

Visual Responses in Teleosts

Electroretinograms, Eye Movements, and Circadian Rhythms

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ABSTRACT We have recorded ocular potentials in response to brief flashes of light from two teleosts, the white perch (*Roccus americana*) and the green sunfish (*Lepomis cyanellus*). The animals were respired and maintained in an alert state for up to 2 d. Responses were recorded with corneal and transcleral electrodes. The responses of green sunfish were composed of electroretinogram (ERG) and eye movement potentials, whereas the responses in white perch contained only the ERG. Injection of curare abolished the sunfish eye movement potentials, unmasking the ERG. Observation under infrared illumination established a direct relationship between eye movements and the fast potentials which could be abolished by curare. We found no evidence of circadian changes in the amplitude of the ERG b-wave of either species. However, our results combined with those of a previous study of sunfish ocular potentials (Dearry, A., and B. Barlow, Jr. 1987. *J. Gen. Physiol.* 89: 745-770) suggest that the sunfish visual system exhibits rhythmic changes in oculomotor responses, which appear to be controlled by a circadian oscillator.

INTRODUCTION

In a number of vertebrate species visual system physiology and morphology have been observed to vary with time of day. Daily rhythms thus far reported include changes in visual sensitivity (human: Bassi and Powers, 1986; rat: Terman and Terman, 1985), changes in the electroretinogram (ERG) (rabbit: Brandenburg et al., 1983; pigeon: Barattini et al., 1981; lizard: Fowlkes et al., 1984), retinal melatonin metabolism (chicken: Hamm and Menaker, 1980; *Xenopus*: Besharse and Iuvone, 1983), retinal dopamine metabolism (rat: Wirz-Justice et al., 1984), and photoreceptor disc shedding (rat: Lavail, 1976). In teleost fish there are circadian rhythms in the sensitivity of visual responses (Bassi and Powers, 1987) and in cone retinomotor movements (John et al., 1967; Ali, 1975; Levinson and Burnside, 1981). The teleost retina is an attractive preparation for examining circadian changes in retinal

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physiology because its well-studied dopaminergic interplexiform system may play a role in retinal rhythmicity (Dearry and Burnside, 1985). Interplexiform cells may modulate retinal physiology in response to both intraretinal and efferent signals. Their effect on the synaptic physiology of the outer plexiform layer of the retina is well established (reviewed in Dowling, 1989) and their influence on the retinomotor movements of cone photoreceptors has been suggested (Dearry and Burnside, 1986). They receive signals from retinal amacrine cells (Dowling and Ehringer, 1975) and efferent fibers originating in the nervous terminalis of the olfactory bulb (Zucker and Dowling, 1987).

The ERG, a readily recorded light-evoked ocular potential, is a robust indicator of retinal sensitivity. It has been used to measure circadian changes in the retinal physiology of a number of vertebrates (Barattini et al., 1981; Brandenburg et al., 1983; Fowlkes et al., 1984) and invertebrates (Jahn and Crescitelli, 1940; Arechiga and Wiersma, 1969; Arechiga et al., 1974; Fleissner, 1974; Koehler and Fleissner, 1978; Barlow, 1983). Two teleost species have previously been examined for rhythmicity in their ERG, the Japanese dace (Barlow, 1985) and the green sunfish (*Lepomis cyanellus*, Dearry and Barlow, 1987). While no rhythmic changes were found in the dace ERG, it was reported that the green sunfish exhibits a circadian rhythm in the amplitude of a corneal light-evoked potential identified as the ERG b-wave. We set out to extend these observations with the intent of investigating the possible role of the interplexiform system in rhythmic retinal phenomena. We examined the ERG b-wave of the white perch (*Roccus americana*) and responses recorded from the eyes of green sunfish. Our findings indicate that the responses of sunfish are composed of both ERG and eye movement potentials, while perch exhibit only the ERG. We find no evidence for rhythmicity in the ERG b-wave of either species, and suggest that the rhythmic element in sunfish responses is related to eye movements.

METHODS

Two types of electrodes were used to record flash-evoked potentials. One type of electrode was a conducting thread electrode identical to that used in previous studies (Barlow, 1985; Dearry and Barlow, 1987), and the second type of electrode was made from a Teflon-coated silver wire. The conducting thread electrode was placed in contact with the cornea, while the Teflon-coated silver wire was inserted into the vitreous through a small puncture in the sclera made with a 26-gauge needle. Electrodes were connected to the positive inputs of differential amplifiers (gain of 10,000, bandpass 3–300 Hz: Electronics Laboratory, Rockefeller University, New York, NY; or gain of 1,000, band pass 10–1,000 Hz: World Precision Instruments, Sarasota, FL). An indifferent electrode was placed on the skin near the eye and connected to the negative inputs of the amplifiers. The water in the aquarium served as ground.

