are warranted to assess the danger to the human retina.

Key words: DNA, repair synthesis, retina, ultraviolet, 300 nm, thymidine incorporation, nucleus, inner segment

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References


Light Exposure Can Reduce Selectively or Abolish the C-Wave of the Albino Rat Electroretinogram

Adrienne L. Groves, Daniel G. Green, and Leslie J. Fisher

The ERG (electroretinogram) of the albino rat is reported to lack a c-wave. Observations of our own suggested that the conditions of light-rearing are important. Consequently, the authors recorded c-waves in two groups of albino rats. One group was reared from birth in dim illumination (dark-reared) and the other in 12/12 cyclic light (light-reared). Rats were tested after birth from 22 days to about 1 year. All dark-reared animals had a c-wave. Rats reared in cyclic light typically had no detectable c-wave. Physiologic and anatomic evidence suggests this consistent difference, c-waves present in dark-reared animals but absent or diminished in light-reared animals, is probably not due to extensive light induced retinal damage. No consistent differences between the two groups were seen in a- or b-wave thresholds, a- or b-wave intensity-response functions, and in the time-course of b-wave dark adaptation. Invest Ophthalmol Vis Sci 26:388–393, 1985

The c-wave, a slow cornea-positive potential that appears after the b-wave, is a prominent feature of most vertebrate electroretinograms (ERGs). Steinberg, Schmidt and Brown have shown that a component of the c-wave is produced by the retinal pigment epithelial (RPE). Faber provided evidence that the vitreal c-wave is the difference between the epithelial response and slow PIII. Both responses are caused by a light-evoked decrease in concentration of extracellular potassium in the subretinal space. The decrease in potassium seems to be secondary to the hyperpolarizing response of the rods to light. Thus, the c-wave reflects a very fundamental aspect of the rod photoreceptor’s response to light stimuli.

Previous investigators have reported that the c-wave is absent in albino rats. Dodt and Echte reported that while pigmented rats had measurable c-waves, albino rats did not. Weidner specifically tested this finding and also concluded that the albino rat lacks a c-wave.

Several years ago in the course of doing other experiments, we observed striking differences in the c-waves recorded from two groups of albino rats. Animals reared with minimum exposure to light had ERGs with c-waves, while the rats of the same strain in another colony that had been reared in a normal animal room with bright, cyclic lighting did not. Unknown to us, Pautler and Noell had made similar observations.

The present study is directed at uncovering the cause for this interesting difference. One possibility immediately comes to mind. The absence of the c-wave in albino rats might be due to retinal light damage during rearing. This is a reasonable hypothesis, since the albino rat retina is known to be
susceptible to damage by light. Rearing albino rats in darkness may prevent that damage and leave the c-wave intact. The present study examines ERGs in albino rats reared in different lighting conditions and presents evidence suggesting that photoreceptor damage does not explain the absence of c-waves in light-reared animals.

**Materials and Methods.** Two groups of albino rats (Sprague-Dawley) were used in this study. All procedures described herein conform to the ARVO Resolution on the Use of Animals in Research. One group (n = 16) was raised from birth in the dark and experienced only occasional dim red illumination. The other group (n = 20) was raised in cyclic lighting, on a 12 on/12 off light cycle. The fluorescent lights in this animal room produced 15–40 ft cd of illumination. Rats were tested from 22 days of age to about 1 year. Six of the above animals (three in each group) were cross-fostered littersmates tested at 6 weeks of age. Before a testing session each was dark-adapted for 12 hr or more and then anesthetized with sodium pentobarbital (5 mg/100 g, intraperitoneally). The pupils were dilated with atropine (1% atropine sulfate) and a topical anesthetic (tetracaine) was applied to the cornea. The rat was positioned in a small cut beside the nose. Electrical potentials were DC amplified and monitored on a storage oscilloscope (Tektronix, Model RM565). Single responses were checked for orientation to insure that the deflections from the baseline determined prior to the flash. Negative responses such as those in Figure 1 shows ERGs recorded under identical conditions in two older rats (2 months). No c-wave is seen in the dark-reared animal (bottom). Figure 1B (center) shows ERGs recorded from two 27-day-old albino rats. Both responses were obtained using the same standard 1.0 cd/m² white stimulus. The top trace is the ERG of a light-reared rat, and the bottom trace is a dark-reared rat. Note that in the dark-reared rat there is a prominent c-wave following the a- and b-waves; measured from baseline, it is approximately 300 /µV. In the ERG of the light-reared rat, however, the c-wave is absent. Figure 1C (right) shows ERGs from two adult rats, recorded under the same conditions. Note again that the c-wave is absent in the light-reared rat but has been recorded in the dark-reared rat.

