The effects of prolonged dark exposure on visual thresholds in young and adult rats.

DAVID G. BIRCH AND GERALD H. JACOBS.

A comparison of electroretinogram (ERG) thresholds measured on rats reared in continuous darkness and under cyclic lighting conditions shows that by 30 days of age the dark-reared animals have achieved significantly lower thresholds than the animals reared under cyclic illumination. Ten days of continuous dark exposure produces this same increase in sensitivity in adult rats reared in cyclic lighting. These changes in sensitivity appear to reflect structural changes occurring within the rod outer segments.

Perhaps the most basic capacity of the vertebrate visual system is its ability to adjust sensitivity in accord with changes in the photic environment. This adjustment, adaptation, is based on the operation of several mechanisms—fluctuations in pupil size, photomechanical changes within the retina, photopigment bleaching and regeneration, and functional alterations within neural networks. The relative contributions of these mechanisms depend on the details of the visual environment and the species under study. In addition to these mechanisms, several results strongly suggest the possibility that changes occurring in photoreceptor structure in response to changes in environmental light conditions could also be reflected in alterations in the sensitivity of the visual system. Thus it has been recently found that light influences the rates of photoreceptor disc synthesis and shedding. For instance, in rats, disc shedding in rods occurs primarily during a brief interval of time following light onset. In the absence of light, both rhodopsin content and outer-segment length increase. Furthermore, there is a lower phospholipid/opsin ratio following 2 weeks of complete darkness, suggesting that this change in photic environments leads to a closer packing of rhodopsin in the lipid matrix of the outer segment membrane.

Increase in photoreceptor length and photopigment concentration could reasonably be expected to alter the range of light conditions over which the organism can respond. We report here measurements of visual sensitivity in the rat which appear to be directly traceable to the structural changes in the photoreceptor.

Methods

Subjects. Pregnant albino rats were obtained from Simonsen Labs, Gilroy, Calif. Three of these were housed in breeding cages in a large animal colony. After weaning, the offspring were maintained singly in suspended cages (20 by 25 by 20 cm) in standard overhead cage racks. The colony room was illuminated by overhead incandescent lights which were cycled to produce days of 12 hr light: 12 hr dark. The illuminance measured at the floor of these cages with the detector of a Weston low-level illumination meter pointed upward averaged 13 lux with a cage-to-cage variation of <7 lux. The room was maintained at a temperature of 24°C and a relative humidity of 50%.

Three other pregnant rats were placed in constant darkness within environmental chambers (Industrial Acoustics Co.). Offspring were kept with their mothers in large (46 by 36 by 18 cm) group cages until weaning, after which they were housed in individual cages (20 by 25 by 20 cm). Routine animal maintenance was conducted in total darkness whenever possible or with a dim red light whenever necessary. The temperature and humidity within the chambers were the same as those within the main colony room.

Electroretinograms (ERGs) were recorded from animals reared under both conditions at a variety of postnatal ages. In addition, the effects of prolonged dark exposure on adult rats (50 days of age or over) was assessed by placing five adult albino rats and three adult hooded rats in continuous darkness for at least 10 days before the recording session.

The animals were transported to the laboratory in lightproof cages, and all the recording preparations were conducted under dim red lights. As a result, the first significant visual stimuli received by the dark-reared animals were during the recording sessions. Animals reared under cyclic illumination were totally dark-adapted for a period of 24 hr prior to the recording session.

Apparatus and procedure. Rats were anesthetized with sodium pentobarbital and placed in a modified stereotaxic head holder. Normal body temperature was maintained during the recording session through the use of a circulating hot water heater. Pupils were dilated with a topical application of Cyclogyl (1% cyclopentolate hydrochloride). ERG responses were recorded with an insulated tungsten electrode inserted through the cornea at the margin of the limbus. The placement of
Fig. 1. ERG responses from representative albino rats reared in the dark and in cyclic light. Each trace is the average of 10 responses. The cyclically-reared animals were dark-adapted for 24 hr prior to the recording session. Note the changes in calibration scale.

the tip of the electrode into the posterior chamber of the eye was verified visually. An indifferent electrode was sewn into the skin above the orbit. Visual stimuli were presented through a Maxwellian view optical system. The test stimulus was derived from the output of a Bausch & Lomb monochromator (tungsten-iodide source) with exit pupils adjusted to yield a chromatic light having a half-energy passband of 10 nm. A neutral density wheel (0.3 log unit steps) was used to vary the intensity of this light. The image of the source was formed on the rat's pupil by a doublet lens located 9 cm from the eye. The filament image measured 2.1 mm in the plane of the pupil and illuminated approximately 40 degrees of the rat's retina.

A high-speed electromagnetic shutter and digital timer were used to produce 100 msec test stimuli. ERG responses to these stimuli were differentially amplified (amplifier bandpass = 0.2 to 1000 Hz) and then averaged with an Ortec 4623 averager. The averaged response to at least 10 stimulus presentations was read out on an X-Y plotter for further analysis.

