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EFFECT OF MONOCHROMATIC LIGHT UPON THE ERG CIRCADIAN RHYTHM DURING ONTOGENY IN CRAYFISH (*PROCAMBARUS CLARKII*)

MARIA LUISA FANJUL-MOLES,*† MANUEL MIRANDA-ANAYA* and BEATRIZ FUENTES-PARDO‡
*L. Invertebrados, Departamento de Biología, Facultad de Ciencias, U.N.A.M., Apdo, Postal 70-371, 04510, México, D.F., Mexico; ‡L. Cronobiología, Departamento de Fisiología, División de Posgrado e Investigación, Facultad de Medicina, U.N.A.M., Apdo, Postal 70-250, 04510, México, D.F., Mexico

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Abstract—1. The aim of this work was to investigate if the asymmetric development of the two sets of visual photoreceptors responsible for short wave and long wave sensitivity during crayfish ontogeny influences the characteristics of the electroretinogram (ERG) amplitude rhythm.

2. The parameters of the ERG rhythm of juvenile (2–12 weeks) and adult stages were studied in free-running conditions under blue, red or white light flash test.

3. It was found that monochromatic light produces significant changes in the ERG circadian rhythm parameters in juvenile stages and has a synchronizing effect on the ultradian rhythm prior to the ERG circadian rhythm.

4. Based on these results we propose the existence of two sets of circadian photoreceptors related to two independent systems involved in the synchronization of the ERG circadian rhythm (a short wave and a long wave detection system).

INTRODUCTION

In the course of ontogeny in crayfish, the electroretinogram amplitude rhythm appears as an ultradian rhythm which gives rise to a circadian rhythm. Some parameters of the circadian rhythm, such as the $\alpha : \rho$ ratio, relative amplitude, period and phase, change until the characteristics of the adult animal appear in the last stages of juvenile animals (Fanjul-Moles *et al.*, 1987). On the other hand, Sánchez and Fuentes-Pardo (1977) have shown that the eyestalk of the adult crayfish may show a circadian rhythm but this rhythm is superimposed by ultradian oscillations which have been interpreted as resulting from the lack of coupling of possible circadian oscillators.

It was recently reported (Fanjul-Moles and Fuentes-Pardo, 1988) that the eye sensitivity of the crayfish *Procambarus clarkii* changes in the course of development. This seems to indicate that the three sets of photoreceptors in the adult animal's eye undergo an asymmetrical development during ontogeny. Younger animals show greater sensitivity at short wavelengths (blue, UV) and this sensitivity shifts to long wavelengths (green, red) when the animal approaches the adult stage.

It is well known that circadian rhythms can be synchronized by external light cycles due to the existence of circadian photoreceptors. This type of photoreceptor has been found both in the eyes of invertebrates (for review see Page, 1981, 1982) and vertebrates (Hoffmann, 1981).

Some papers have provided detailed reports on the effect the spectral composition of the light stimulus has in the synchronization of different circadian rhythms (Nuboer *et al.*, 1983; Joshi and Chandrashekaren, 1985). One way to validate the existence of circadian photoreceptors with a differential spectral sensitivity, that could synchronize sets of independent circadian oscillators, may be to use the spectral characteristics of light as a stimulus to obtain an ERG amplitude rhythm in free-running conditions throughout the different stages of development, in which there seem to be different degrees of putative oscillators coupling.

In this work, the ERG rhythm was obtained with two test monochromatic lights (blue and red), and with white light throughout development. Both significant changes in the ERG rhythm parameters and a synchronizing effect by the monochromatic light on the ultradian rhythms prior to the ERG circadian rhythm were found. Based on these results, we propose the existence of at least two sets of photoreceptors that bring about different characteristics to the ERG circadian rhythm during ontogeny.

MATERIALS AND METHODS

Thirty juvenile and five adult *Procambarus clarkii* were used. The ages of the juvenile animals ranged between 10 and 90 days after hatching. Three age groups were formed: 2–4, 4–8 and 8–12 weeks. Animals were kept in a well-ventilated indoor aquarium on LD 12:12 (light turned on at 7 a.m. and turned off at 7 p.m.) before any experimental procedure. Test animals, partially immersed in water, were fixed in acrylic cages and individually housed in a chamber under constant temperature (15°C) and darkness. The eyestalk was immobilized and a steel microelectrode (1–5 μm at the tip) was implanted through the cornea, near the basement membrane, to record the ERG. Retinal potentials relayed to a DC preamplifier (Grass 7P122) were registered with a polygraph (Grass mod. 79) for 9, 12 or more

