Elevated Dark-Adapted Thresholds in Albino Rodents

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Albino mice and rats have elevated dark-adapted thresholds compared to normally pigmented animals. The absolute dark-adapted incremental threshold for black mice is about 1.5 log units lower than the threshold for albino mice when measured by single-unit recordings from the superior colliculus. Cell counts from the outer nuclear layer in albino mice are not significantly different from those in black mice, indicating that the elevated dark-adapted thresholds are not due to light damage of photoreceptor cells. No photoreceptor outer segment damage was found in these albino animals at the light or electron microscopic level. These experiments have been repeated in hooded and albino rats. The thresholds from albino rats were about 2 log units higher than the thresholds from pigmented rats in the dark-adapted state. The proximity of the retinal pigment epithelium (RPE) and the pigmented choroid in these animals suggests that a reduction in ocular melanin in hypopigmented animals may be causal to their elevated thresholds. Invest Ophthalmol Vis Sci 29:544-549, 1988

The white rodent is among the most commonly used animals for biomedical and psychological research. Many authors assume that these animals have normally functioning visual systems. Others have been bothered by the variability in dark-adapted light sensitivity in pigmented versus albino animals, but in general this variation has been attributed to experimental artifact.1 Results reported here show that albino rodents are defective in their absolute dark-adapted light sensitivity.

In general, hypopigmentation mutants suffer from defects in cellular organelles, ie, melanosomes, lysosomes and platelet-dense granules.2-4 The idea that a reduction in dark-adapted light sensitivity is associated with hypopigmentation was first suggested by electrophysiological recordings in the mutant mouse pearl.2 This defect originates within the retina and behavioral experiments have shown that it is not caused by anesthetics or other drugs commonly used during the recording sessions.5-6 When the retina from a pearl mouse is isolated from its pigment epithelium, the dark-adapted light sensitivity is indistinguishable from the black mouse.6,7 This result is consistent with the idea that one of the major sites of defective gene expression in the pearl visual system is in the retinal pigment epithelium. In addition, morphological abnormalities (unrelated to light damage) have been found both in the basal portions of the retinal pigment epithelium and in the photoreceptor terminals of the pearl mutant.8,9 If pearl mice have reduced pigment in their RPE and elevated thresholds, then albino mice and rats which totally lack ocular melanin should also have elevated thresholds.

Materials and Methods

Mice were either C3BL/6J or the congenic control C57BL/6J +/+ . Rats were either albino (CD) or hooded controls (Long Evans) from Charles River (Wilmington, MA). All animals were within a few weeks of being weaned and kept in dim (1 cd/m²) cycling 12 hr dark/12 hr light. All investigations involving animals conformed to the ARVO Resolution on the Use of Animals in Research.

Electrophysiology

A mild tranquilizer was given (Taractan, 0.13 mg, i.m.) to induce anesthesia and atropine sulphate (0.1 mg, s.c.) to reduce mucus secretions. Each animal was anesthetized with a single dose of pentobarbital (60 mg/kg). Xylocaine was applied to all pressure points and the animal fixed in a head holder. A craniotomy was performed over the superior colliculus. Visually driven single units were isolated using tungsten-in-glass electrodes in the superficial layers of the superficial colliculus in both black and albino animals.

It was usually necessary to give one supplemental
A PDP-11/73 computer was used with the 2.9BSD UNIX® (University of California, Berkeley, CA) system and kernel modifications for real-time data acquisition. The computer program controlled the entire experiment including stimulus presentation, data acquisition, real-time analysis of the response and subsequent adjustment of the source intensity. In all cases the waveform of the response histogram was constantly monitored to ensure that a center dominated response was evoked. Typically, the responses from three different stimulus presentations were collected (250 msec of before the stimulus, 500 msec of stimulus, and 250 msec after the stimulus). If the cell did not respond well under these conditions, then the stimulus parameters were changed. The number of action potentials that occurred before the stimulus multiplied times two (because the duration is half of the stimulus duration) was subtracted from the number that occurred during the stimulus presentation. This difference is the number of extra spikes due to the stimulus. The computer program then interpolated for a stimulus luminance that would result in three extra spikes, and the stimulus intensity adjusted. This criterion stimulus was then presented 10 times and if the resulting criterion response was within 20% of three extra spikes the criterion stimulus was interpolated for a value of three extra spikes, otherwise the above process was repeated. Each criterion stimulus value was plotted against the respective background luminance resulting in the incremental sensitivity curves shown in Figures 1 and 2. When the stimulus luminance was reduced to a value that resulted in a response 50% of the stimulus presentations, then this was defined as the threshold of this cell. The criterion response of three extra spikes was chosen as a compromise between the cell’s linear response range and the threshold of that cell.