Results. Representative ERG responses recorded from animals reared in cyclic light and in constant darkness each to 30 days of age are shown in Fig. 1. These responses were elicited by 500 nm test flashes over a range of different intensities (expressed in log quanta per rod per flash). With the aid of computer averaging, responses could be recorded from dark-reared animals when fewer than one in 100 rods absorbed a quantum of light. Although the cyclically reared animals were dark-adapted for at least 24 hr prior to the recording session, stimuli had to be approximately 10 times more intense in order to elicit a minimal response in these animals than in the dark-reared animals.

It is clear from Fig. 1 that the amplitudes of the ERG responses were smaller for the cyclically reared animals than for the dark-reared animals at each stimulus level tested. The amplitudes of these responses were measured from the lowest point of the negative a-wave to the peak of the positive-going b-wave. Intensity-response functions were derived by plotting these values as a function of stimulus intensity. With the relatively low noise levels in these recordings, 10 µV represented a sizable signal, and so this was used as the criterion response amplitude. The mean threshold and standard error for five dark-reared albino rats was $-1.6 \pm 0.3 \text{ log quanta/rod/flash}$. For five cyclically reared animals, the equivalent value was...
ERGs could first be recorded from albino rats at 10 days of age. The time course of the increase in sensitivity in the two groups of albino rats was determined by recording from animals at a number of postnatal ages. The mean threshold (10 μV criterion) and standard deviation for animals (number in parentheses) at each age are plotted in Fig. 2. The interesting features of these results is that the thresholds of the animals in the two groups were similar until close to 20 days of age and diverged thereafter. Beyond 20 days of age, thresholds for the cyclically reared animals remained constant, whereas the thresholds for the dark-reared animals continued to drop until approximately the thirtieth postnatal day.

Decreases in absolute threshold in the rat were not restricted to those animals which were dark-reared. Similar changes were also found in adult rats that had been reared in cyclic illumination but which were then kept in total darkness for 10 days or longer. In fact, the thresholds of four adult albino rats following 10 days of darkness were not significantly different from the thresholds of the dark-reared animals [t (7) = 0.16, p > 0.5]. Both dark-rearing and long-term dark maintenance therefore substantially lowered the absolute threshold of the albino rat.

The effects of long-term dark exposure were not investigated in detail in hooded rats. Preliminary results, however, suggested that the hooded rat showed a relatively smaller, but still clearly apparent, increase in sensitivity after 10 days in darkness. The hooded rats examined under these conditions showed at least a 0.3 log unit decrease in absolute threshold.

Discussion. Noell and Albrecht5 suggested that dark-rearing leads to a "nonphysiological condition of light deprivation" and reported that such animals are more susceptible to light-induced retinal degeneration. The results of this study indicate that this susceptibility is coupled to a substantial lowering of the visual threshold. Although the thresholds for the dark-reared animals are considerably lower than those for the cyclically reared animals, the maximum ERG amplitudes that can be elicited from these two groups of animals may not differ. Thus a recent study6 reports that the amplitudes of the ERG responses to a stimulus light intense enough to saturate the ERG are not
significantly different in dark-reared and cyclically reared albino rats.

There are strongly suggestive parallels between the changes in threshold described here and the structural changes that are known to occur in the photoreceptors of rats when they are kept in total darkness. It seems likely that changes in receptor metabolic activity underlie both effects. Thus, in the absence of light, disc shedding from rod outer segments is reduced to a greater degree than is the rate of disc addition. The net effect of this is a growth in the length of the outer segment and an increase in rhodopsin content in the retinas of rats maintained in darkness. These two processes reach a new steady state after approximately 10 days. The magnitude of the threshold change we observed is impressive, but so too is the structural change—albino rats reared in darkness from birth show a 51% to 64% increase in rhodopsin content and an 18.1% increase in outer segment length.

The effects of total light deprivation are less dramatic in the pigmented rat than in the albino. The retinas of the pigmented animals are shielded from excessive light exposure by the presence of ocular pigmentation. Probably because of this, total light exclusion results in only a 9.0% increase in rhodopsin content and approximately a 0.3 log unit decrease in ERG threshold. These differences in the effects of photic environment on albino and hooded rats may help to explain the conflicting outcomes of studies designed to compare the sensitivity of albino and pigmented eyes. In man, higher amplitude ERG responses are found in albinos than in normals, whereas in rats, b-wave amplitudes have been reported to be smaller in the albino. In the present study, we found comparable thresholds in albino and pigmented rats when these animals were kept in darkness for 10 days or longer. On the other hand, cyclically reared albino rats had considerably higher thresholds and smaller amplitude ERG responses. Light history is therefore an important variable to consider when contrasting the relative sensitivities of albino and pigmented eyes.

The development of the visual system of the albino rat has been studied previously, and in each case the investigators report changes in the amplitude of the ERG over time. These changes correspond to those we find in the absolute thresholds of the cyclically reared animals. However, Weidman and Kuwabara concluded that the ERG becomes "about the same as that of the normal adult rat" by the fourteenth postnatal day. In contrast, the present results (Fig. 2) indicate that thresholds continue to decline until about the twentieth day before becoming indistinguishable from those of the adult animal.

