RHYTHMIC MOVEMENTS OF CONES IN THE RETINA OF BLUEFISH, POMATOMUS SALTATRIX, HELD IN CONSTANT DARKNESS

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Rods, cones and retinal pigment in a number of fishes undergo alterations of shape and position in response to light and darkness (Arey, 1916; Parker, 1932; Walls, 1942; Nicol, 1965). Several fresh-water and anadromous fish species kept in continuous darkness exhibited well-defined rhythms of photomechanical changes in the retina, implying an internal control mechanism (Welsh and Osborn, 1937; Wigger, 1941; Arey and Mundt, 1941; Ali, 1961; John and Haut, 1964; John, Segall and Zawatzky, 1967; John and Gring, 1968).

We undertook the following study to determine whether the retina of the bluefish *Pomatomus saltatrix* (Linnaeus), a marine pelagic species, undergoes photomechanical changes ordinarily associated with dark- and light-adaption in the absence of light cues. The facts which prompted the study were: (a) under a natural light regime, the bluefish is diurnal and its swimming speed is associated with daily light changes but not entirely controlled by them; (b) rhythms of bluefish swimming activity are evidently in part controlled by internal mechanisms; and (c) the bluefish seems to depend on vision for capturing prey and is particularly active during morning twilight forage (Olla, 1966a, b).

The above facts led us to hypothesize some internal control for adaption in the retina. To investigate this we measured cone and pigment epithelial movement in the retinae of bluefish which we had sampled periodically while the fish were being kept in constant darkness.

Materials and Methods

We used bluefish 12 to 17 cm. long captured in Sandy Hook Bay, New Jersey, for this study. We held experimental fish in two 75-gallon recirculating aquaria with water temperature at $22 \pm 1°$ C. and salinity at 25‰. Each aquarium was illuminated by two 24-inch, 20-watt fluorescent lamps. A frosted plate glass was placed between the lamps and water surface to diffuse light evenly. Each aquarium with its lighting system was enclosed in a light-proof box. A photo-timer controlled artificial day length.

Before we could establish the presence or absence of a retinal rhythm, it was necessary to determine the range of photomechanical changes in the bluefish retina under both darkness and light and the approximate time necessary to complete these changes. For dark-adaption we subjected five fish to total darkness and removed one fish at intervals of 15, 30, 60, 120, and 180 minutes after the natural

seasonal time of sunset (1930 hr.). For light-adaption we subjected five fish to a light intensity of 50 ft.-c. and removed one fish at intervals of 15, 30, 60, 120, and 180 minutes after the natural seasonal time of sunrise (0630 hr.).

Our procedure was as follows: we removed a fish at random from an aquarium, immediately decapitated it, punctured the cornea and fixed the head in Held's solution. This procedure took less than one minute. For fish sampled under darkness, we performed the procedure under illumination from a red-tinted, 15-