**Appearance of the Stimulus**

The background stimulus was a constant luminance of the entire tangent screen centered on the receptive field of the unit (80–100 degrees). The spot stimulus was a brief flash centered on the receptive field of the cell with a diameter slightly less than the receptive field center. In the initial experiments Kodak gelatin neutral density filters were used to attenuate the source. In the later experiments a video display unit (DSD frame grabber/frame buffer, with a RGB color monitor) was used to present the stimuli. In all experiments the luminance of the test spot and background was verified with a photometer (United Detector Technology, Hawthorne, CA).

**Anatomy**

Animals were anesthetized with pentobarbital and perfused with saline followed by 2%/2% buffered paraformaldehyde/glutaraldehyde. The eyecups were removed, hemisected through the optic disc, dehydrated in alcohol, and embedded in JB4 plastic (Sorvall, Newtown, CT). Care was taken to position the eyecup so the initial sections would be taken from the para-disk region and subsequent sections towards the periphery of the eyecup (see Balkema and Bunt-Milam, 1983, for details). Each section included both superior and inferior retina. The blocks were cut at 4 μm and stained with Richardson’s stain. Counts of the number of outer nuclei layer cells were compared in albino and pigmented mice. A calibrated 100 μm bar was positioned parallel to Bruch’s membrane and all adjacent nuclei within the outer nuclear layer over a length of 100 μm were counted. Ten samples from each retina were averaged and expressed as the total number of nuclei/100 μm of retina. In addition to the outer nuclear layers, the photoreceptor layers were examined for morphological evidence of light damage at ×1000 oil immersion. Tissue from the same animals was also processed for electron microscopy (for details see Balkema and Bunt-Milam, 1983).
Fig. 1. Incremental sensitivity plots from both normal and albino mice. Mice were of the C57BL/6J strain either control (+/+) or albino (c2J/c2J). A total of 24 cells from seven black mice were averaged and 21 cells from three albino mice were examined. Error bars indicate the standard error of the mean.

(rat10,12,13, mouse2). The mean threshold for black mice is 0.008 cd/m² (SEM = 0.0078 < 0.008 < 0.0082) (see Fig. 1). All albino (C57BL/6J c2J/c2J) units are less sensitive than units in black mice in the dark-adapted state. The average absolute threshold from the albino mice is almost 25 times or roughly 1.5 log units elevated compared to the average of the black mice. The mean threshold of albino mice is 0.23 cd/m² (SEM = 0.19 < 0.23 < 0.28). As background luminance increases the difference between black and albino mice decreases (see Fig. 1). At backgrounds above 1 cd/m² the difference is not significant (see Fig. 1).

To examine whether the deficit observed in albino mice is found in other species, the absolute dark-adapted threshold in the albino rat was compared to the hooded rat. Thresholds from albino rats are elevated by 1.95 log units compared to the thresholds from the pigmented hooded rats in the dark-adapted state (Fig. 2). The mean threshold for the hooded rat is 0.0012 cd/m² (SEM = 0.00082 < 0.0012 < 0.0016), while the mean threshold for the albino rat is 0.073 cd/m² (SEM = 0.049 < 0.073 < 0.11). Thus albino mice and rats both show significant elevation in their absolute dark-adapted threshold.

One of the easiest ways of explaining a threshold elevation of this magnitude in the albino animals is that the hypopigmented animals have severe light damage of their retinas compared to the pigmented animals. However, no morphological evidence for light damage in the photoreceptor layer was found in the albino animals compared to the normally pigmented mouse at the light microscope level (see Fig. 3). In addition, estimated numbers of outer nuclear layer nuclei/100 μm² of retina in black mice (mean = 136 ± 22) compared to albino mice (mean = 144 ± 11) were not significantly different. No abnormalities were found in the photoreceptor layer at the ultrastructural level.

Is it possible for light damage sufficient to cause a 1.5–2.0 log unit elevation in threshold to go undetected at the light microscope level? To answer this question mice were placed in constant light for 4–5 days and then tested for possible threshold elevation. The amount of threshold elevation was less than 0.2 log units (not significantly different from pigmented mice) and the retinas from these animals showed severe damage at the light microscope level.

