LOCAL CONTROL OF RETINOMOTOR ACTIVITY IN THE FISH RETINA

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Abstract—Small circular spots of light were focussed on otherwise unilluminated retinas of paralyzed fish, Cichlasoma biocellatum. The retinas were subsequently examined histologically, and a small circular region was found within which the cones and pigmented retinal epithelium were photomechanically light adapted; rods could not be resolved. The same result was obtained with the optic nerve cut. The region was circular and about the same size as the adapting spot. There existed a transition zone between light- and dark-adapted regions within which the photomechanical state was intermediate. These results exclude the hypothesis that light-induced retinomotor phenomena are systemically controlled, and favor local control. An analysis of the transition zone favors the view that photomechanical light adaptation of a given cell may be caused by light caught by that cell or its neighbors.

Key Words—retina; retinomotor; photomechanical; fish. Animal Classification—Cichlasoma biocellatum (Euteleostei, Perciformes, Cichlidae).

INTRODUCTION

In many fish and amphibia, the relative positions of photoreceptor outer segments and retinal epithelial pigment depend upon the ambient light level. While in the dark, the myoids of the rods contract such that their outer segments assume a position close to the outer limiting membrane. The cone myoids lengthen, displacing their outer segments sclerad, near the melanin granules in the pigment epithelium. During light adaptation, these positions gradually reverse; rod outer segments move sclerad while cone outer segments and melanin granules move vitread. These photomechanical movements are usually interpreted as adaptations of the retina to its photic environment (Ali, 1975; Arey, 1915).

Recent work (Burnside, 1976; Murray and Dubin, 1975) has contributed toward an understanding of the molecular basis of some of these phenomena. In contrast, our work was intended to elucidate the locus of control of the retinomotor activity. Our results will be interpreted according to the following three general hypotheses.

(1) Systemic control. The movements might be controlled by a circulating hormone or by central neural influences. According to this hypothesis, some center in the brain (or elsewhere) would receive information about ambient light levels, and distribute signals (chemical or neural) to the photoreceptors and pigment epithelium. The early experiments of Arey (1916) suggested that systemic control might be mediated by efferent fibers to the retina. Although in Arey's time the existence of efferent fibers was uncertain, it is now more strongly established (Sandeman and Rosenthal, 1974; Vanegas, Amat and Millan-Essayag, 1973; Wittkowsky, 1971). The systemic hypothesis gains further support from the observation that retinomotor activity follows a circadian rhythm, even in total darkness (John, Segall and Zawatsky, 1967).

(2) Intercellular local control. The photomechanical activity of an individual cell might result from light received by itself and its neighbors. Nearby cells would intercommunicate synaptically or via secretion of some locally diffusible substance. This hypothesis is made plausible by the discovery of functional receptor contacts in several vertebrates (Baylor, Fuortes and O'Bryan, 1971; Copenhagen and Owen, 1976; Fain, Gold and Dowling, 1976) and by the intraretinal presence of melatonin (Bubenik, Brown and Grotta, 1976).

(3) Intracellular local control. The photomechanical activity of an individual cell might result only from the light received by that cell.

Our experimental approach has been to shine a small focussed spot of light on the retina, and then to measure, in histological sections, the spatial extent of the adapted region. An abstract of our findings has appeared elsewhere (Easter and Macy, 1976).

METHODS

This work was first begun at the Bermuda Biological Station, using a reef fish, Haemulon sciurus, the white-striped grunt. Upon returning to Ann Arbor, the experiments were continued on a different species, Cichlasoma biocellatum, the "Jack Dempsey", 7-13 cm long, readily available at local pet stores. All the results reported here were obtained on the latter fish, but similar observations were made initially on the grunt. All successful experiments were carried out between March and mid-July of 1976; late summer experiments yielded uninterpretable results.