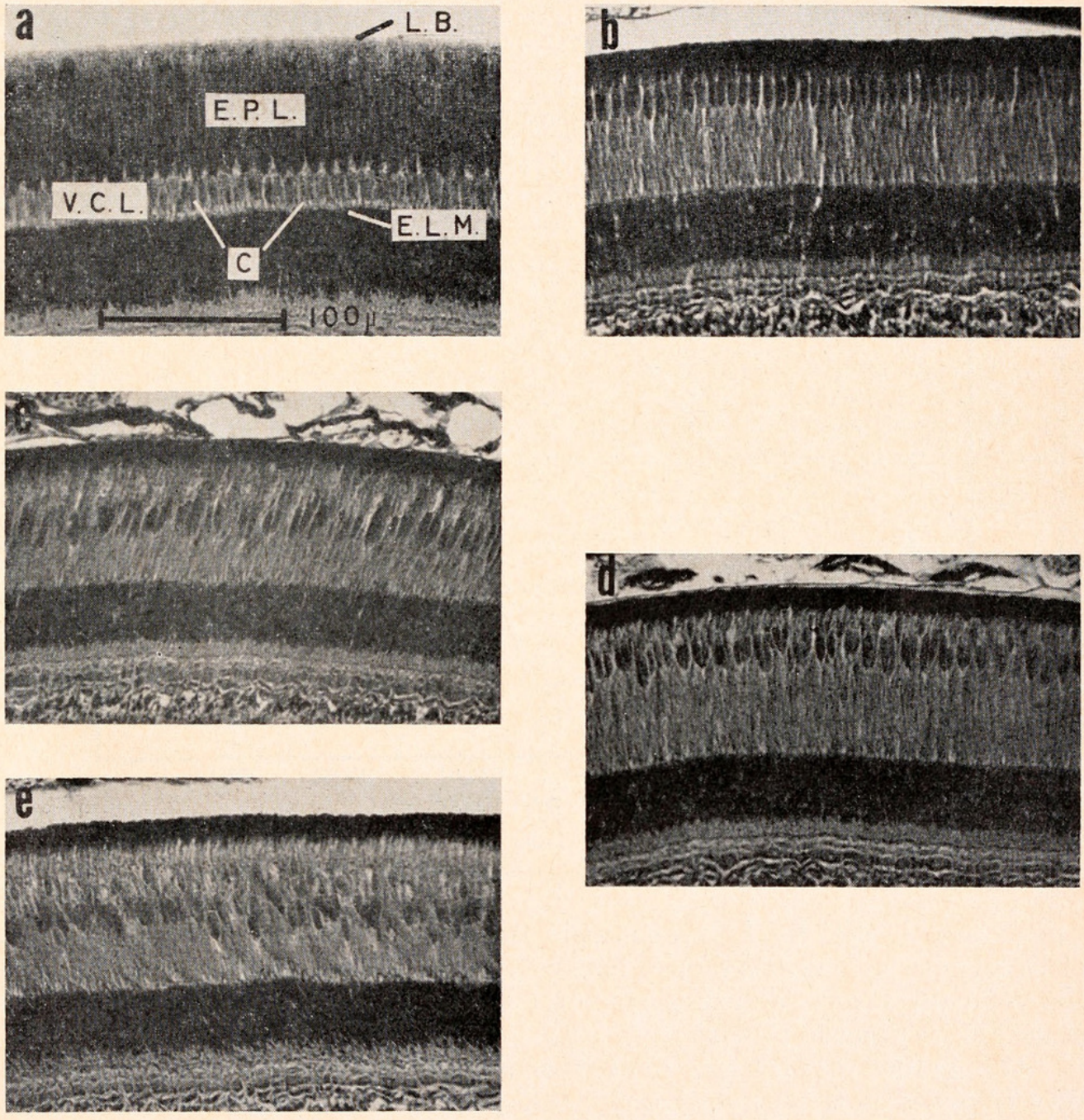

FIGURE 1. Photomicrographs showing the comparison between transverse sections of fully light- and dark-adapted bluefish retinae with those from fish sampled under constant darkness. a. Fully light-adapted retina. b. Fully dark-adapted retina. c. Retina of bluefish sampled at 1200 hours (16½ hr. after light offset). d. Retina of bluefish sampled at 2400 hours (28½ hr. after light offset). e. Retina of bluefish sampled at 1200 hours (40½ hr. after light offset). L.B., lamina basalis; E.P.L., epithelial pigment layer; V.C.L., visual cell layer; E.L.M., external limiting membrane; c, cone ellipsoids.

watt tungsten light bulb. To ensure complete penetration of the fixative, we slit the eyes at the sclerocorneal junction after they had been fixed for 24 hours in darkness. After an additional 24 hours of fixation, we enucleated the heads and removed corneas and lenses. We then dehydrated the eyes in an ethyl alcohol series, cleared them in toluene and embedded them in paraffin (mp 56.5° C.). We sectioned the embedded eyes serially in a transverse plane at 8 μ and stained with Harris' hematoxylin and eosin.

