Circadian Rhythm in Goldfish Visual Sensitivity

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To determine whether rod-mediated vision in goldfish is regulated by a circadian clock, absolute threshold was measured psychophysically in animals maintained in constant darkness. Responses were recorded approximately every 4 hr to a diffuse 532 nm stimulus, 5 sec in duration, with no background light present. Visual threshold tended to be lowest at the time of transition from light to dark, as experienced by the fish before it was placed in constant darkness. Threshold tended to be highest at the time of transition from dark to light. The average peak-to-trough fluctuation for five fish was 0.5 log unit in amplitude, and its period was about 24 hr. The results show that the rhythm in visual threshold can still be detected after 7 days of darkness, and that it can be entrained to a new light-dark cycle. These properties are characteristic of regulation by a circadian oscillator. Invest Ophthalmol Vis Sci 28:1811-1815, 1987

Visual characteristics of many animals, including human, vary with time of day. In most cases the mechanisms underlying such periodic changes are unknown. Various aspects of retinal morphology and physiology in several species are modulated by circadian clocks, and such clocks have been implicated in regulating dark adapted visual threshold in Limulus. Behavioral data from vertebrates are more limited, but physiological data suggest that some teleosts may provide model systems for explaining the mechanisms of modulation in vertebrates. In this paper we provide evidence that thresholds for rod-mediated stimuli in goldfish are regulated by an endogenous circadian oscillator. Because in this species rod outer segment (ROS) shedding is controlled by the ambient light cycle and not by endogenous circadian processes, any daily changes in psychophysical threshold should reflect mechanisms other than ROS shedding.

Materials and Methods

Five goldfish (Carassius auratus, Ozark Fisheries, Stoutland, MO) 9–10 cm standard body length (tip of nose to base of tail) were maintained on a LD 12:12 lighting schedule for at least 2 weeks before being used (lights on at 0800 hr, off at 2000 hr; intensity during the light phase 340 lux). They were fed at irregular intervals during the light phase of the cycle. Absolute visual threshold (the minimum detectable light stimulus with no background present) was measured by means of a psychophysical procedure described in full elsewhere. With this procedure it is possible to measure the absolute threshold of both the rod and long wavelength sensitive cone systems in goldfish by selecting stimuli of appropriate wavelengths. In the present experiment the stimulus conditions were designed to influence preferentially the rod system.

Training and Testing

In training sessions, a fish was placed in a light-proof aquarium, dark-adapted for at least 1 hr, and presented with a visual stimulus followed by a mild tail shock in a classical conditioning paradigm. Respiration was monitored by a thermistor positioned in front of the fish's mouth. Pre-stimulus breathing rate was determined before each trial by computing the average number of breaths per 5 sec interval, over a total of 30 sec. A trial consisted of presentation of a 5 sec, 532 nm, 140° diffuse light stimulus, rear-projected on a screen tangent to the right eye. Light offset was contiguous with the onset of a 100 msec shock to the tail (5–15V DC). A response was defined as the slowing of breathing rate to ≤50% of pre-stimulus rate during the 5 sec light stimulus. Training sessions consisted of 10 trials per day at an intensity approximately 3.5 log units above absolute threshold. All training sessions took place between 1000 hr and 1400 hr during the light phase of the LD cycle. Test sessions began after the fish had responded to ≥80% of the training trials on 2 consecutive days.

A tracking procedure was used to determine
Experimental Procedures

Each fish was trained during the light phase of the LD cycle, and its threshold was first measured between 1000 hr and 1400 hr, also during the light phase. The purpose of the preliminary measurement of threshold was to show that thresholds typical of the rod system were obtained before animals were placed in DD. For these baseline tests the fish were dark-adapted for 1 hr (between 1000 hr and 1400 hr), threshold was determined with the tracking procedure, and the fish were immediately returned to their home aquaria in cyclic light (LD). They remained in LD, undisturbed, for at least 1 week to ensure that they were fully entrained to the cyclic light. Then four of the fish were placed in DD and the other fish was placed in a room with a reversed light-dark cycle (lights on 2000 hr, off at 0800 hr); this manipulation was designed to determine whether the rhythm could be phase-shifted.