The apparatus for maintaining the fish and delivering light stimuli was essentially identical to that used by Dearry and Barlow (1987). Fish were anesthetized in ice water, spinalized with a 20-gauge needle, fitted with a recording electrode, and then placed in a holding apparatus. During experiments they were maintained in a plexiglass tank within a light-tight faraday cage with the ventral half of their body submerged and the dorsal half kept moist with a paper towel. Oxygenated water was circulated by a pump with the outlet tube directed at the mouth, and the fish respired on their own. For curare experiments the inlet was placed in the mouth of the fish so that the oxygenated water ran over the gills. The number of experiments in which curare was used was kept to a minimum. Sunfish were maintained in tap water, while perch were

maintained in brackish water taken from their native pond, or a mixture of 80% tap water and 20% seawater which approximated the salinity of their native ponds. On the rare occasion when an animal exhibited any behavior which may have indicated distress the experiment was immediately terminated.

Light stimuli were delivered through an optical fiber aimed at a diffuser screen placed ~ 1 cm from the eye. Two different light sources were used during the course of these experiments. One source was used for all perch experiments (data shown in Figs. 1 A, 5 C, and 6) and the curare experiments with sunfish (Figs. 2 and 3), while the other was used for all other sunfish experiments (Figs. 1 B, 4, and 5, A and B). Both light sources contained a tungsten lamp and provisions for neutral density and chromatic filters. The unattenuated irradiance of the second light source was measured at the cornea using a calibrated silicon photodiode (PIN 10DFP; United Detector Technologies, Santa Monica, CA) and found to be $41 \mu\text{W}/\text{cm}^2$ (1.1×10^{14} photons/s per cm^2) for white light and $0.75 \mu\text{W}/\text{cm}^2$ (2.4×10^{12} photons/s per cm^2) using the 650-nm filter. These calibrations suggest that the stimuli used in our circadian experiments with sunfish were photopic because the white light is about half of normal room illumination and the 650-nm light is >3 log units brighter than the absolute cone-mediated threshold for ERGs in another teleost (goldfish: Fig. 3 of Nussdorf and Powers, 1988). We estimate that the intensity of the first light source was <1 log unit below that of the second, and thus the stimuli used in the perch circadian experiments may also have been photopic. Flash duration was controlled by an electronic shutter. Experiments were performed at room temperature (20°C).

For circadian experiments, sunfish (obtained from Fender's Fish Farm, Baltic, OH) were maintained on a light-dark (LD) 14:10 light cycle, with dawn at 6 a.m. and dusk at 8 p.m., which approximated the prevailing local light cycle, for at least 2 wk before experimentation. Perch were collected from Oyster and Dam Ponds in Falmouth, MA and maintained in traps in their native pond until 1 or 2 d before experimentation, at which time they were transferred to the same LD cycle as the sunfish.

RESULTS

Ocular Potentials

Fig. 1 shows light-evoked potentials recorded from the eyes of white perch (*Roccus americana*) and green sunfish (*Lepomis cyanellus*). At relatively low intensities, the responses from both species recorded with vitreal electrodes were composed of a slow positive component followed by a slow negative component (Fig. 1, A and B, left). The positive component is the ERG b-wave, while the slow negative component apparently represents differentiation of the signal by the bandpass amplifier rather than slow pIII since it was eliminated in dc-coupled recordings (not shown). At intensities only slightly higher than those necessary to elicit a b-wave, most sunfish responses exhibited an additional fast negative component which followed the onset of the positive-going b-wave (Fig. 1 B, right). The fast negative component was also present in responses recorded with a corneal conductive thread electrode identical to that used by Dearry and Barlow (1987). Fig. 1 B shows simultaneous recordings from the eye of a sunfish using both corneal and vitreal electrodes. Note that the amplitude of the b-wave is $\sim 100\%$ greater when recorded with the vitreal electrode, in agreement with a previous comparison of vitreal and corneal ERG recordings (Brown and Wiesel, 1961). Similar results were obtained in four experiments in which corneal and vitreal responses were recorded simultaneously from sunfish. Responses to flashes of monochromatic light of 650 nm contained b-waves and fast components similar in

form to those evoked by flashes of white light in Fig. 1 (see Fig. 4, *A* and *B*, insets). At moderate intensities such as those in Fig. 1 *A*, recordings from perch contained only the slow positive b-wave. At higher intensities the perch b-wave was preceded by a negative-going a-wave (not shown).