To determine the presence of a retinomotor rhythm under continuous darkness, we held the fish for seven days under an artificial photoperiod of 13 hours light of constant intensity (50 ft.-c.) beginning at 0630 and 11 hours dark beginning at 1930. We assumed that by then the fish had become acclimated to the light cycle. Then beginning at the time the light went off on the seventh

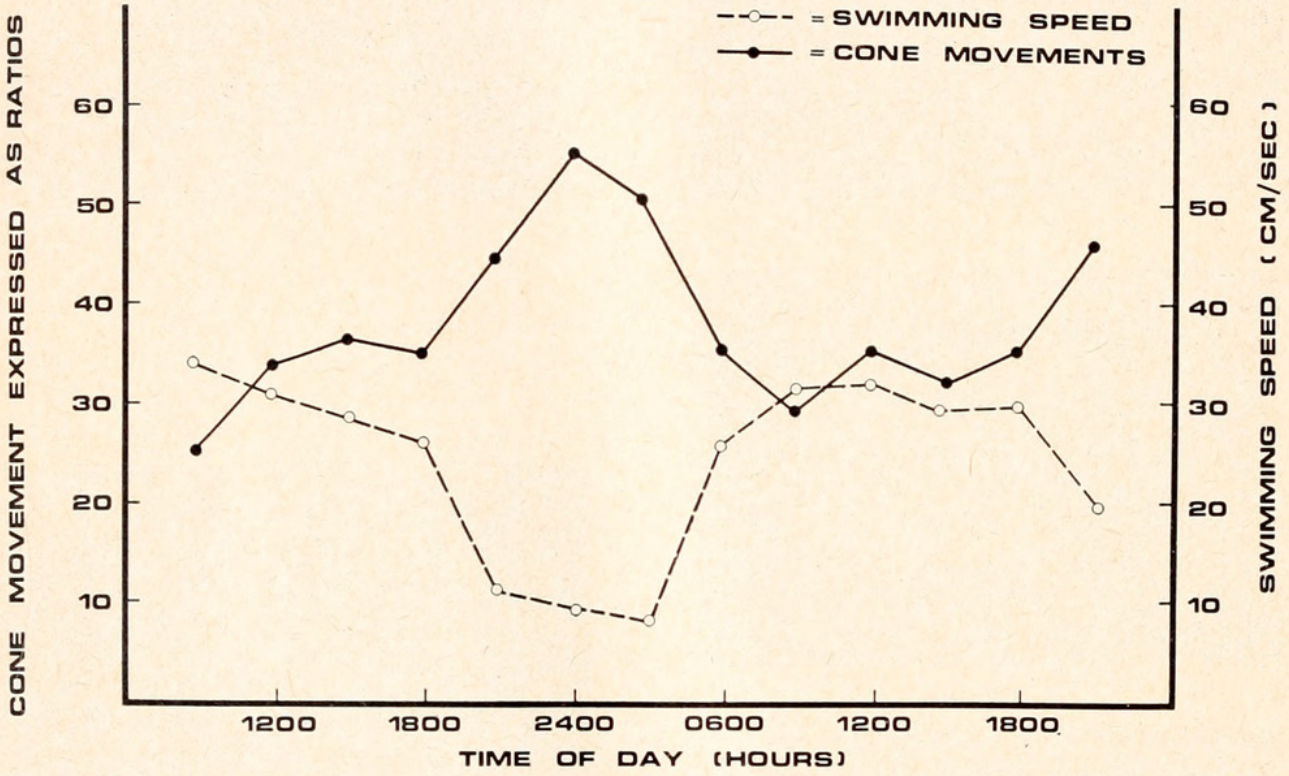

FIGURE 2. Mean values of cone position from fish held in constant darkness compared with swimming activity under constant low illumination (9 ft.-c.).

day (1930) we held the fish in constant darkness for 49½ hours. At 0900 hours of the eighth day and every three hours thereafter for 36 hours, we sampled two fish from the aquaria and prepared the eyes for histological examination in the same way as described above.