To measure thresholds in DD, a fish was transported in a lightproof container from its aquarium in DD to the dark testing apparatus (at different times of day for each fish). The fish remained in the testing apparatus for 1.5–7 days while threshold was measured with the tracking procedure every 4 hr. The only light available during this time was from the dim scotopic stimulus (2–3 log units above absolute threshold, at maximum). Water temperature in the test aquarium was monitored, and did not vary systematically during the course of the experiment (range 22–24°C).

The results are reported as log relative sensitivity (the reciprocal of threshold) over time of day; time 0000 hr on Figures 3 and 4 represents the first midnight test session for any given animal. Times are reported as the closest hour at the start of each test session. To examine the possibility that a rhythm in breathing rate might have influenced the results, we computed the correlation between pre-stimulus respiration rate on trials near threshold and the value of absolute threshold.

Results

Figure 1 gives absolute thresholds measured approximately every 4 hr for a fish maintained in constant darkness for 7 days. No systematic changes in threshold occurred during the first 2 days. On days 3–7, however, this fish was most sensitive between 1600 hr and 2200 hr and least sensitive between 0000 hr and 1200 hr. The peak in sensitivity thus occurred at about the time of transition from light to dark in the entrainment cycle, and the trough occurred at about the time of transition from dark to light. During the 5 days when a rhythm was present, its amplitude varied from 0.2 to 0.7 log unit, peak to peak. On day 4, when the amplitude was the largest observed for this animal, a stimulus that never evoked a response at 1200 hr was responded to at 2000 hr every time it was presented (Fig. 2). A statistical test of the difference in sensitivity between 1200 hr and 2000 hr over all 7 days during which threshold was measured showed that such fluctuations would occur by chance with very low probabilities ($t = 3.50$, $df = 6$, $P < 0.01$).

The period of the rhythm for this fish during the last 5 days in DD appears to be close to 24 hr (Fig. 1) but the exact free-running period cannot be deter-
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Fig. 2. Psychometric functions for detection of the 532 nm stimulus during two test sessions on day 4 of DD for the animal shown in Figure 1. At 1200 hr (open symbols) this fish was 0.8 log unit less sensitive than at 2000 hr (filled symbols). Stimuli that were detected with high probability at 2000 hr failed to elicit any response at 1200 hr. The same curve has been fit by eye to both sets of data.

This fish was maximally sensitive at 0800 hr and minimally sensitive at 2000 hr, 12 hr out of phase with the fish in Figure 1. The peak again occurred near the onset of subjective night, and the trough near the onset of subjective day. This result demonstrates that the rhythm in visual sensitivity can entrain to a new light-dark cycle, as is characteristic of endogenous circadian rhythms.

The mean absolute threshold (n = 5) for the baseline tests conducted in darkness during the light phase of the LD cycle before exposure to DD was 4.6 log q sec\(^{-1}\) cm\(^{-2}\), measured at the cornea (range: 4.5–4.9). These values are similar to those obtained earlier in fish of similar size and light exposure history, tested in darkened tanks during the light phase.

Fig. 3. Visual sensitivity varied over time of day on the first day of testing for three additional goldfish. Points are mean log sensitivity (±1 SEM), relative to the low point at 0400 hr, where only one fish was successfully tested. For all other points, n = 3. Zero on the ordinate is 4.6 log quanta sec\(^{-1}\) cm\(^{-2}\). Figures 3 and 4 are double plotted to facilitate visualization of the rhythms. The shaded bar at the top shows the time of light on- and offset during LD entrainment for 2 weeks prior to testing in DD.

Fig. 4. The rhythm in absolute sensitivity can be phase-shifted. After training, this fish was maintained on a light-dark cycle that was reversed relative to the animals in Figures 1–3. Then it was placed in DD and its threshold was measured every 4 hr. After entrainment to the reversed cycle, this fish showed a peak in sensitivity shifted 12 hr relative to the other fish, to coincide with the new subjective night. The dashed line in the figure replots the averaged data from Figure 3, phase-shifted 12 hr.

mined because of the relatively long times between samples. Nonetheless, the demonstration that clear and consistent changes in threshold persist after many days in constant conditions is evidence for the existence of an endogenous circadian rhythm in visual sensitivity.