ERGs were recorded with two silver-silver chloride electrodes. A cotton wick from the active electrode was placed on the cornea and kept moist with a Ringer solution. A wick from the indifferent electrode was positioned in a small cut beside the nose. Electrical potentials were DC amplified and monitored on a Tektronix RM565 oscilloscope. Single responses were either photographed or measured directly from a storage oscilloscope (Tektronix, Model RM564). The stimulating light (a 150W Xenon arc lamp) was focused on a ping-pong ball diffuser placed over the eye. Flash intensity was controlled by placing neutral density filters in the beam. Flash duration was controlled with an electromagnetic shutter (Uniblitz). The unattenuated stimulus produced between 2.2 and 3.0 log cd/m² on the inside of the ping-pong ball, depending on the experiment. After determining b-wave threshold (a 50-gmv response) a b-wave intensity-response series was collected. B-wave amplitudes were measured from the bottom of the a-wave to the peak of the b-wave. C-waves were measured as positive deflections from the baseline determined prior to the flash. Negative responses such as those in Figure 1 from the light-reared animals were equated with zero. Flashes were delivered every 30 sec or at longer intervals. The threshold of the b-wave was monitored between flashes. For brighter flashes the interstimulus interval was increased until sufficient time had elapsed for the dark-adapted threshold to be reestablished.

Histology was done on some animals as follows. After a recording session, the eyes were sutured with surgical silk thread at the dorsal-most aspect (for orientation) and then excised. After hemisection and lensectomy the eyes were fixed, stained, embedded and sectioned according to previously reported procedures. Sections 2 µm thick were stained with methylene blue and examined at X1,008 magnification. Thicknesses of RPE were measured using an AO filar micrometer at a total magnification of X1,260. Sections were checked for orientation to insure that the thicknesses measured were normal to the plane of the RPE by selecting only those sections that passed through the length of individual outer segments. Sections 900–1,200 nm thick were used for electron microscopy.

**Results.** Rearing conditions and c-waves: The first experiment shows that while c-waves are attenuated or absent in light-reared albino rats, they can be recorded in dark-reared albino rats.

Figure 1A (left) shows ERGs recorded from two 27-day-old albino rats. Both responses were obtained using the same standard 1.0 cd/m² white stimulus. The top trace is the ERG of a light-reared rat, and the bottom trace is a dark-reared rat. Note that in the dark-reared rat there is a prominent c-wave following the a- and b-waves; measured from baseline, it is approximately 300 /µV. In the ERG of the light-reared rat, however, the c-wave is absent. Figure 1B (center) shows ERGs recorded under identical conditions in two older rats (2 months). No c-wave is recorded in the light-reared animal (top), but the c-wave is seen in the dark-reared animal (bottom). Figure 1C (right) shows ERGs from two adult rats, recorded under the same conditions. Note again that the c-wave is absent in the light-reared rat but has been recorded in the dark-reared rat.

Figure 2 summarizes our data on the amplitudes of the c-waves that were recorded. The stimulus was our standard 1-sec xenon white flash, I = 1.0 cd/m². All of the dark-reared animals (filled symbols) had measurable c-waves. Most c-waves from dark-reared animals were several hundred /µV (x = 210 /µV, n = 18), although there was variability in amplitude. Most light-reared animals (open symbols) do not have c-waves (x = 16 /µV, n = 20).

**B-waves:** The likely explanation for the findings from the first experiment was that light damaged the retina. The following experiments were designed to test this idea by comparing ERG a- and b-waves of
light- and dark-reared animals for a variety of stimulus conditions. Typical results for ERGs recorded from cross-fostered littermates at various b-wave intensities are shown in Figure 3a. In addition, B-wave intensity-response functions were obtained from 20 animals between 30 days and 48 weeks of age. B-waves in light-reared animals were comparable with those in dark-reared animals. Paired t-tests of b-wave amplitudes at each of 10 intensities showed no differences at the $P < 0.05$ level. B-wave maximum amplitudes in the two groups were $x = 1219 \mu V$ (n = 10) for the light-reared, $x = 1289 \mu V$ (n = 10) for the dark-reared. B-wave thresholds also were comparable ($I = -3.70 \log cd/m^2$ for the dark-reared; $I = -3.79 \log cd/m^2$ for the light-reared). Thus, these data provide no evidence for an effect on b-wave amplitudes of light rearing which eliminated the c-wave.

A-waves: Figure 3A shows intensity-response functions for a-waves and b-waves obtained from the six cross-fostered littermates. No significant differences were found in a-wave thresholds or intensity-response relationships in these animals (or in the other animals on whom such measurements were made).

Dark adaptation: As a measure of the functional integrity of the pigment epithelium, we measured b-wave dark-adaptation curves after 1.0 second exposure to the full intensity 1000 cd/m$^2$ white stimulus, an exposure estimated to bleach about 10% of the rhodopsin.10 These data are shown in Figure 3B, in which each point marks the threshold intensity required to elicit a 50 $\mu V$ b-wave response. Again there were no differences between the light- (open symbols) and dark-reared animals (closed symbols).