†To whom all correspondence should be addressed.

consecutive days and delivered every 15 min to a PC computer to be processed. White or monochromatic light test flashes of fixed intensity and duration of 10 ms were delivered to the eye every 3 min from a photostimulator Grass PS22 provided with a xenon flash lamp. Light intensity was adjusted by neutral density filters. Monochromatic light was provided by Kodak wratten narrow hand interference filters, with half-width bands about 100 nm each and transmittance wavelengths of 400–500 and 600–700 nm. In addition, UV and i.r. cut-off filters were placed between the light source and the interference filters when required. The eye was always placed at a distance of 5 cm from the light source. The intensity of the stimulus and the attenuation values of both the neutral density and the spectral filters were calibrated with respect to the crayfish position using a Li-Cor 185 quantum-radiometer-photometer with a Li-190 SB Quantum sensor. The flash intensity was fixed to a value of 5.1 sec^{-1} m^{-2} μE. Experimental animals, both juvenile and adult crayfish, in free-running conditions were submitted to white test flashes during the first 3 days of recording. Afterwards, the test flash was changed to monochromatic light (blue or red) during the following 6 days, 3 days for each color and if possible, due to the survival of the preparation, they were changed back to the initial condition for 3 more days. The results were analysed by constructing graphs of the ERG voltage vs time which allowed the measurement of the free running oscillation period for the ERG amplitude (τ), activity (α) and rest (ρ) ratio, as well as the relative amplitude of the cycles (night:day amplitude ratio), and the duration and phasing of α for each one of the three light conditions employed. We defined the phase as the onset of activity, the time during which the cycle amplitude is over 50% of its maximum value until the moment when the same value had returned. The data records from 30 juvenile and five adult crayfish that fully completed the experimental protocol were submitted to a variance analysis (Scheffé test) in order to test the statistical significance of the difference among the age ranges for each of the circadian parameters analysed. Due to the great variability exhibited by the ERG oscillations in the very young animals (2–4 weeks), the period which had the greatest statistical significance according to the iterative procedure of the power spectrum technique was calculated (Enright, 1981) (power spectrum analysis obtained with Statgraphics computer program).

RESULTS

Effect of monochromatic light on ERG rhythm parameters

The use of monochromatic light to obtain the ERG rhythm during the different stages of ontogenic development in the crayfish showed that there are differences in the spectral sensitivity of the visual receptors. Such differences were detected by the lack of response to red light by animals younger than 4 weeks, on the one hand, and on the other by the differences in the parameters of the ERG circadian rhythm in each experimental situation. Figure 1 shows that the length of the period was already circadian in the animals aged 2–4 weeks ($N = 10$). $\tau = 24.7 \pm 1$ hr was obtained with blue light and the value with white light was 17 ± 6 hr. After stimulation with blue light flashes, the 4–8 weeks age group decreased its period to 20.8 ± 4 hr and showed a rhythm period of 22.2 ± 1 hr under red light, and an increase to 23 ± 4.4 hr with white light. The 8–12-weeks age group showed a period of 26 ± 1 hr with blue light, 22.2 ± 0.5 hr with red light and 22.7 ± 3 hr with white light. On approaching the adult stage, the differences between the three colors used were substantially reduced: 24.3 ± 3 hr for blue light, 22.3 ± 0.6 hr for red light, and a stable period of 22.6 ± 1.2 hr for white light.

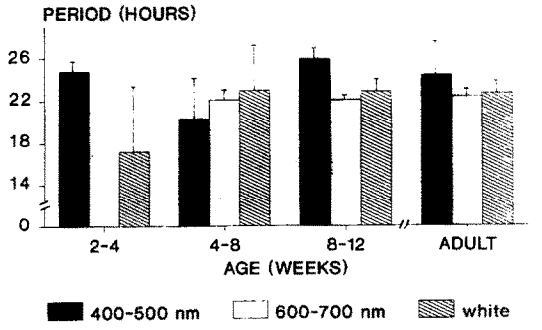

Fig. 1. Changes in the period length of the ERG rhythm (ordinate) obtained under white and monochromatic light from 2 weeks to the adult stage (abscissa). F calculated showed $P < 0.05$ when the period length of the rhythms obtained with white and blue light in animals aged 2–4 weeks were compared. The height of the bar is the average of 10 recordings plus the standard deviation in juvenile animals and the average of five recordings in adult animals.