Discussion

It has been demonstrated that rodents with hypopigmented coats have elevated dark-adapted thresholds (albino mice and rats, present findings; pearl mice2; albino rabbits14). Previous work with the pearl mouse shows that this defect originates within the distal half of the retina.2,6,15 Light-induced damage to the distal half of the albino retina16 would be an obvious explanation for these findings; however, light damage was not seen in the albino mouse retina when
compared qualitatively and quantitatively with normally pigmented controls. Furthermore, the c21 albino mutant is relatively resistant to light induced photoreceptor damage compared to other albino mouse mutants. Finally, the cyclic lighting level (<1 cd/m²) used for this study was much lower than that associated with light damage to photoreceptors.

Other investigators who work with albino rodents have noticed that these animals are less light-sensitive than those that are normally pigmented, but have attributed this to light damage, experimental artifact or to melanin being a photochrome which is lacking in albino animals.18,19 Although the possibility that melanin can either produce an electrical response or augment another response at extremely bright stimulus levels cannot be ruled out, it is doubtful that it plays a role at the threshold light levels used in this study.

At present the substrate for the light sensitivity defect in albino animals is not known. Is the sensitivity defect caused by a pleiotropic effect of the albino mutation or is it a result of decreased ocular pigmentation in these animals? One way to answer this question is to record from a selection of hypopigmented, non-allelic mutants with defects in different aspects of ocular melanization. Preliminary results show that regardless of the cause of the hypopigmentation, mutants have sensitivity defects proportional to the loss of melanin.2,14 Thus it seems that that the loss in sensitivity in hypopigmented mutants does not depend on a specific allele, but rather the effect of that allele on ocular melanin.
Where in the visual pathway is the defect in hypopigmented animals? Recordings from retinal ganglion cells in both pearl and albino animals have shown that the defect originates within the retina, and the proximity of the melanin in the choroid and RPE to the photoreceptors suggests that it is in the distal retina. When the retina of hypopigmented animals is isolated from the RPE and the photoreceptors bathed in a normal Ringers solution, recordings from ganglion cells then show normal light sensitivity. This result is consistent with the idea that a diffusible ion is involved in the restoration of normal sensitivity. It has been shown that the retinal pigment epithelium of albino animals has reduced calcium binding due to the low level of melanin. The melanin (both choroidal and RPE) in pigmented animals may serve to buffer the subretinal Ca2+ to lower levels than in albino animals which lack melanin. Although it seems clear that Ca2+ does not act as an internal messenger within photoreceptor outer segments, Ca2+ may influence adaptation by regulating the activity of the guanylate cyclase and the cGMP levels in a feedback stabilization cycle. Modulation of the activity of the cGMP within the rod outer segment may contribute to a light adaptation mechanism. This makes calcium a candidate for the diffusible ion involved in the maintenance of normal dark-adapted light sensitivity. Thus, the defect in the hypopigmented mice would appear to be an abnormal ionic environment in the subretinal space that is caused by the decrease of ocular melanin.

But why would the pearl mouse, with pigmentation intermediate between pigmented and albino mice be less sensitive electrophysiologically than the albino mutant? This may be due in part to structural abnormalities that are peculiar to the pearl mouse. The basal RPE of the pearl mouse has a significantly lower number of infoldings compared to normally pigmented mice. This reduction in basal RPE area may cause a reduction in the rate of ion transport across the RPE in the pearl mouse. Thus, even though the albino mouse lacks a pigmented RPE, it still has adequate transport to a large ion sink in the choroid. The pearl mouse, on the other hand, has reduced RPE melanin in addition to reduced ion transport to the choroid. Preliminary evidence that the albino mouse has a normal RPE at the EM level supports this interpretation.

These findings in the albino rodent appear to represent a phenomenon that may be generalized to many hypopigmented mammals. Initial recordings from hypopigmented mouse mutants with varying amounts of RPE melanin (beige, pale ears, pearl and steel) indicate that the deficit in dark-adapted light sensitivity is proportional to the amount of hypopigmentation and not some other gene effect because these four independent mutations are regulated by separate genes at separate loci. Albino rabbits also show a large elevation of threshold compared to their pigmented controls. Finally, because melanin often accompanies sensory receptors in other systems, for example the auditory system, other sensory defects might be expected in hypopigmented animals.

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

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References


