Longterm Dark Induced Fine Structural Changes in Crayfish Photoreceptor Membrane*

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Summary. 1. Fine structural effects of longterm continuous darkness (2–16 weeks) have been quantitatively measured in five rhabdom parameters of adult crayfish (Procambarus) using transmission (Figs. 3–6) and freeze fracture (Figs. 13–15) electron micrographs. The most striking modifications took place during the first four weeks.

2. During the first two weeks in darkness four changes occurred: a) the diameter of rhabdom microvilli increased significantly (Figs. 9, 10), b) the diameter of particles (one or more rhodopsin molecules) visualized on the protoplasmic face of the receptor membrane by freeze fracture (Figs. 13–15) decreased significantly, whereas c) their number increased (Fig. 16) and d) lysosome related bodies near the rhabdom (Figs. 3, 4) in all five retinular regions studied strongly decreased in number (Fig. 12).

3. During weeks 3 and 4 in darkness two further changes occurred: a) the normally regular microvillus pattern of the photoreceptor membrane (Figs. 1, 2) was significantly disrupted and b) the number of membrane particles then fell to about half their initial count (Fig. 16) except in retinular cell eight where they maintained control levels for up to two months in the dark.

4. Most of these effects of prolonged darkness have clear functional implications. Disruption of microvillus pattern and sustained decrease in visual pigment concentration after more than two weeks without light reflect deterioration in vision. Alterations in microvillus diameters and lysosome density imply changes in the membrane turnover steady state resulting from protracted darkness. The data demonstrate that normal photoreceptor membrane could not be maintained in this eye for long in the absence of light.

5. The dark-induced disorganization of microvillus regularity confirms the earlier, disputed demonstration but also shows regional differences in susceptibility which might explain different conclusions drawn from local samples.

6. New distinctive features of retinular cell eight were found in its minimal sensitivity to microvillus disruption compared with the seven regular retinular cells and its maintenance of control densities of membrane particles despite two months continuous darkness.

Introduction

To function effectively photoreceptor membranes must counterbalance exquisite photon sensitivity with recuperative and adaptive processes which sustain optimal responsiveness. These secondary factors must involve regulated cyclic synthesis and breakdown at several levels (e.g., Waterman, 1975; Cosens, 1976; Young, 1976; Holtzman et al., 1977; Röhlich, 1977). Basically the supportive mechanisms provide the differentiation, turnover and replacement of the functional receptor membrane including its component visual pigment molecules. Adaptive mechanisms involve not only adjustments to ambient stimulus conditions but also intrinsic controls modulated by diurnal and perhaps other biological rhythms.

Among the many factors involved both darkness and light seem to play key roles directly or indirectly in such photoreceptor membrane regulation (e.g., Besharse et al., 1977; Hollyfield et al., 1977). Obviously they usually alternate daily in the natural environmental 24 h light and dark cycle. In excess either

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Abbreviations: i, irregularity index; mv, microvillus(i)
can lead to fundamental or even irreversible derangements in vision yet both are essential in appropriate amounts. Thus light induces a wide range of adaptive and disruptive changes in photoreceptor mechanisms of many organisms (e.g., Noell et al., 1966; Kuwabara and Gorn, 1968; Brammer and Clarin, 1976; Brandenburger et al., 1976; Loew, 1976; Pecci Saavedra and Pellegrino de Iraldi, 1976; Robison and Kuwabara, 1976; Bruno et al., 1977; Kong and Goldsmith, 1977).

Darkness also evokes a considerable variety of visual modifications (e.g., Chow et al., 1957; Eguchi, 1964; Röhlch, 1968, 1977; Carpenter et al., 1974; Eakin and Brandenburger, 1974, 1975; Roach and Wiersma, 1974; Besharse and Brandon, 1976; Barnes and Goldsmith, 1977; Besharse and Holleyfield, 1977; Brandenburger, 1977; Blest, 1978; Yamamoto and Yoshida, 1978; Nässel and Waterman, 1979). The well known depolarizing effect of darkness on vertebrate photoreceptors (e.g., Tomita, 1970, 1976) testifies to the fundamental importance of dark effects, as do the familiar but manifold responses of dark adaptation in all kinds of eyes. Massive synthesis of photoreceptor membrane may be induced by darkness in certain spiders (Blest, 1978) and crabs (Nässel and Waterman, 1979).

