

LETTER TO THE EDITOR

## Electroretinograms and Circadian Rhythms in Green Sunfish

Dear Sir,

The article by McMahon and Barlow (1992) in this issue seeks to reinterpret some results reported in a prior publication in this journal (Deary and Barlow, 1987). McMahon and Barlow (1992) suggest that a "fast" response observed in the previous electrical recordings originates from eye movement, and that this potential was incorrectly interpreted as the b-wave of the electroretinogram (ERG) in the prior publication. In response, this letter addresses several pertinent points: (1) observation of ostensibly movement-related artifacts during the previous recording sessions; (2) ERG characteristics; (3) effects of optic nerve section; and (4) circadian rhythm.

(1) During the original experiments in 1985, occasional responses obtained from green sunfish maintained in constant darkness evidenced a fast negative deflection, possibly related to eye movement. Of more than 100 fish used in these experiments, only 8 exhibited this type of fast negative response. Furthermore, responses from each of these eight fish were recorded every half hour during a 12–48-h period (i.e., 24–96 responses per fish), and no indication of this type of fast potential was found in more than three responses from a given fish. Other responses recorded from these fish exhibited normal positive b-waves. Thus, these fast negative responses were atypical. This type of response was most often observed during generation of an intensity–response function and was rarely observed at the lower intensities used in circadian experiments. It should be emphasized that such responses were not used to assess ERG amplitude or rhythm. They were not included in the original publication since this type of response, whatever its origin, was thought to be artifactual, was not an issue in its own right at the time, and other responses better demonstrated the difference between subjective night vs. subjective day. In appearance, these responses exhibited a time course and waveform similar to those shown in the right-hand panels of Fig. 1 of McMahon and Barlow (1992). Thus, as in the latter, b-waves in Deary and Barlow (1987) were characterized by positive, upward deflections; conceivably movement-related responses were downward and negative. The occurrence of this negative response and its potential relationship to movement are not in dispute. However, the presence of this fast negative response in recordings having the same polarity as those reported by Deary and Barlow (1987) is inconsistent with the idea proposed by McMahon and Barlow (1992) that the original authors misidentified the ERG b-wave.

(2) McMahon and Barlow (1992) state that, ". . . the intensity response function of the fast component reported by Deary and Barlow (1987) differs from that of the b-wave." In the curarized fish used by McMahon and Barlow (1992), a 10-fold increase in illumination intensity ( $I$ ) elicited an approximately fourfold increase in response amplitude ( $V$ ). In the report by Deary and Barlow (1987; Fig. 6 therein), a

10-fold increase in  $I$  elicited an  $\sim 10$ -fold increase in  $V$ . Results from other laboratories studying vertebrate retinas indicate that a 10-fold increase in  $I$  generally results in a 6- to 10-fold increase in  $V$  (e.g., Birch, 1987; Sandberg et al., 1987; Nussdorf and Powers, 1988). In addition, McMahon and Barlow (1992) state that, "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." However, the response to which they refer (Fig. 3 DAY of Dearry and Barlow, 1987) resembles ERG responses recorded by Witkovsky (1968) from carp exposed to background illumination. In both instances, the ERG recording consists of a relatively small a-wave followed by a b-wave of approximately equal amplitude. The similarity of these responses is consistent with other results presented in Dearry and Barlow (1987) suggesting that a circadian oscillator acted to induce a photopic condition in the green sunfish eye during subjective day. The change in waveform between subjective day and subjective night may also be a consequence of this oscillator. The responses of Dearry and Barlow (1987) possess a time course, waveform, and spectral sensitivity function similar to those observed for ERG responses recorded from a variety of fish (e.g., Witkovsky, 1968; Easter and Hamasaki, 1973; Cohen et al., 1977; Hoffert and Ubels, 1979; Powers et al., 1990). Together, these findings suggest that the potentials recorded by Dearry and Barlow (1987) exhibit the characteristics expected for a vertebrate ERG.

(3) McMahon and Barlow (1992) state that, "The waveform of the negative component [of Dearry and Barlow (1987)] was unchanged by optic nerve section, but the waveform of positive component was altered compared with intact preparations." However, the results to which these authors refer (Fig. 9 of Dearry and Barlow, 1987) show that optic nerve section significantly altered the waveforms of both the negative (a-wave) and positive (b-wave) components of the ERG. Hence, "constancy of the waveform of negative slow component" is not a valid argument for suggesting that the negative component of the ERG recorded by Dearry and Barlow (1987) is a b-wave. Moreover, it should be noted that sectioning the optic nerve damped subjective night responses proportionally more than subjective day responses (Fig. 10 of Dearry and Barlow, 1987). This would not be the expected result if these responses were generated by scattered light impinging upon the contralateral eye as proposed by McMahon and Barlow (1992). If that were the case, both subjective night and subjective day responses should have been equally reduced. Instead, this difference in the degree to which cutting the optic nerve affected night vs. day responses suggests that some circadian signal within the eye is differentially modified. This result is again inconsistent with the idea that Dearry and Barlow (1987) misidentified the ERG b-wave.

(4) McMahon and Barlow (1992) state that, "... 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." The authors' observation of a variation in movement response amplitude (their Fig. 5 B) has a period of 20 h. This is not suggestive of a circadian rhythm, nor is it at all similar to the "rhythm in ERG amplitude" observed by Dearry and Barlow (1987). However, it appears that their b-wave data may be suggestive of a rhythm with a

period of ~24 h. Their ERG responses increase during subjective night over a period of 10–12 h, much too long a time to attribute to regeneration of bleached visual pigment. This suggests that some additional process is occurring to influence retinal sensitivity. It should also be noted that McMahon and Barlow (1992) maintained fish under an artificial light/dark cycle for 2 wk before use, whereas Dearry and Barlow (1987) maintained fish under a natural light/dark cycle for at least 3 wk before use. This difference between an abrupt light/dark transition and naturally occurring dawn and dusk has recently been found, in preliminary results, to influence the expression of a number of retinal rhythms (e.g., Bush et al., 1990; Dahl, 1990). In addition, Dearry and Barlow (1987) demonstrated that circadian retinomotor movements occurred in the population of fish used for their ERG studies, whereas McMahon and Barlow did not test for the presence of such a rhythm. It may be that the expression of circadian oscillator(s) was diminished in the population of fish used by McMahon and Barlow (1992).

Finally, Powers et al. (1990) have demonstrated that goldfish possess a circadian rhythm in ERG b-wave response. The amplitude, intensity–response, and spectral sensitivity functions of this rhythm are similar to those parameters of the rhythm in green sunfish as reported by Dearry and Barlow (1987). These results are consistent with the idea that teleosts possess a circadian oscillator controlling retinal sensitivity. Both Dearry and Barlow (1987) and Powers et al. (1990) reported that the circadian rhythm in ERG amplitude is not directly related to retinomotor movements. In addition, Powers et al. (1992) have recently suggested that retinomotor movements do not greatly influence teleost visual sensitivity. Thus, the mechanisms underlying these retinal rhythms and their possible relationship remain to be determined.

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