With a calibrated ocular micrometer we measured the position of 20 consecutive cone ellipsoids from one eye of each fish beginning 50 μ dorsad and 50 μ ventrad to the optic nerve insertion. We expressed measurements as percentages of the width of the combined pigment epithelial and visual cell layers to compensate for variations in retinal cell layer thickness. We did this by dividing the distance from the external limiting membrane to the proximal end of the cone ellipsoid by the distance from the external limiting membrane to the lamina basalis and multiplying the quotient by 100.

Results

We found that the retinal changes associated with light- and dark-adaption followed the classical pattern for the cones and pigment epithelium (Arey, 1916; Parker, 1932; Walls, 1942; Nicol, 1965). Complete light-adaption of the bluefish retina took between 15 and 30 minutes, complete dark-adaption between 30 and 60 minutes. When the fish had become light-adapted, the cone ellipsoids lay close to the external limiting membrane and the pigment epithelium was expanded and remained in that position until the light phase ended (Fig. 1a). When the fish had become dark-adapted the cones lay close to the pigment epithelium which was in a contracted state (Fig. 1b). Thus under a regime of alternating phases of light and dark, the cones and pigment epithelium moved from one extreme to the other with each change of phase. However, under constant darkness the cones continued to move according to the natural photoperiod from the position normally associated with dark-adaption to that normally associated with light-adaption; but they did this gradually in a rhythmic pattern (Fig. 1c–e; Fig. 2). But whereas the pigment epithelium had expanded and

Table I

Medians of retinal cone positions expressed as the ratio of the distance between the ELM and the proximal end of the cone ellipsoid/distance between the ELM and the lamina basalis, × 100; and results of the Tukey-Duckworth End Count Test

Time	Fish A		Fish B		Average of medians	End count
	Sampling location		Sampling location			
	Dorsal	Ventral	Dorsal	Ventral		
			Clock daytime			
0900	23	24	29	26	26	−
1200	31	31	34	34	33	−
1500	34	37	39	38	37	
1800	39	26	33	34	36	
			Clock nighttime			
2100	45	41	43	48	44	+
2400	53	56	56	53	55	+
0300	55	41	50	54	50	+
0600	40	37	32	29	35	
			Clock daytime			
0900	26	23	33	34	29	−
1200	35	35	38	30	35	
1500	40	41	25	24	33	−
1800	35	36	38	32	35	
			Clock nighttime			
2100	43	46	44	45	45	+

\+ = Clock nighttime values larger than the largest clock daytime value.

− = Clock daytime values smaller than the smallest clock nighttime value.

Total end count 8 = 0.025*

* = 0.025 signifies that the differences between the clock daytime and the clock nighttime readings are significant at the 2.5% level.

TABLE II

Sign Test, comparing hourly differences of cone position between fish and between location sampled

Hour	Median: Fish B—Fish A		Median: Ventral sample—Dorsal	
0900	Dorsal,	+	Fish A,	+
1200	Day 1	+	Day 1	0
1500		+		+
1800		−		−
2100		−		−
2400	Dorsal,	+	Fish A,	+
0300	Day 2	−	Day 2	−
0600		−		−
0900		+		−
1200		+		0
1500		−		+
1800		+		+
2100		+		+
0900	Ventral,	+	Fish B,	−
1200	Day 1	+	Day 1	0
1500		+		−
1800		−		+
2100		+		+
2400	Ventral,	−	Fish B,	−
0300	Day 2	+	Day 2	+
0600		−		−
0900		+		+
1200		−		−
1500		−		−
1800		−		−
2100		−		+
No. of +		14		11
No. of −		12		12

contracted with change from light to darkness, it remained contracted throughout the whole period of constant darkness.