Figure 2 shows the changes shown by another circadian parameter: the activity and rest time ratio ($\alpha : \rho$ ratio) during the different development stages using blue light flashes (dark bar), red light flashes (white bar) and white light fishes (striped bar). It is evident that in the very young animals (2–4 weeks) a blue light brings about a period of much more activity than rest, in contrast to the low $\alpha : \rho$ ratio that shows the circadian rhythm when white light is applied to these animals. With further development, applying blue light flashes to the crayfish brings about a shorter activity phase, reaching stability at a value close to 1.5 when the animal reaches the adult stage. It must also be noted that the color red produces an $\alpha : \rho$ ratio which is close to 1 in the younger animals (4–8 weeks) that respond to this color. This value is twice as high in the 8–12 weeks age group and decreases to 1.6 in adult animals. On the other hand, white light leads to a small increase in this ratio, ranging from a value of 1 for the younger animals to 1.7 for the adult group.

Figure 3 shows the changes that take place during development in the third circadian parameter: the rhythm's relative amplitude, i.e. the relation between the ERG voltage during the activity phase and the ERG voltage during the rest phase. Again, this shows the greatest difference in the circadian rhythm parameters in 2–4 week animals when they are stimulated with a blue light (2.8, dark bar) and with a white light (1.3, striped bar). The value of this parameter for the three colors used is notably similar for 4–8 weeks juvenile animals (which already show a response to red), but this is not true for the 8–12 weeks group in which sensitivity to the color red is translated in a relative amplitude of the rhythm with proportionally very high values (3.3). Adult crayfish show a higher night:day ratio with red (3.0) than with blue (2.25) or white (1.6).

In juvenile animals (4 weeks of age) that already show a circadian rhythm under a white light (even if

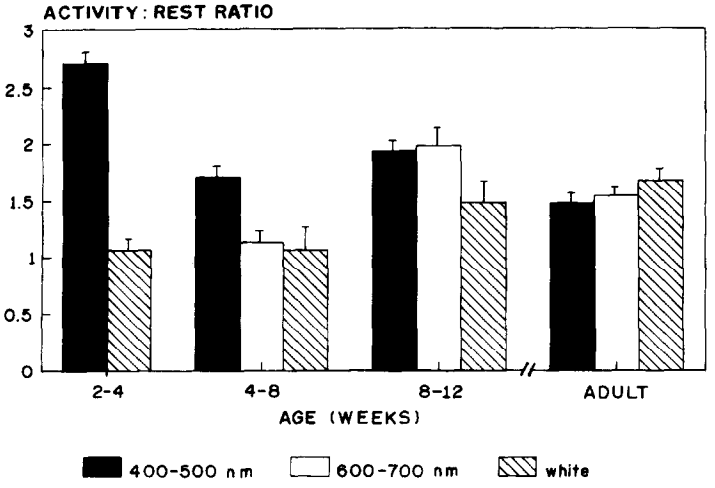

Fig. 2. Changes in the $\alpha:\rho$ ratio (ordinate) during crayfish development from 2 weeks to adult stage (abscissa). Note the significant differences ($P < 0.05$) in this parameter in age 2–4 weeks comparing blue vs white light, in age 4–8 blue vs red and white light, age 8–12 white vs blue and red light. In the adult animals there are no significant differences. Note the significant difference (5% level) among ages: blue light (2–4 weeks vs other ages) and red light (8–12 weeks vs other ages). The height of the bar is the average of 10 recordings plus standard deviation in the juvenile animals and the average of five recordings in adult crayfish.

there are superimposed ultradian oscillations), the greatest amplitude of the rhythm is usually during the geophysical day. When the test flashes change to monochromatic light, the phase measured at the onset of activity may move backwards or forwards. This makes the rhythm's acrophase move towards the geophysical night.

Figure 4 shows the changes in activity time (α) during a typical experiment in which a 4-week crayfish was successively stimulated with white, blue and red light. During the first 4 days of recording, stimulation with a white light produced a circadian rhythm with a period of 22.6 ± 0.4 hr and $\alpha = 9$ hr. The shift to blue light after the fifth day resulted in a circadian rhythm with a 23.6 ± 0.5 hr period. The activity phase began with a delay of 10 hr (since its beginning was expected at 6 hr but actually began at 16 hr) and had a duration of close to 15 hr. The shift to red light beginning on day 8 resulted in a slight delay in the onset of the activity phase and led to a shift to a circadian period to 25 ± 0.8 hr. In an equivalent experiment using an adult animal, changing from white light to blue light and red light only led to small changes in the time of the α onset but no significant changes in the duration of α or in the duration of the period that showed values of 22.6,