In contrast to the animal in Figure 1, all other fish in this experiment did show variations in threshold during the first day of testing (Figs. 3, 4). Figure 3 shows average threshold values during the first day in DD for 3 fish that had been entrained to the same light cycle as the fish in Figure 1. (The data for one day of testing are plotted twice in Figures 3 and 4, to make the rhythms easier to visualize.) Figure 4 is a plot of threshold measurements during the first day in DD from a fish entrained to a different light cycle (discussed below). Both figures show clear fluctuations over the 24 hr period. The magnitude of the change in Figure 3 was about 0.5 log unit on average, with a peak in sensitivity near the beginning of the subjective night. Testing continued for 1.5, 2 and 3 days respectively in these three fish, and all three continued to show rhythmic changes in absolute sensitivity throughout the test periods. Thus, daily changes in absolute threshold were observed in every goldfish we tested, and in four of the five fish these changes were apparent on the first day in DD.

Figure 4 shows results for the animal that was maintained on a reversed light-dark cycle for 3 weeks prior to testing in DD. The dashed line is the data from Figure 3 phase-shifted 12 hr on the time axis.
of the cycle. At these intensities, approximately one photon was incident per 3500 rods. We conclude that the thresholds reported in this paper represent absolute threshold for the rod system of goldfish.

Discussion

The rod-mediated absolute threshold fluctuates daily in goldfish. The average difference in threshold measured in this study was 0.5 log unit, or a factor of about 3; this is larger than the daily change in human rod threshold and is comparable in magnitude to circadian changes in b-wave sensitivity reported for the green sunfish. Goldfish appear to be most sensitive near the time of subjective dusk and least sensitive near subjective dawn. Reynolds et al report that this species is crepuscular, with peaks of locomotor activity near dawn and dusk in cyclic light. Perhaps the daily change in visual sensitivity is related to activity cycles.

The daily fluctuation in rod vision has characteristics typical of rhythms controlled by circadian oscillators: (1) it persists under constant conditions; (2) its period is close to 24 hr; and (3) it can be entrained to a new light cycle. We do not know why threshold in one of the five subjects in this study did not vary systematically during the first 2 days of exposure to constant darkness. It is interesting, however, that one of the four human subjects in another study from our laboratory also demonstrated no consistent fluctuations in rod threshold during the first 24 hr of DD (subject MP, Table 2, ref. 3).

What mechanisms might be responsible for the circadian rhythm in threshold? The rhythm reported here appears to be independent of ROS shedding; goldfish ROS shed following light onset in LD cycles, but they do not continue to shed in DD. Retinomotor movements are also unlikely to be involved, because rods (which were preferentially stimulated in this experiment) are reported not to undergo such movements in goldfish. However, it is possible that a rhythm of even larger amplitude could be uncovered by the use of longer wavelength, cone-mediated stimuli. Dearly and Barlow suggest that part (but not all) of the fluctuation in b-wave sensitivity in the green sunfish may be due to the influence of cone retinomotor movements when ERG amplitude is measured under conditions that favor detection by cones. These conditions were not used in our experiment.

Efferent neural pathways to the retina exist in teleost fishes and these may provide circadian feedback from the brain to regulate sensitivity. Alternatively, circadian variations in melatonin could influence threshold. Melatonin production in the retina increases during the night, and melatonin has been shown to regulate the release of dopamine in the rabbit retina. Dopamine regulates other physiological processes such as horizontal cell receptive field sizes. A circadian rhythm in dopamine could certainly result in daily changes in sensitivity. It is also possible that rhythms originating in higher visual centers (eg, the optic tectum) may contribute to the rhythm in visual threshold. Further research is warranted to elucidate the mechanism of the changes in sensitivity described here.

Key words: circadian rhythms, scotopic threshold, goldfish

Acknowledgments

We thank Drs. Terry L. Page and Robert B. Barlow, Jr. for helpful discussions.

References