The waveform of the ocular potentials we have recorded in sunfish are similar to those shown by Dearry and Barlow (1987). Their "day" responses contain a slow potential similar in duration (> 100 ms) to the b-wave we recorded (Fig. 1 *B*, left), while their "night" responses are similar to the triphasic responses we recorded (Fig. 1 *B*, right), containing both the b-wave-like slow component and a fast component (see Dearry and Barlow, 1987, p. 751). As in our records, the fast component recorded by Dearry and Barlow occurred after the onset of the slow component, and was relatively short in duration (< 50 ms). Although the form of the flash-evoked response is similar in both studies, the polarity is not. In the previous report the slow component is displayed as negative (downward) and the fast component as positive (upward). We

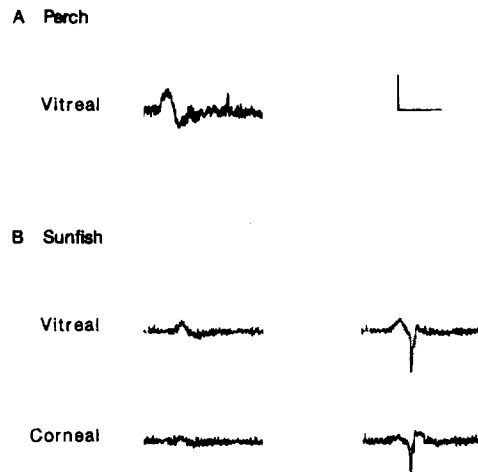


FIGURE 1. Flash-evoked ocular potentials. (*A*) White perch response to a 100-ms flash of white light, $\log I = -6.0$ recorded with a vitreal electrode. (*B*) Sunfish responses recorded simultaneously with corneal and vitreal electrodes. Responses to 10-ms flashes of white light of $\log I = -3.2$ (left responses) and $\log I = -2.9$ (right responses). Calibration bar in *A* is for both panels. Calibrations are $100 \mu\text{V}$ vertical in *A*, $50 \mu\text{V}$ vertical in *B*, and 400 ms horizontal in both panels.

are confident of our assignment of polarity: the b-wave, which is the slow component, is positive and upward, while the fast component is negative and downward. The temporal properties of the fast negative component we recorded (Fig. 1 *B*) are identical to the fast component that Dearry and Barlow (1987) interpreted as the b-wave of the ERG. To understand better this element of sunfish visual responses we sought to identify its origins.

Curare Abolishes the Fast Component

One possible source of the fast component is eye movement. To investigate this possibility we injected sunfish with curare, a treatment that blocks neuromuscular transmission and eye movements (Johnstone and Mark, 1969), but leaves the ERG intact (Witkovsky, 1968). Fig. 2 *A* shows responses to light recorded with a vitreal electrode after injecting curare. The left panel shows the response to a bright flash recorded before curare injection. The sunfish was then injected with 2.5 mg/kg of *d*-tubocurarine into the dorsal musculature. This dose was sufficient to abolish all

observable movement, including gilling, after a few minutes. As shown in the right panel, curare also abolished the fast component of the response, leaving only the slow positive potential with a time course and waveform typical of the ERG b-wave. Similar results were obtained with four sunfish that received curare injections of ~ 2.5 mg/kg.

Fig. 2 *B* shows the responses recorded from another sunfish after injection of curare. The positive component has characteristics typical of an ERG b-wave and is similar to the b-wave recorded intravitreally from goldfish (DeMarco and Powers, 1989). Its time course is slow (> 100 ms) and its amplitude is relatively large (~ 180 μ V) at saturation ($\log I = -1.0$). The three highest stimulus intensities in the series evoked a negative-going potential typical of an ERG a-wave preceding the positive

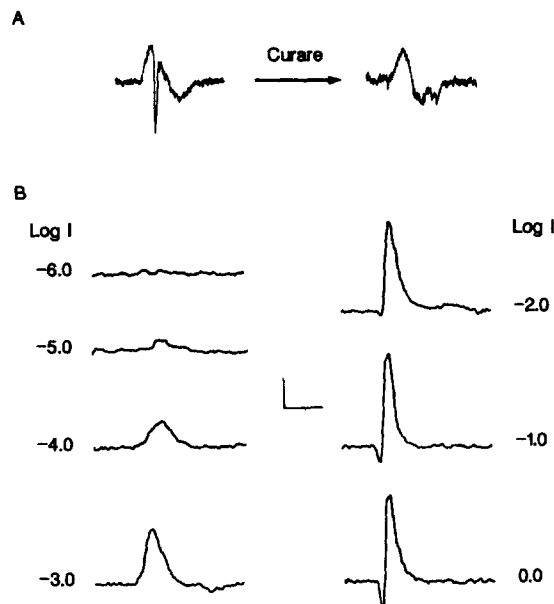


FIGURE 2. Effect of curare on sunfish ocular potentials. (*A*) Effect of curare on potentials evoked by 100-ms flashes of unattenuated white light ($\log I = 0.0$). After the response on the left the recording cage was opened, *d*-tobucurarine HCl of 2.5 mg/kg dissolved in physiological saline was injected intramuscularly under red light, and the right-hand response was recorded ~ 20 min after injection. Vertical calibration is 100 μ V and horizontal calibration is 400 ms. (*B*) Post-curare intensity-response curve from another curare-injected sunfish. These responses were recorded after ~ 2.5 h of dark adaptation and show the usual increase in

sensitivity relative to the preparation in *A*, which had not been extensively dark adapted. Flashes are 10 ms of white light at the indicated attenuation. Vertical calibration is 50 μ V and horizontal calibration is 200 ms.

component. The threshold of this potential is 3–4 log units greater than the b-wave, and it is relatively short in duration (< 50 ms) and small in amplitude (50 μ V) relative to the b-wave.