A diagram of the Maxwellian view stimulator is shown in Fig. 1. An image of aperture A2 (0.5 mm dia) is brought to focus in the center of the entrance pupil (2.1±3.0 mm dia) of the fish's right eye. The circular aperture, A1, was imaged at the back focal plane of LS and therefore on the fish's retina, assuming that the eye is emmetropic. This is a controversial assumption (Schwassmann, 1975), and our use of a small pupillary aperture was intended to...
A local region of photomechanically light-adapted cells was found in the fundi of retinas receiving stimulation at intensities within the range of $2 \times 10^6 - 10^7$ quanta/sec mm$^2$. An example is shown in Fig. 2(a). Within the adapted region, cone ellipsoids have moved vitread and are lined up against the outer limiting membrane. Pigment granules in the pigment epithelium have also moved vitread while in the rest of the retina, they are concentrated sclerally. Rods could not be resolved. Figure 2(b) illustrates the transition zone, about 7-10 cone diameters wide, between light- and dark-adapted retina, within which the cone ellipsoids and epithelial pigment granules occupy positions intermediate between the two states.

The shape of a locally adapted region was determined by examination of all those sections which included a part of it. The width of the light-adapted region (excluding the transition zones) was measured. In Fig. 3, the half widths are plotted on the ordinate; the abscissa shows the space between successive sections. The points fit a semicircular contour, to be expected if the light-adapted zone were circular. The diameter of the best fitting semicircle in Fig. 3 is 600 $\mu$m. Corrected for a linear shrinkage factor of 30% (Johns and Easter, 1977), this corresponds to a light-adapted disk of 857 $\mu$m in diameter in the live fish, close to the computed value of the retinal image in this animal, 830 $\mu$m.

A local circular light-adapted patch was also obtained from the animal whose optic nerve had been cut prior to stimulation.

**RESULTS**

A local region of photomechanically light-adapted

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**Fig. 1. Stimulator.** Four rays are traced from the quartz halogen source (S) to the fish's eye (EL). The filament is imaged at lenses L$_1$ and L$_4$. The aperture A$_2$ is imaged at the entrance pupil of the fish's eye. The plane at L$_1$ is imaged at lens L$_3$. The aperture A$_1$ is imaged at the back focal plane of lens L$_4$; on the fish's retina. F$_1$, heat absorbing glass; F$_2$, neutral density filters; F$_3$, interference filter.

**Fig. 2.**

- **Fig. 2(a):** A local circular light-adapted patch in the retina. The half-width of the light-adapted region over a radius of 20 $\mu$m, half bandwidth = 20 nm. The energy of the beam was measured at this wavelength with a radiometrically calibrated photodiode (United Detector Technology, PIN90 Cal DF) at the plane of the pupil, in air. Intensity was altered by insertion of calibrated neutral density filters, F$_2$.
- **Fig. 2(b):** Illustrates the transition zone, about 7-10 cone diameters wide, between light- and dark-adapted retina, within which the cone ellipsoids and epithelial pigment granules occupy positions intermediate between the two states.

**Fig. 3.** Reconstruction of the light adapted region. The half-width of the light-adapted region, uncorrected for shrinkage, is plotted against section number. The distance between successive sections is 50 $\mu$m. The data obtained with and without knowledge of section number are given by open and filled circles, respectively. All data from one fish.
Fig. 2. Retinal photomicrographs. (a) Low magnification view of a locally light-adapted region, surrounded by dark-adapted retina. Within the light-adapted region, cone ellipsoids and epithelial pigment are vitread. Outside it, they are more sclerad. (b) Higher magnification view of the right boundary of the region shown in (a). Note the transition zone between light- and dark-adapted regions. Convention: sclerad upwards. Calibrations: 100 μm. Abbreviations: PRE, pigmented retinal epithelium; OLM, outer limiting membrane; ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.
Apparentiy, the circadian modulation of cone lengths is intraretinal. We therefore reject the first hypothesis proposed in the Introduction.