These developmental changes in the ERG parallel changes seen in retinal anatomy. The retina of the rat is unusually immature at birth as compared with man, corresponding in degree of development to the retina of a 4-month human fetus. At 9 days of age, the nuclear layers of the retina have formed, and small inner segments extend beyond the external limiting membrane. At the distal end of the inner segments the connecting cilia are frequently seen extending toward the pigment epithelium, but little, if any, outer segment material is seen. At 10 days of age, the outer segments are developing rapidly, and it is at this time that the ERG can be first recorded from the retina (Fig. 2). The initial electrical activity is of small amplitude and is cornea-negative. The outer segments grow rapidly in length during the next few days. In the mouse, which has a similar developmental sequence, the rate of disc synthesis far exceeds the rate of disc disposal until the twentieth postnatal day. This period is characterized by a rapid drop in the ERG threshold (Fig. 2). Disc synthesis and disc disposal rates converge at approximately the time that thresholds stabilize in the cyclically reared animals. In the dark-reared animals, however, the convergence of the rates of these two processes is presumably delayed, since their thresholds do not stabilize until 30 days of age.

From the Department of Psychology, University of California, Santa Barbara. Supported by grant EY-00105 from the National Eye Institute. Submitted for publication Jan. 26, 1978. Reprint requests: Dr. David G. Birch, Department of Ophthalmology, College of Medicine, University of Florida, Gainesville, Fla. 32610.

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REFERENCES
Retinomotor activity and the c-wave of the hypoxic trout retina. J. RUSSELL HOFFERT AND JOHN L. UBELS.

The teleost retina exhibits retinomotor activity in response to changing light intensity. We have shown that hypoxia interferes with normal retinomotor activity in the dark-adapted rainbow trout, so that the retina assumes an essentially light-adapted configuration with the response to changing light intensity. We have shown that cones contracted, the rods extended toward the pigmented epithelium, and epithelial pigment expanded. The a-wave is not immediately affected by hypoxia, this in marked increase in ERG c-wave amplitude which we observed during hypoxia in trout. Since the amplitude of the a-wave (receptor potential) is not immediately affected by hypoxia, the increase in c-wave amplitude may be related to the movement of the rods toward the pigmented epithelium, which would cause a greater than normal change in extracellular potassium ion ($[K^+]_o$) and increase in the c-wave amplitude.

Previous reports from our laboratory concerning the electroretinogram (ERG) of the dark-adapted rainbow trout (Salmo gairdneri) have shown that retinal hypoxia caused by acetazolamide, ischemia, or decreased ventilatory flow results in a transient increase in c-wave amplitude of as much as 400%. In our study of the cause of this increase in c-wave amplitude we have considered the retinomotor activity of the teleost retina and the physiological origin of the c-wave. Retinomotor activity has been described in detail by Al and is the mechanism by which the teleost, which has a fixed pupil, can adjust to changes in light intensity via movements of the photoreceptors and retinal epithelial pigment (REP) granules. The c-wave of the ERG is due to a rod-dependent hyperpolarization of the apical membrane of the pigmented epithelium in response to decreasing extracellular potassium ion ($[K^+]_o$).

The myoids of the rods contract in the dark, pulling the outer segments away from the pigmented epithelium. In a previous paper we suggested that hypoxia could interfere with the contractile mechanism of the rods, causing them to extend toward the pigmented epithelium. Since the amplitude of the a-wave (receptor potential) is not immediately affected by hypoxia, the increase in c-wave amplitude may be caused by the close proximity of the rods to the pigmented epithelium which would result in a greater $[K^+]_o$ change near the apical membrane upon photostimulation, thus increasing the degree of hyperpolarization of the apical membrane.

Experiments which combine both electroretinographic and histologic techniques were designed to determine whether the rod movements suggested above actually occur during hypoxia. In this paper we report the changes in the positions of the rods, cones, and REP granules which were observed during retinal hypoxia in light- and dark-adapted rainbow trout. These movements are correlated with simultaneously observed changes in the a- and c-waves of the ERG.

Materials and methods

Experimental protocol. Rainbow trout (S. gairdneri), weighing 200 to 300 gm, were obtained from Midwest Fish Farming Enterprises, Inc. (Harrison, Mich.) and maintained in aerated filtered tap water at 12°C. The photoperiod was 16 hr light and 8 hr dark. To facilitate handling during preparation for the experiment, the fish were lightly anesthetized with tricaine methanesulphonate (MS-222). ERGs were recorded by a method similar to that of Forner et al. The fish were not anesthetized during the experiment, however, they were paralyzed with 2 to 4 U of tubocurarine chloride, and the local anesthetic procaine hydrochloride was applied to the cornea prior to the insertion of the recording electrode into the vitreous humor. Aerated water (12°C) was pumped over the gills at 535 ml/min, and ERGs were recorded at 5 min intervals during 50 min of dark adaptation, which was a sufficient period of time to allow the ERG to reach a constant ampli-