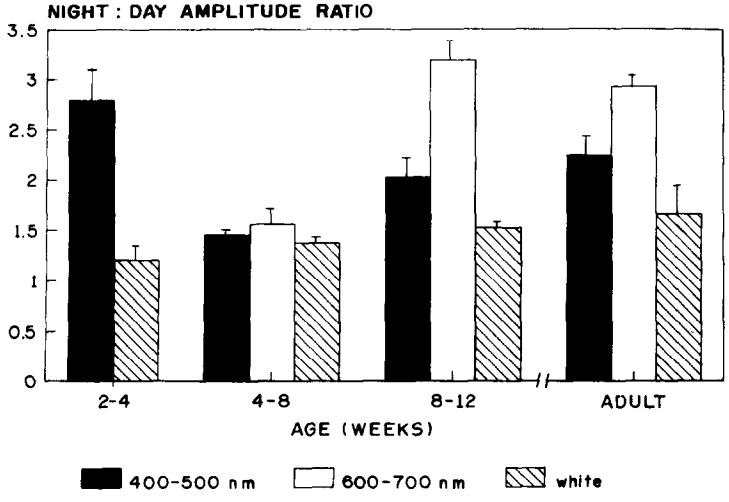

Fig. 3. Changes in the night:day amplitude ratio (ordinate) in the ERG circadian rhythm from 2 weeks to the adult stage (abscissa). Note the significant difference ($P < 0.05$) when comparing blue vs white light at 2–4 weeks; when comparing red vs white and blue light at 8–12 weeks and adult stage when comparing red vs blue and white light. There is also a significant difference among ages (5%): blue at 2–4 weeks and other ages and red at 8–12 weeks and adult stage vs other ages. The height of the bar is the average of 10 recordings plus the standard deviation for the juvenile stages and five recordings for the adult stage.

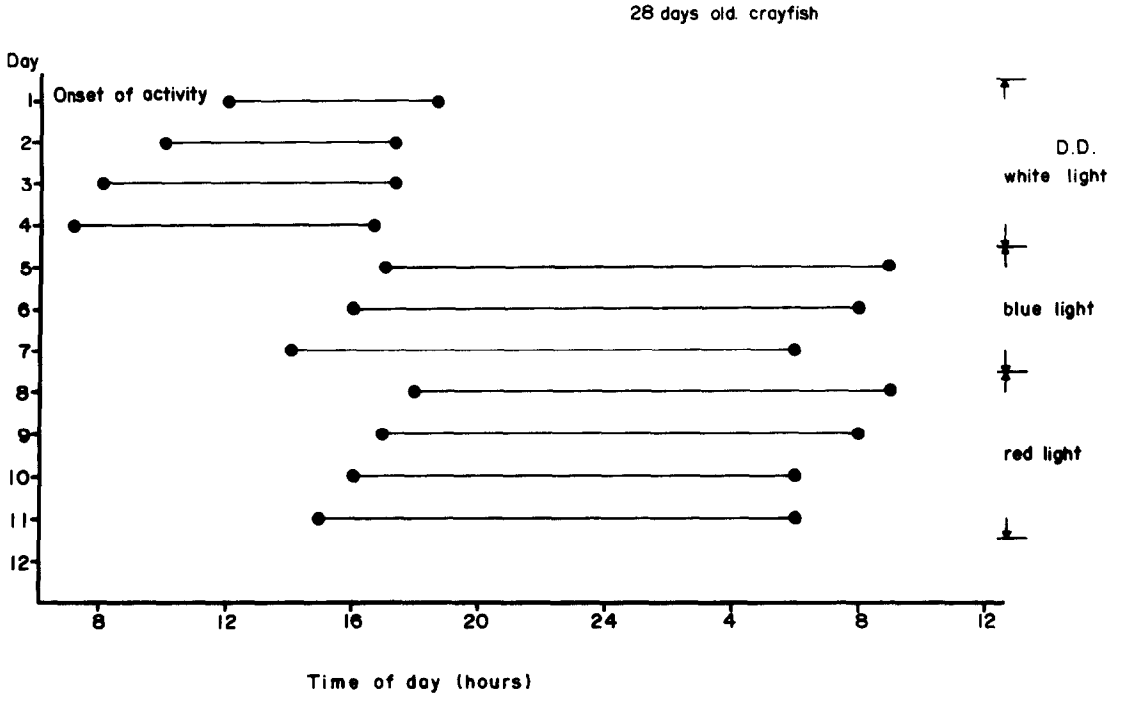

Fig. 4. Activity time during 11 days of recording in the free-running ERG rhythm obtained under white, blue and red light in a 4-week-old crayfish. The onset of activity indicates the phase of the rhythm. Note how this phase changes from day to night time when the rhythm is obtained under monochromatic light.