Our conclusions conflict with earlier reports, those of Ali (1964) and Arey (1916). Ali (1964) illuminated one eye of a goldfish and found weak light adaptation in the pigment epithelium of the other. It is possible that his experiment was contaminated by spread of light from one eye to the other. When a bright light is projected into one eye of a goldfish, the optic disc of the contralateral eye, viewed through the pupil, becomes luminous (unpublished observations). We suggest that in Ali's experiment, part of the bright light sent through the pupil of the experimental eye passed through the optic disk of that eye, into the translucent skull and brain, and into the control eye through its optic disk. We acknowledge, however, that there might exist some minor systemic influence which our experiments could not rule out. Or it may be that the different results represent real differences between the two kinds of fishes. Arey (1916) reported that optic nerve section in *Amietius* blocked photomechanical light adaptation. But he also reported many instances in which the movements were not completely blocked by this procedure. In addition, he showed that photomechanical movements were not blocked by optic nerve section in *Abealz or Fundulus*. Our work therefore confirms and extends most of his results, if not his interpretations and conclusions.

The rest of our results seem consistent with both of the remaining hypotheses, but we favor the second, control by intercommunicating neighborhoods of cells, over the third, intracellular control. Our bias emerges from an analysis of the transition zone. Its occurrence could be reconciled with the intracellular hypothesis by invoking either of two assumptions. The first is mechanical linkage, at or near the myoids of neighboring cells. According to this explanation, an illuminated cone could contract and physically pull its unilluminated neighbors down with it. We think this is unlikely since no interreceptor contacts have been demonstrated at so distal a level. A second way to account for the transition zone is by invoking poor image quality. If, for example, the step from high to low intensity were blurred over 50 pm or so, we might suppose that the incomplete responses in the transition zone were graded according to the intensity received. Such an interpretation seems unlikely for two reasons. First, it is inconsistent with behavioral data on other species of fish (Schwassmann, 1975) whose acuity has been shown to correspond to intercone spacing. If the step from high to low intensity required 10 cone diameters (see Fig. 2b), such high acuity would be impossible. To be sure, our use of a small pupillary aperture may have degraded the image, owing to diffraction. But the radius of the Airy disk, even with our small aperture, is still less than 1 pm, much too small to account for the transition zone. Second, if graded responses to low intensities were common, we would expect to have observed them over substantial regions in some of our animals exposed to low intensities, but we did not. Thus, we believe that the presence of the transition zone argues against a purely intracellular control system, and we favor control by intercellular communication. The radius of the photomechanical adaptation pools is
probably about the width of the transition zone, 7–10 cones. This is roughly equivalent to the range of electrophysiological interactions between cones in turtle retina (Baylor et al., 1971).

One final comment concerns contrast of the retinal image. If we accept that photomechanical light adaptation occurs only when light above a certain threshold intensity is received in the adaptation pool, then Fig. 4 suggests that contrast is quite high. When the intensity of the central part of the stimulus exceeded threshold (2 x 10^6 quanta/mm^2 sec) by a factor of 1000, the diameter of the adapted region had not changed significantly. This implies that regions adjacent to the stimulated areas received intensities only 1/1000 (or less) that of nearby regions. This very high contrast is roughly the same as reported for the mechanoreceptors (reviewed by Brown, 1961, pp. 405–408).

In summary, we have shown that the photomechanical responses (light-induced retinomotor activity) by cones and pigment epithelium are locally controlled. Our data suggest that the control is derived from signals received by a small neighborhood of cells, on the order of tens of μm in diameter, and that quanta caught by some cells may indirectly affect neighbors. Local control has also been demonstrated in the pigment migrations in the eyes of insects (reviewed by Goldsmith and Bernard, 1974, pp. 407–415) and squid (Daw and Pearlman, 1974). These results and ours, stand in marked contrast to the systemic control by a circulating factor, which crustaceans possess (reviewed by Brown, 1961, pp. 405–408). We refrain from speculating on why some animals should control systemically what others manage locally.

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REFERENCES


