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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; Witkovsky, 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 interreceptor 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 Grota, 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 L5 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

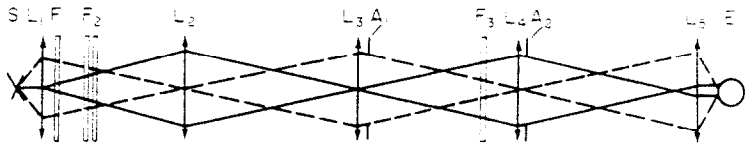

Fig. 1. Stimulator. Four rays are traced from the quartz-halogen source (S) to the fish's eye (E). The filament is imaged at lenses L_2 and L_4. The aperture A_2 is imaged at the entrance pupil of the fish's eye. The plane at L_1 is imaged at lens L_3. The aperture A_1 is imaged at the back focal plane of lens L_5 and on the fish's retina. F_1, heat absorbing glass; F_2, neutral density filters; F_3, interference filter.

diminish the possible blur due to refractive error. Wavelength was set by interference filter, F3 (Optics Technology Monopass, $\lambda_{max} = 538$ nm, half bandwidth = 20 nm). The energy of the beam was measured at this wavelength with a radiometrically calibrated photodiode (United Detector Technology, PINIO Cal DF) at the plane of the pupil, in air. Intensity was altered by insertion of calibrated neutral density filters, F2.

Fish were paralyzed by intramuscular injections of 2–3 mg of gallamine triethiodide (Flaxedil, Davis & Geck), fitted with a mouthpiece, and placed in the stimulation chamber. The right eye looked through water and a clear acrylic window into the beam of the stimulator. Aerated water flowed over the gills at a rate of 150–200 ml/min. A light-tight box was placed over the stimulation chamber, the beam and room lights were turned off, and the animal was allowed to dark adapt for 2 hr. At the end of this time, the beam was turned on and a small window in the light-tight box was opened to let the beam in. If necessary, it was recentered in the pupil, and the eye was exposed for 1.25 hr. At the end of this time, the position of the beam was again checked to make sure that the fish had not shifted. The animal was then removed and blood vessels in the tail were examined microscopically for blood movement. If the animal had shifted during exposure, or if no blood movement was seen, the animal was discarded. Otherwise, the fish was decapitated and the right eye removed and placed in Bouin's fixative. These final steps took only 5–7 min, not long enough for significant photomechanical adaptation by the room lights. The left eye was also removed and the diameter of its lens measured. After 1–2 days of fixation, the lens and the anterior half of the right eye were removed and the tissue returned to the fixative for another 1–2 days. The tissue was then dehydrated through a series of alcohols, embedded in paraffin, and sectioned at 10 μm. Every fifth section was saved, mounted and stained with hematoxylin and eosin.

Under tricaine methanesulfonate anesthesia, the optic nerve of one animal was cut intraorbitally with scissors. The nerve was clearly seen to have been sectioned. The animal was allowed 3 days to recover and was then stimulated in the manner described above. The postoperative interval was too brief for any significant regeneration of optic nerve fibers (Attardi and Sperry, 1963).

The size of the retinal image of the spot was calculated for each fish with geometrical optics using the following information and assumptions. The image of A1, in the back focal plane of L5, was determined to subtend 17° 8′ at the center of the fish's lens (value corrected for refraction at the air–plastic–water interfaces). The retinal image was assumed to subtend this same amount with respect to the lens center. The distance from the lens center to the retina was computed as 2.46 × the radius of the lens measured in the contralateral eye (Easter, Johns and Baumann, 1977).

RESULTS

A local region of photomechanically light-adapted cells was found in the fundi of retinas receiving stimulation at intensities within the range of 2×10^6–10^{11} 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 μm. Corrected for a linear shrinkage factor of 30% (Johns and Easter, 1977), this corresponds to a light-adapted disk of 857 μm in diameter in the live fish, close to the computed value of the retinal image in this animal, 830 μm.