Fig. 3 shows an intensity-response curve for the responses shown in Fig. 2 *B* plotted on a log-log scale. The logarithm of b-wave amplitude grows roughly linearly with the logarithm of intensity for ~ 3 log units from threshold to $\log I = -3.0$ and ceases to be graded with intensity 5 log units above threshold at $\log I = -1.0$. The wide range between threshold and saturation of the sunfish b-wave is markedly different from the intensity-response characteristics of the fast component as previously reported by Deary and Barlow (1987, p. 753). This response was graded with intensity over only 1.5 log units.

The Fast Component Is Related to Eye Movements

The results obtained with curare injections suggest that the fast component of sunfish visual responses could be related to eye movements. To further investigate this possibility we observed eye motions while recording the flash-evoked potentials. Eye movements were observed and recorded on videotape using a dissecting microscope equipped with infrared illumination and an infrared converter and fitted with a video camera. Light stimuli and recording conditions were the same as in other experiments except that the diffusing screen was removed to allow visualization of the eye, and the faraday cage was open with the room darkened to allow access to the preparation; thus these preparations were partially light adapted. Stimulus intensity was varied and the presence or absence of flash-evoked eye movement was assessed.

Examples of eye movements and ocular potentials are shown in Fig. 4. In Fig. 4 *A* the first three panels are a series of still photographs from videotape showing eye position before, during, and after a flash that was judged to have evoked eye movement. From left to right the panels show the eye before the flash, during the

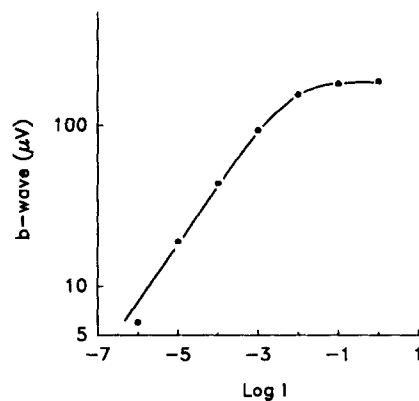


FIGURE 3. Sunfish b-wave intensity–response function: log-log plot of the responses in Fig. 2 *B*. Curve is fit by eye. A linear fit by least squares of the section of the curve from $\log I = -6.0$ to -3.0 gave a calculated slope of 0.40 log units increase in the amplitude of the b-wave per log unit increase in intensity.

flash (note reflection on the lower left aspect of the cornea), and 100 ms (three frames) after the flash. The eye is still in the first two panels, but changes position in the third panel. The fourth panel shows the ocular potentials recorded in response to a similar flash that elicited such an eye movement. Both a b-wave and a fast negative component are evident, typical of responses recorded without curare (Fig. 2 *A*). Fig. 4 *B* shows a similar sequence of photographs for a case in which no eye movement was detected. Note that when no eye movements were detectable only the b-wave was recorded (fourth panel), similar to recordings after curare injection (Fig. 2 *A*).

We examined the correlation of eye movements with the waveforms of ocular potentials in 52 trials using two fish. An observer blind to the chart recorder responses judged that 22 of 35 trials evoked eye movements. Upon inspection we found that the recorded responses of all 22 trials exhibiting eye movements contained both the b-wave and the fast component, as in Fig. 4 *A*. All remaining trials ($n = 13$) failed to induce eye movements and lacked the fast component, as in Fig. 4 *B*. Similar observations under less formal experimental conditions revealed that 13 trials that evoked eye movement produced fast potentials, and 4 flashes that failed to

evoked movement did not. In sum, all detectable eye movements were associated with fast components in the ocular potentials.