Histology: Light micrographs from two cross-fostered littermates are shown in Figures 4A and B. Qualitatively, the retinas from both conditions are similar. Measurements of the thickness of RPE, however, revealed a quantitative difference. The thickness of light-reared and dark-reared specimens was 5.6 $\pm 0.34 \mu m$ and 4.8 $\pm 0.29 \mu m$, respectively ($P < 0.01$, df = 46).

The retinas were examined electron microscopically. Slight disruption of the outer segments and apical processes consistent with mild light damage was noted in one of the specimens from the light-reared animals. All other specimens appeared qualitatively normal.

Discussion. Our results confirm previous studies reporting that the ERG c-wave was absent in the albino rat.5,6 Most albino rats reared in cyclic lighting had no c-waves. We also find, as did Pautler and Noell,8 that dark-reared albino rats consistently have c-waves.

Several possible mechanisms could account for the absence of the c-waves in light-reared animals. Light exposure might (1) damage the photoreceptors and affect their ability to evoke a decrease in extracellular K$^+$; (2) affect the electrical properties of the pigment
epithelium; (3) enlarge the subretinal space sufficiently to dilute the magnitude of the change in K⁺; (4) produce photoreceptor debris that forms a diffusional barrier between the outer segments and the pigment epithelium; (5) cause a larger slow PIII (and consequently a smaller corneal c-wave).

The first possibility seems unlikely because we find that the intensity-response relationships, maximum amplitudes and thresholds of the a- and b-waves are very similar in the light- and dark-reared animals. Furthermore, histologic observation at the light microscopic level reveals no evidence of photoreceptor damage in some light-reared animals.

The pigment epithelium also appears to be normal and undamaged in the light-reared animals. However, there may be changes that cannot be seen microscopically.

What about the space between the photoreceptors and pigment epithelium? This space could become enlarged or a diffusional barrier here could prevent the potassium change from reaching the pigment epithelium. Hoffert and Ubels¹ on trout retina suggest that the distance between the neural retina and the pigment epithelium does affect c-wave amplitudes. We do not believe this explains our results. The size of the subretinal space appears no different in our light- and dark-reared animals. Likewise, the hypothesis that there is a diffusional barrier is not supported by our histology. Debris forming a physical barrier between the photoreceptors and pigment epithelium is not present in the light-reared animals.

Faber² showed in the rabbit that the c-wave was the sum of a positive pigment epithelial and negative retinal potential. Faber called the retinal potential slow PIII. Slow PIII is probably generated by the Müller cells responding to the light-evoked decrease in extracellular potassium. An increase in the amplitude of slow PIII in the light-reared animals also could explain our results. The only way this could occur without a concomitant increase in the epithelial component is for the Müller cells to become more sensitive to potassium. How light could have this action on Müller cells in the light-reared animals is not at all clear. Moreover, to the extent that the b-

![Fig. 2. C-wave amplitudes are shown for light-reared (○) and dark-reared (●) albino rats. The abscissa is the age of the animal. Flash intensity 1.0 cd/m² flash duration 1.0 sec.](image)

![Fig. 3. A, ERG a-wave and b-wave intensity response functions from dark- and light-reared littermates (six animals). Each point is the mean of measurements on three animals. The error bars indicate ± SD of the mean. B, Dark-adaptation curves obtained from cross-fostered dark- and light-reared littermates (four animals). Each point is a single measurement of b-wave threshold intensity (50-μV criterion response). The threshold stimulus intensity is indicated in log cd/m². The points on the left are the dark-adapted thresholds.](image)
wave also reflects Müller cell activity, the absence of enhanced b-wave responses is inconsistent with this idea.

In summary, we can record c-waves in dark-reared albino rats but not in light-reared albino rats. As far as we can determine, on the basis of a-waves, b-waves, the time course of dark adaptation and retinal histology, the neural retina is not affected by these two rearing conditions. Our evidence suggests that light exposure has not affected photoreceptor function or the space between the photoreceptors and pigment epithelium. The two remaining possibilities are that the light may be affecting the pigment epithelium and/or Müller cells. Since damage to Müller cells would be expected to reduce slow PIII and thus enhance, rather than depress, the corneal c-wave, the
likely site of action is the pigment epithelium. If so, these experiments provide evidence for an effect of light on the pigment epithelium without any corresponding changes in the neural retina. Given this, it seems possible that more intense exposure may exert its damaging effect on photoreceptors by first damaging the pigment epithelium.

Key words: ERG, rat retina, c-wave, light exposure, a-wave, b-wave

References