th>
<th>Pigmented</th>
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<tr>
<td>Dott &amp; Echter, 1961</td>
<td>−4.2</td>
<td>−4.2</td>
<td>b-wave</td>
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<tr>
<td>Cone, 1963</td>
<td>−4.7</td>
<td></td>
<td>b-wave</td>
</tr>
<tr>
<td>Cicerone &amp; Green, 1980</td>
<td>−3.8*</td>
<td>−4.4*</td>
<td>ganglion cell</td>
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<tr>
<td>Green &amp; Powers, 1982</td>
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<td></td>
<td>b-wave</td>
</tr>
<tr>
<td>Graves, Green &amp; Fisher, 1985</td>
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<td>−4.0*</td>
<td>colliculus,</td>
</tr>
<tr>
<td>Balkema, 1988</td>
<td>−4.0</td>
<td>−3.9</td>
<td>ganglion cell</td>
</tr>
<tr>
<td>Present Study</td>
<td>−4.1*</td>
<td>−4.0*</td>
<td>pupil</td>
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</table>

* Assumes a 100-msec critical duration.

Note: Comparison of pigmented and albino dark-adapted thresholds. The numbers give the threshold energy in units of log cd/m²·sec. These were calculated by multiplying the luminance by the flash duration. For example, in this study the albino ERG threshold was −2.7 log cd/m² and the flash duration was 50 msec (−1.3 log sec) giving a threshold of −4.0 log cd/m²·sec. In those instances where a flash duration longer than 0.1 sec was used, we assumed a 100 msec critical duration in calculating the dark threshold. The values arrived at in this way are starred.

Key words: albino rat, hypopigmentation, night blindness, threshold, light sensitivity

References

8. Torre V, Matthews HR, and Lamb TD: Role of calcium in regulating the cyclic GMP cascade of phototransduction in retinal rods. Proc Natl Acad Sci U S A 83:7109, 1986.