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Phase Shifting the ERG Amplitude Circadian Rhythm of Juvenile Crayfish by Caudal Monochromatic Illumination

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ABSTRACT

The objective of the present work was to determine the physiological mechanisms underlying the synchronization of the ERG amplitude rhythm. Chronic ERG recordings were obtained from juvenile instars of crayfish. Changes on the ERG amplitude rhythm produced when 30 min blue light illuminated the telson were determined. The PRC obtained with these data showed advances in the early subjective night and delays in the late subjective night. These phase shiftings resemble the features of curves obtained by dark pulses in other species. The relation of this curve with PRCs generated in the crayfish and other animals species are discussed.

Abstracting keywords: Circadian rhythm, extraretinal photoreceptor, blue light, phase-response curve, juvenile crayfish.

INTRODUCTION

In a previous work on the development of crayfish rhythmicity(Fanjul-Moles et al., 1992) it was proposed the existence of two independent systems involved in the synchronization of the electroretinogram (ERG) circadian system, i.e. a short and a long wavelength detection systems. Both, retinal and extraretinal photoreceptors seem to be involved in the entrainment of the ERG rhythm of adult crayfish (Page and Larimer, 1976; Fuentes-Pardo and Inclán-Rubio, 1987; Sandeman et al., 1990). Hence neural integration from different circadian photic inputs might occur in the central circadian phase shifting system, as has been proposed for other animal species (Horne and Renninger, 1988; Brad Hanna et al., 1988.) The present work was undertaken to test synchronization of the ERG amplitude rhythm in juvenile instars of crayfish *Procambarus clarkii* when blue monochromatic light, by iluminating the telson, stimulates the caudal photoreceptor system (CPS), one of the structures proposed as a photoreceptor for entrainment of the pacemaker in the adult crayfish .

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METHODS

Thirty-one crayfish aged between two and six months after eclosion were used. The retina's electrical response (eletroretinogram-ERG) to a fixed white light was recorded individually in animals maintained in darkness and constant temperature (Fanjul-Moles et al., 1992) for at least 8 days. Each animal was maintained under free running conditions during 3 days. On the fourth day, and at different circadian times (CT), a 30 min. and 5.1 microeinstein $sec^{-1}m^{-2}$ blue light pulse was applied on the transparent ventral region of telson. To confine illumination to the sixth abdominal segment, the telson was inserted in a special device made of black acrylic plastic, allowing only the entrance of an optic fiber light provided with a Kodak wratten interference filter (transmittance wavelength of 450 nm.). To avoid the heat an infrared cut-off filter was placed between the light source and the interference filter. The effect of the luminous stimulus on the phase of the ERG oscillation was determined on the fourth day after stimulation. CT was calculated by normalizing each cycle with respect to the 24-hour period. The phase reference point was the onset of activity, CT 12 (CT 12 corresponds to the moment at which 50% of the maximum amplitude of the cycle is reached). The difference between the observed and expected times of the onset of activity i.e when the ERG rhythm attained its steady state (4th day), was the criterion used to measure a phase shift, which was used to construct a phase-response curve.

RESULTS AND DISCUSSION

The ERG amplitude rhythm of juvenile crayfish kept in constant darkness was recorded at 15 min intervals. It was plotted against the external time and analyzed over the eight days of the experiment. The average circadian period of all the animals studied was 23.45 ± 2.01 hrs. A significant reduction of the ERG amplitude was elicited when the blue light reached the telson. The ERG reduction was obtained from the first ERG measure after stimulation until 30 min after light supression. The effect of the stimulus on the ERG amplitude appeared to be both phasic and tonic. The average reduction of ERG varied between 2 and 30 % depending on the external time. This change seems to follow circadian pattern (maximal decrement between 14 and 17 CT). Although this pattern was not analyzed in the present work, it appears to indicate an important functional relationship between the eyes and CPS, as has been reported by other authors (Inclán-Rubio and Fuentes-Pardo, 1987). Figure 1 shows the phase response curve (PRC) obtained by applying the blue light on the telson. This PRC shows mostly insensivity in the subjective day, advances (A) are seen at the time of the expected dusk and in the early subjective night, delays (D) occur around the time of the expected dawn and in the late subjective night, the D/A ratio is almost 1. The features of this curve resemble the characteristics of the curve obtained by dark pulses in other animal species (Klein et al., 1985). The PRC's of the ERG obtained in juvenile instars of crayfish through the application of a 15 min of white light to the whole animal of the same age (Fuentes-Pardo et al., 1992) indicates a zone of both delays and advances of moderate amplitude in the early subjective night as well as a zone of delays in the late subjective day. The unimodal curve obtained in the present work, resulting from iluminating just the CPS, shows shorter shifts mostly restricted to the subjective night. Although these results are preliminary, the features of both PRCs seem to indicate that the magnitude and direction of the shifts depend on the number as well as the characteristics of the light inputs converging on the juvenile crayfish pacemaker system. This phenomena could be related to similar mechanisms as proposed for antiphasic families of PRCs generated in other animals and particularly for *Bulla gouldiana* ocular pacemaker (Block et al., 1995).

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