Circadian Rhythms in Ocular Potentials

Results from our curare and videotape experiments indicate that visual responses in sunfish contain potentials that are related to eye movements as well as the ERG. To further investigate the origin of the rhythmic responses we recorded ocular potentials from sunfish maintained in darkness. Flashes were delivered at 15- or 30-min intervals and were attenuated with neutral density filters to minimize the occurrence of fast potentials and obtain b-waves that were easily measured and below saturation (30–70 μV). The responses recorded here lacked a-waves so the amplitude of the

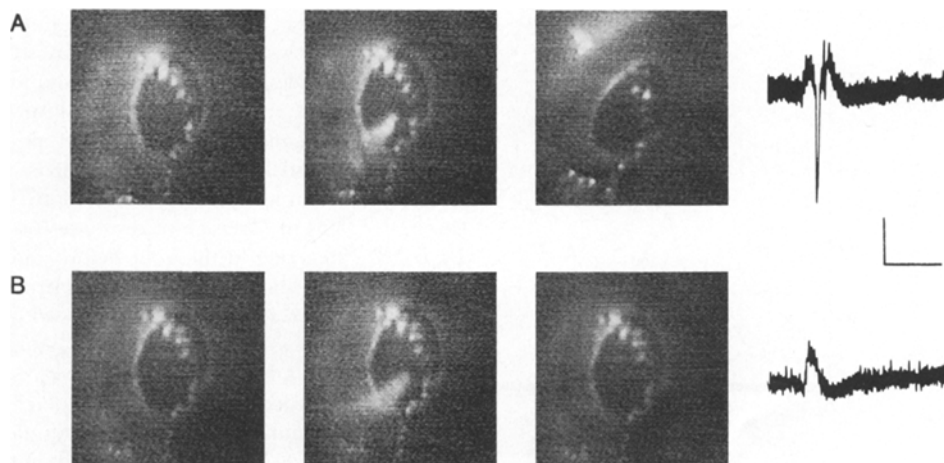


FIGURE 4. Correlation of sunfish eye movements and fast potentials. Frames are from a videotape recording using infrared illumination and an infrared image converter attached to the videocamera. The grain in the frame reflects that of the image converter. Frames show the eye before, during, and after flash-evoked eye movements. Traces show flash-evoked ocular potentials. See text for details. Stimuli were 10-ms flashes of white light, $\log I = 0.0$. Vertical calibration was 50 μV and horizontal calibration was 400 ms.

b-wave was measured as the positive deflection from baseline. Experiments were considered satisfactory if the recording period included at least one subjective dusk or dawn transition.

Five experiments with sunfish yielded an adequate number of responses lacking the fast component to allow assessment of the b-wave amplitude over time. These recordings were initiated in the late subjective day and ranged in duration from 11 to 46 h with a mean duration of 23.4 h. In four of the recording sessions the light stimuli were 10-ms flashes with a wavelength of 650 nm. The other recording session used 100-ms flashes of white light. The pattern of changes in the amplitude of the ERG b-wave was similar in all five experiments. Typically, the amplitude increased during the first few hours of recording in darkness, plateaued for a variable period, and then declined. The times at which response amplitude began to decline were not