On the other hand damaging or disruptive effects of darkness on visual systems are widespread. Thus in some invertebrate rhabdomeric eyes photoreceptor membrane disruption and microvillus pattern disorganization have been found to occur in the dark phase of normal daily light dark cycles (isopods, Nemanic, 1975; flatworms, Bedini et al., 1977). But usually more than 24 h or even several weeks of continuous darkness are required before damage becomes obvious.

However, there are widespread ontogenetic differences in susceptibility of photoreceptor systems to darkness both in various invertebrates (Eguchi and Waterman, 1966; Roach and Wiersma, 1974; Röhlch, 1977) and in vertebrates (e.g., Wiesel and Hubel, 1963; Chow, 1973; Besharse and Holleyfield, 1977).

The present report describes a number of marked effects of long term darkness (2-16 weeks) on the fine structure of rhabdomeric membranes in the compound eye of the crayfish Procambarus. Disruption of normally regular microvillus organization had previously been reported to result from extended periods in darkness (Eguchi, 1964, 1965; Eguchi and Waterman, 1966). But these results had become rather controversial since several other workers without presenting any new quantitative data have attributed these selective disruptive results to fixation artifacts even though the original crayfish results had later been confirmed and extended (Roach and Wiersma, 1974).

Partly to settle this ambiguity, but more importantly because of accelerating general interest in receptor membrane turnover, we have reexamined this phenomenon with new experiments and additional techniques. Our results show that darkness does in fact induce quantitatively significant increases in microvillus irregularity as well as significant modifications in the other four parameters studied.

**Materials and Methods**

Adult crayfishes (Procambarus clarkii) about 7-8 cm in body length kept individually in aerated fresh water at 20 °C were used. Control animals were maintained under normal fluorescent room lights controlled on a 12L:12D cycle. Experimental animals were kept in constant darkness for periods of 2, 4, 8, 12, or 16 weeks. Their only illumination was a few minutes of dim darkroom red light per week for feeding and cleaning. At fixation the compound eyes of these dark adapted crayfish were prepared under photographic red light but immediately returned to darkness for the first 30 min in fixative solution.

We concluded from continued normal feeding and otherwise apparently normal health that retinal changes in the experimental animals were primarily due to the absence of light. A really decisive control is difficult to design but no evidence suggested that any of the five structural alterations in the eye were secondarily induced by poor nutrition or failing health in the dark kept crayfish.

For transmission electron microscopy the retina was fixed for two hours at 4 °C in 5% glutaraldehyde buffered at pH 7.4 with 0.1 M Sorenson's phosphate solution. After fixation the tissue was washed with buffer, post-fixed for two hours with 2.5% OsO4, dehydrated and finally embedded in epoxy resin. Thin sections were stained first with uranyl acetate and then with lead citrate.

For freeze fracture electron microscopy retina tissues were fixed from 4 to 5 h at 4 °C in 5% glutaraldehyde with 0.1 M Millonig phosphate solution containing 1% sucrose. Dark adapted eyes were fixed under a darkroom light as described above. The tissues were washed for 12 h in buffer, then immersed successively for 1 h each in 10%, 20% and 30% buffered glycerol. Subsequently retinas were quickly frozen in liquid freon for 1 min and immediately transferred to liquid nitrogen.

Fracturing was done with a razor blade at −110 °C under a vacuum of 10−6 Torr in a Balzer's high vacuum freeze-fracture apparatus. Platinum was obliquely evaporated onto the fractured specimen followed by a coating of carbon from above. Observations of both types of preparation were made with a Philips 200 electron microscope.

To begin with inspection of the electron micrographs showed that substantial differences were present not only with regard to period in the dark but also as a function of location in the rhabdom. To specify these we divided the photoreceptor organelle for reference into five successive axial regions (Fig. 1).

Starting at the distal end these are: 1) the small ovoid rhabdome of R8 (Fig. 3); 2) a distal neck region comprising some microvilli from R1–R7 arranged in alternate bands (Fig. 3); then the ellipsoidal rhabdom “proper” made up of about 25 regular orthogonal platelets of microvilli from R1–R7 divided for analysis into: 3) distal; 4) middle; and 5) proximal regions. Each of the last three comprises about a third of the total axial length of the whole ellipsoid. Lysosome counts were made adjacent to all five of these rhabdom elements (Fig. 4).

While preliminary estimates of retinal changes were indicative of objective measurements were necessary to assess the microvillus disorganization (Figs. 5, 6). To do this all appropriate electron micrographs were examined under a dissecting microscope with...