Variation in the positions of the cones relative to the external limiting membrane in any given part of the retina could result from one or both of at least two causes: (a) differences in the part of the retina sampled, and (b) differences between specimens. To examine these points we subjected the data to the following non-parametric statistical analysis: (1) We tabulated the medians of each set of 20 measurements made dorsad and ventrad to the optic nerve insertion for each fish and each sampling hour (Table I). (2) We then tabulated in Table II the signs of the hourly differences in the position of the cones between specimens and between samples taken either dorsad or ventrad to the optic nerve insertion. Applying the Sign Test of Dixon and Mood (1946), we found no significant differences in the position of the cones between fish and sampling location. (3) Therefore, we added the median cone positions for each sampling hour in Table I and computed the averages of the totals. (4) To compare the positions of the

cones in specimens sampled during daytime and nighttime, we applied the Tukey-Duckworth End Count Test shown in Table I (Tukey, 1959). The results of this test showed the positions of the cones to differ significantly between the two groups, indicating a movement of cones in specimens kept in total darkness. This could be accounted for only by some degree of internal control. (5) We plotted the mean values of cone positions referred to in (3) above (Fig. 2) to examine the pattern of movement over the period of the experiment and for comparison purposes plotted a curve of the activity rhythm of a group of adult bluefish held under constant low illumination of 9 ft.-c. (Olla, 1966a, b).

It is apparent from examination of the two curves in Figure 2 that the dark-adapted position of the cone ellipsoids and low swimming activity of bluefish occur concurrently during the hours of the natural dark phase and conversely, a light-adapted position of the cone ellipsoids and increased swimming activity occur during the time of the natural light phase. These results provide further evidence for the existence of an internal control mechanism which can act independently of light.

Discussion

Swimming activity and photomechanical changes in the retina are both related to light, are diurnal, and are under some degree of internal control. Previous work on bluefish activity rhythms (Olla, 1966a, b) compared with the present results bears out this relationship (Fig. 2). The fact that an overt response such as swimming speed and a covert response such as cone migration in the retina are in part internally controlled suggests a common synchronizing system for phasing rhythmicity. Internal control of retinal adaption for a predator dependent on vision for prey capture has an obvious selective advantage. It would predispose the retina for light and dark. Such a predisposition would effectively lessen the time for light and dark adaption.

We made no attempt to study the longevity of the retinal rhythm. Previous results of bluefish activity rhythms showed persistence of the rhythm for at least five days. In the absence of daily light cues, an eventual desynchronization of the rhythm might occur. Several investigators have described a reduction in adaption as the length of time under constant darkness increased (Welsh and Osborn, 1937; Wigger, 1941; Ali, 1961; John *et al.*, 1967; John and Gring, 1968).

Although Wigger (1941) in his work on goldfish, *Carassius auratus,* Ali (1961) in Atlantic salmon, *Salmo salar,* and John and Gring (1968) in the bluegill, *Lepomis macrochirus,* found an indication of a rhythm in the pigment epithelium of specimens kept in constant darkness, we found no such rhythm in the bluefish. In some species the pigment epithelium may be controlled internally but evidently in the bluefish it responded only to an external stimulus. A predisposition for light and dark in the epithelial pigment layer may not be as functionally critical as in the visual cells.

Nicol (1965) showed differences in responses between double and single cones in the plaice, *Pleuronectes platessa,* merry sole, *Microstomus kitt,* and sole, *Solea solea,* John *et al.* (1967) in the goldfish, *Carassius auratus,* and John and Gring (1968) in the bluegill, *Lepomis macrochirus.* We observed similar differ-

ences between double and single cone movement in the bluefish but did not differentiate between them in our measurements.

Th question of whether retinal rhythmicity in the bluefish becomes changed by manipulation of the light cycle remains for further experiments.

We wish to express our grateful appreciation to Mr. Enoch B. Ferrell for his advice on statistical treatment of the data.

Summary

1. The retina of bluefish, *Pomatomus saltatrix,* undergoes photomechanical changes in response to light and darkness. Complete light-adaption requires between 15 and 30 minutes, complete dark-adaption between 30 and 60 minutes.

2. Retinal cones of juvenile bluefish held under continuous darkness for two days exhibited a diurnal retinomotor rhythm. The epithelial pigment layer remained in a dark-adapted condition throughout the two days and displayed no discernible expansion.

3. Results were related with activity rhythms of bluefish and suggested that internal control of a retinomotor rhythm may predispose the eye to environmental changes in light intensity.

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