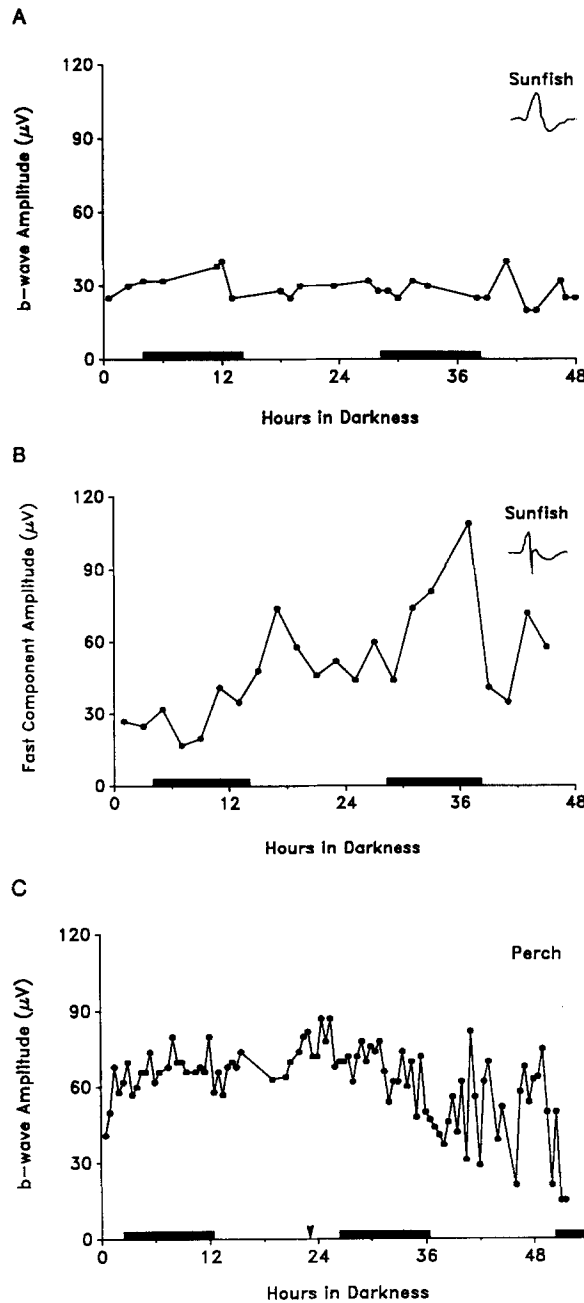


FIGURE 5. Amplitude of flash-evoked potentials vs. time in darkness. (A) Amplitude of responses that contained only the ERG b-wave from a sunfish maintained in darkness. Stimuli were 10-ms flashes of 650-nm light, $\log I = -0.5$ delivered at 30-min intervals. Individual responses are plotted. Inset shows example ERG response. Scale is 50 $\mu\text{V}/\text{cm}$ vertical and 250 ms/cm horizontal. (B) Amplitude of eye movement potentials from the same preparation as in A. Points are plotted as the average of responses occurring in the hour before and the hour after the point. Inset shows example response, with the same scale as in A. (C) Amplitude of the ERG b-wave recorded from a white perch maintained in darkness. Stimuli were 100-ms flashes of 650-nm light, $\log I = -3.0$, delivered at 30-min intervals for the first circadian cycle and 100-ms flashes of white light, $\log I = -5.0$ for the second (change at arrowhead). The shaded bar on the time axis of each recording indicates the time of subjective night during the previous light cycle.

related to the time in darkness or to the time of dawn or dusk of the previous light-dark cycle. In addition, once b-wave amplitude declined, a second sustained increase in amplitude was not observed.

Fig. 5A shows the b-wave amplitude plotted over 46 h in darkness for one preparation which used 650-nm flashes of light. The inset shows a sample b-wave

response. The variation in the b-wave amplitude appears random and not related to time of day. We also recorded the ERG b-wave for extended periods in one of the curare-injected fish (duration 36 h) in which the fast component was completely absent. Here again, the results were similar: no rhythmic changes in b-wave amplitude were detected.

We have not attempted a systematic study of the fast component on a circadian time scale, as did Deary and Barlow (1987). However, in the sunfish preparation shown in Fig. 5 A every test flash did not evoke a fast component and thus we were able to track the amplitude of the fast component and the b-wave. Fig. 5 B plots the time course of the amplitude of the fast component in this preparation for the same recording period as in Fig. 5 A. Here the inset shows a sample response containing both the b-wave and the fast component. There was more variability in the eye movement responses than in the b-waves, and thus we smoothed the data by averaging the fast components recorded in 2-h intervals. The averaged responses appear to change with time of day, suggesting the existence of rhythmic changes of the type reported by Deary and Barlow (1987). In the experiment in Fig. 5 B the

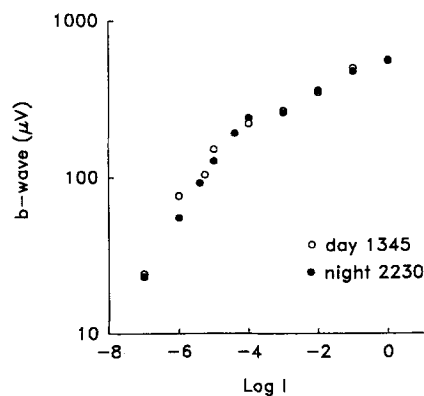


FIGURE 6. Log-log plots of perch b-wave intensity response functions taken in subjective day and subjective night. Stimuli were 100-ms flashes of white light at the indicated attenuations. Points are the average of two responses. Regression fits of the linear portion of the curves ($\log I = -7.0$ to -5.0) yielded calculated slopes of 0.38 log units change in the b-wave per log unit change in intensity for the daytime curve and 0.36 for the nighttime curve. Times are indicated in the lower right.

averaged responses peaked near the subjective dawn on the first day in darkness and in the late subjective night on the second. The results obtained with this preparation are particularly interesting because they show cyclic variation in the amplitude of the fast component contrasted with stability in the b-wave amplitude.

We also obtained satisfactory long-term ERG recordings from seven white perch. Recording conditions were similar to those used in sunfish except that slightly larger responses were used to monitor the b-wave (generally 40–90 μV). Recordings ranged in duration from 22 to 52 h with a mean duration of 32.4 h. Five of these recordings were initiated in the late subjective day, one in the first half of the subjective day, and one in the first half of the subjective night. Several different light stimuli were used in the perch experiments. Three fish were tested with 100-ms flashes of white light, two with 650-nm flashes of 10 or 100 ms duration, one with 650-nm or white flashes of 100 ms, and one with 710-nm or white flashes of 10 ms. Fig. 5 C plots the amplitude of ERG b-wave responses recorded from a perch over 52 h in darkness. We used 650-nm test flashes during the first half of the recording and white test flashes during the second half. The pattern of changes in b-wave amplitude of this experiment is

typical. As with sunfish, the b-wave amplitude increased after the fish was placed in darkness, remained somewhat stable for ~33 h and became variable for ~18 h before decreasing toward the end of the recording. Similar results were recorded from the other six perch regardless of the time of day the experiment was initiated or the light stimulus used: that is, no evidence of circadian changes in the amplitude of the b-wave was found.