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

Figure 4 shows the effects of intensity upon the diameter of the locally light-adapted region over a

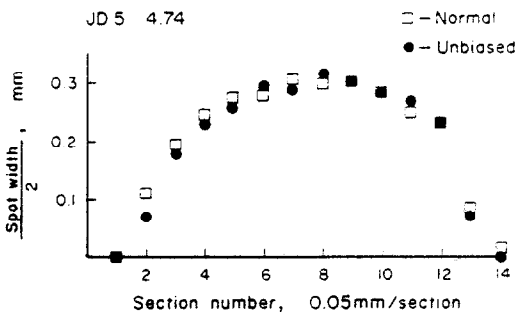

Fig. 3. Reconstruction of the light adapted region. The half-width of the light-adapted region, uncorrected for shrinkage, is plotted against section number. The distance between successive sections is 50 μm. The data obtained with and without knowledge of section number are given by open and filled circles, respectively. All data from one fish.

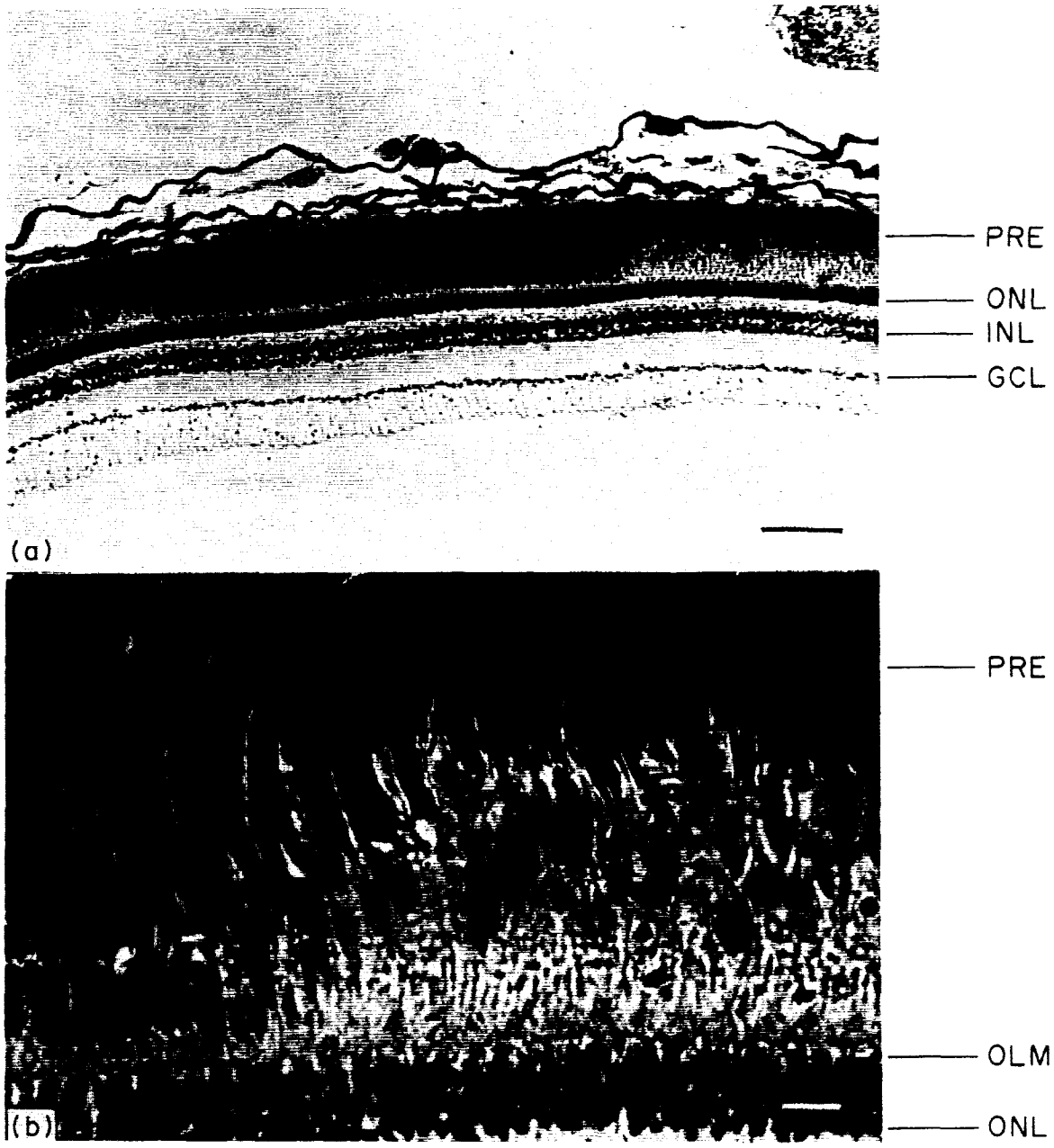

Fig. 2. Retinal photomicrographs. (a) Low magnification view of a locally light-adapted region, surrounded by dark-adapted retina. Within the light-adapted region, cone ellipsoids and epithelial pigment are vitread. Outside it, they are more sclerad. (b) Higher magnification view of the right boundary 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.

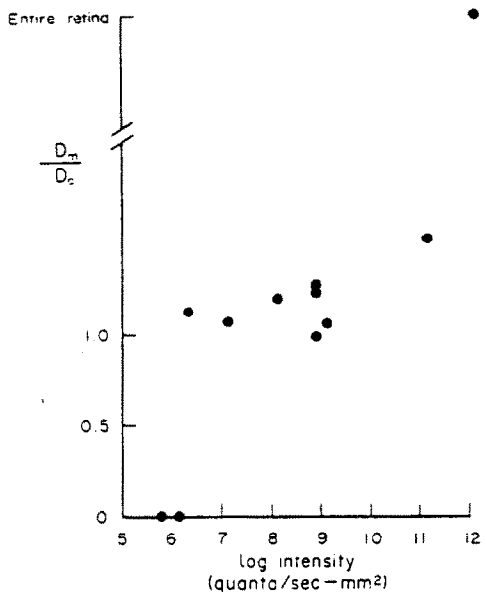

Fig. 4. Size of the locally light-adapted region and the intensity of the stimulating spot. Ordinate: the ratio, D_m/D_c (measured diameter of the locally light-adapted region, corrected for shrinkage)/(calculated diameter of the stimulating spot). Abscissa: log intensity of the stimulating spot at the retina.

range of six decades. The ordinate is the ratio: (measured diameter of the locally adapted region)/(calculated diameter of the retinal image), corrected for histological shrinkage. Each point represents the results from one fish. Below an intensity of 2×10^6 quanta/sec mm^2, no light-adapted region was observed. Over the next four decades, the ratio was essentially constant and close to unity. (The systematic tendency for the ratio to be slightly greater than unity is probably not significant. Our computed image diameter (D_c) was based on the assumption of an intraocular projection distance of 2.46 × lens radius, and this conversion factor may not be exactly correct. A larger value (e.g. 2.5–2.6, Matthiessen, 1880) would have resulted in a better fit. Another possible source of error is the assumption that the tissue shrank by 30% during histological processing. This was derived from work on goldfish, and the eye of *Cichlasoma* may not react identically.) At higher intensities, the adapted region was considerably larger than predicted from the size of the adapting spot.

DISCUSSION

The presence of a local region of photomechanical light adaptation rules out the possibility of control by a substance in the systemic circulation, since nearby retinal regions share a common blood supply. Apparently, the circadian modulation of cone lengths (John, Segall and Zawatsky, 1967), can be overridden by light. These local effects could be controlled by a retinotopic retina–brain–retina pathway, but our results with optic nerve section allow us to reject this alternative. These two results, taken together, indicate that the control of light-induced retinomotor activity 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 *Ameiurus* 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 *Abramis* 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 μm 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 μm, 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 (2×10^6 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 human retinal image (Gubisch, 1967), but comparisons are difficult, owing to the very different methods used. At intensities much higher, the adapted zone increased in size, probably as a result of intraocular scatter from the lens and ocular media.

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. 189–191) 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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