In addition to monitoring the b-wave amplitude, we also measured intensity–response curves for the b-wave at different times of the day in two perch preparations. Both yielded similar results. Fig. 6 shows log-log plots of b-wave amplitude versus intensity for one of these experiments. One set of responses (open circles) was obtained during the subjective day, while the other set (closed circles) was obtained during the subjective night. The intensity–response functions are essentially identical at both times of day, as might be expected from our findings regarding the stability of b-wave amplitude. The test flashes used to monitor b-wave amplitude with time of day in this preparation were white light attenuated to $\log I = -5.0$, giving responses $\geq 100 \mu\text{V}$. These were the largest responses used to track the b-wave in this study and, as can be seen in the figure, they fall in the upper end of the linear range of the intensity–response function.

DISCUSSION

The two principal findings of this study are that (1) flash-evoked potentials recorded from the eyes of green sunfish contain both components of the ERG and potentials related to eye movements, and (2) the amplitudes of ERG b-waves recorded from both green sunfish and white perch do not exhibit detectable circadian rhythms under the conditions used in these experiments. In contrast, an earlier study in the sunfish reported a circadian rhythm in b-wave amplitude (Dearry and Barlow, 1987). We believe that the differences between the results of these two studies can be resolved, and that taken together they provide evidence of a circadian rhythm in the sunfish visual system.

Origin of Visual Responses

The principal difference between the two studies lies in the interpretation of the origins of visual responses recorded from sunfish. The methods used in these two studies are essentially identical, except that we used a vitreal electrode as well as the corneal electrode used by Dearry and Barlow (1987). Both types of electrodes yielded similar potentials in sunfish (Fig. 1). The potentials recorded at moderate stimulus intensities in both studies are similar in form: both contain slow components of > 100 ms duration interrupted by fast components of < 50 ms duration. The potentials from the two studies, however, are opposite in polarity. We are confident of the polarity assigned to the potentials in the present study. In Fig. 2 *B* the vitreal-positive b-wave from sunfish is displayed upward and the vitreal-negative a-wave downward, as is the standard practice.

Our results indicate that the fast component of sunfish potentials is not a component of the ERG, but is related to eye movements. The fast component is prominent in sunfish but not in white perch responses. Intramuscular injections of

curare abolished the fast component of sunfish responses and unmasked the ERG. Our experiments with curare used intense flash stimuli which maximally evoked the fast component, and thus it is unlikely that the elimination of this component with curare could have resulted from a change in the threshold for evoking these responses. It is more likely that it results from curare's blockade of neuromuscular transmission. In addition, the intensity–response function of the fast component reported by Dearry and Barlow (1987) differs from that of the b-wave (Fig. 3). The amplitude of the fast component is graded with intensity over ~ 1.5 log units, whereas the amplitude of the b-wave is graded over ~ 5 log units of intensity change. In addition, the slope of the log intensity versus log response function is significantly higher for the fast component reported by Dearry and Barlow (1987) than for the b-wave (~ 1.0 versus 0.4). Finally, observations under infrared illumination yielded a direct relationship between eye movements and the occurrence of the fast components. We therefore conclude that the fast potential, which in the previous report was interpreted to be the ERG b-wave, is in fact related to eye movements. It is unclear whether this potential is generated by the contraction of ocular muscles or by displacement of the electrode during eye movements. The amplitude of the fast potentials was variable, but their waveform was relatively constant. This may be because these potentials are associated with a distinct type of eye movement: rapid, large-excision eye movements produced in response to flashes of light. In the absence of test flashes these potentials occur infrequently, suggesting that they are a specific response to stimulation.

This interpretation of the origins of the visual responses in sunfish is consistent with the results of both studies. For example, the responses reported by Dearry and Barlow (1987) contained a slow negative-going potential (their polarity), which they interpreted to be the ERG a-wave. This response is long in duration (> 100 ms) and occurs in the absence of significant positive-going potentials in the “daytime” responses (pp. 751 and 757 in Dearry and Barlow, 1987). In contrast, Fig. 2 *B* illustrates that under the dark-adapted conditions of these experiments the sunfish a-wave is evoked by stimulus intensities that are near saturation for the positive-going b-wave, and that the duration of the a-wave (< 50 ms) is brief relative to that of the b-wave (> 100 ms). The waveform characteristics of the response interpreted as the a-wave in the previous report differ significantly from those of the sunfish a-waves we recorded but match those of b-waves with inverted polarity.

Similarly, the results of Dearry and Barlow (1987) with optic nerve section are also consistent with the interpretation that their positive component is an oculomotor response, and their negative component is the ERG b-wave. In these experiments they cut the dorsal ocular muscles, sectioned the optic nerve of the recorded eye, and left intact the nerve to the contralateral eye. Eyes with sectioned optic nerves expressed a rhythm in response amplitude. The waveform of the negative component was unchanged by optic nerve section, but the waveform of positive component was altered compared with intact preparations. In addition, higher flash intensities were necessary to evoke the positive component in operated fish, and at these higher intensities the amplitude of the negative component was increased. One plausible explanation for these results is that, as in intact animals, the positive components are eye movement responses. Movements of the eye with optic nerve section could be

initiated by contralateral eye responses transmitted through crossed input to the oculomotor nuclei and out the cranial nerves to the remaining ocular muscles of the sectioned eye. Indeed, experiments with goldfish show that eyes blinded by optic nerve crush move in response to visual stimuli presented to the contralateral eye (Easter, 1975). Such an interpretation could account for the constancy of the waveform of negative slow component (it was the ERG b-wave and was not distorted by the optic nerve section), the increased flash intensities required to evoke the positive component (eye movements were evoked by scattered light to the contralateral eye), and the distortion of waveform of the positive response (optic nerve section damaged the dorsal ocular muscles).

The Existence of Circadian Rhythms

We recorded the ERG from both green sunfish and white perch for extended periods in darkness and did not detect a circadian rhythm in the amplitude of the b-wave. Similar results have also been reported for another teleost, the Japanese dace (Barlow, 1985). A previous study of sunfish visual responses reported a circadian rhythm in the b-wave (Deary and Barlow, 1987). Our results do not exclude the possible existence of a rhythm in the sunfish ERG. Although we did not examine b-wave sensitivity in sunfish by taking intensity response functions at different times of day as we did in perch, the intensity–response functions we measured in sunfish suggest that the 30–70- μ V responses used to monitor the b-wave were below saturation and their amplitude should have reflected any changes in retinal sensitivity. Nevertheless, this study was by no means exhaustive and more experiments using a wider variety of stimulus conditions will be required to determine if sunfish exhibit a b-wave rhythm. Similarly, we do not wish to exclude the possible existence of a rhythm in the ERG of the white perch, but only to state that under the experimental conditions used in this study we did not detect one.

We did not attempt to determine whether our populations of sunfish and perch expressed circadian rhythms in retinomotor movements, as do many other teleosts, and thus we cannot be certain that the individuals we tested were drawn from a rhythmic population. However, the fact that we recorded a cyclic variation in movement response amplitude (Fig. 5 B), while simultaneously recording a stable b-wave (Fig. 5 A), suggests that the lack of b-wave rhythmicity in our population of sunfish was not due to a complete lack of circadian rhythmicity. Similarly, our observation that sunfish housed in our facility exhibited clear activity rhythms suggests that the light cycle was an effective entraining stimulus.

The previous study of sunfish responses found that the amplitude of the fast component changes with time of day while the animal remains in constant darkness (Deary and Barlow, 1987). Although we did not focus on this rhythmic property, the data in Fig. 5 B are consistent with it. This is the only experiment in which we recorded both b-waves and fast components for a period of 46 h from the same sunfish. The amplitude of the b-wave remained relatively constant over this interval, but that of the fast component did not. The latter varied irregularly over the first 24 h but exhibited a clear nighttime elevation during the second 24-h period. Our results indicate that these responses are related to eye movements. Changes in their amplitude are difficult to interpret because the coupling between retinal activity and

motor output is not known. Modulation could occur at any stage of the process. However, it is possible that the changes in response amplitude do indeed reflect a circadian rhythm in visual sensitivity, consistent with the study of Dearry and Barlow (1987).

Bassi and Powers (1987) reported rhythmic changes in visual sensitivity of goldfish similar to those found by Dearry and Barlow (1987). They measured visual threshold behaviorally in goldfish and found it to be approximately threefold lower during the subjective night. The rhythmic changes in sensitivity do not seem to be related to the circadian rhythms in cone retinomotor movement in teleosts because their sensitivity is highest when the cones are elongated and thought to be less sensitive (Miller and Snyder, 1972; Burnside and Ackland, 1984). Powers and co-workers have extended their goldfish studies to measurements of retinal sensitivity. While this article was under review, they reported in a preliminary study detection of a circadian rhythm in the b-wave of the goldfish (Powers et al., 1990). It is unclear at this juncture whether the differences in our findings and theirs are due to species differences or methodological differences. However, it should be noted that both studies in goldfish examined sensitivity with scotopic light stimuli optimized for rod responses (Powers, M., personal communication) rather than the photopic stimuli we generally used in our circadian experiments.

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