24.2 and 22.3 hr, respectively (Fig. 5). Even though this type of analysis did not make it possible to obtain accurate measurements of the phase changes, since the experimental protocol only contemplates recordings of 3 or 4 days for each one of the experimental conditions due to preparation survival problems, it

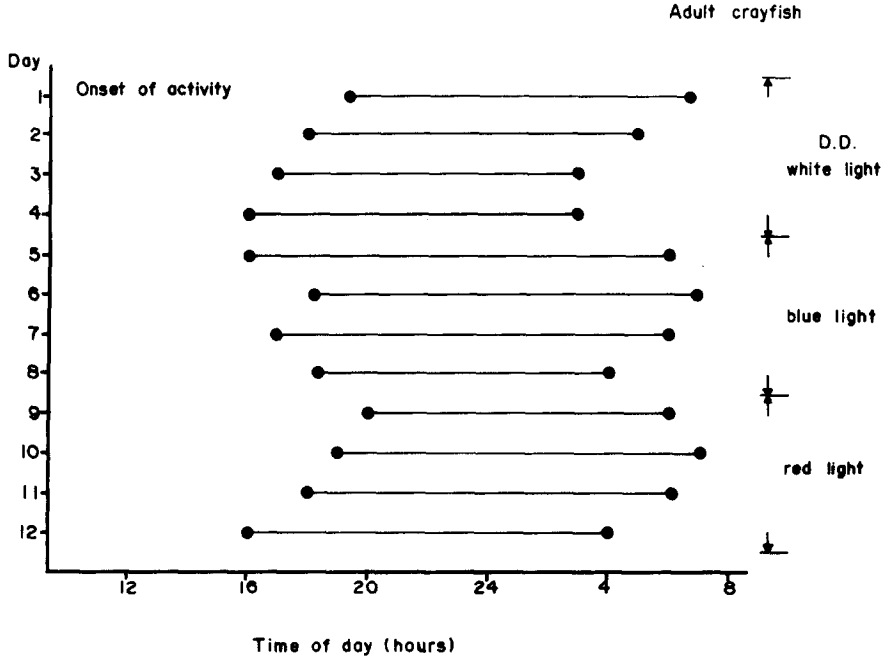

Fig. 5. Activity time during 12 days of recording in the free-running ERG rhythm obtained under white, blue and red light in an adult crayfish. There is no difference in the phase (onset of activity) when shifting from white to monochromatic light flashes. Note the slight increase in the length of the period when the rhythm goes from white ($\tau = 22.6$ hr) to blue light ($\tau = 24$ hr) diminishing again ($\tau = 22.2$ hr) when the rhythm changes to a red light.

seems that this rhythm tends to be delayed when a blue light is used. Consequently, there is a lengthening of the period under this condition even though it tends to move forward again when a red light is used and the period is shortened.

Effect of the monochromatic light on ultradian rhythms

In a previous paper (Fanjul-Moles *et al.*, 1987) it was shown that the ERG amplitude circadian rhythm obtained under white light in free-running conditions is not evident in very young animals (2–28 days after hatching). Rather, there is an ultradian rhythm in which the length of the period ranges from a few minutes to several hours depending on the age of the animal. The circadian rhythm starts becoming evident at 28 days but the phase and amplitude characteristics differ substantially from those of the adult animals. On the other hand, the length of the period shows considerable variation and ultradian rhythms are still superimposed in the incipient circadian rhythm. In this work, long-term recordings of very young animals (2–4 weeks) stimulated with white light showed well-defined ultradian cycles (4–18 hr) and incipient circadian cycles in the ERG amplitude. If under the same conditions a change is made from the test flash to blue light, the onset of evident circadian cycles superimposed by ultradian rhythms showing a notably lower amplitude may be observed. Figure 6 shows one of the 10 experiments of this type. The upper part shows 2 of the 3 days of the ERG rhythm recordings obtained using white light and the respective power spectrum analysis is shown on the right. This chart shows that the frequency peak of greatest statistical significance is 8.9 hr and that there is a 22.3 peak of lower amplitude. The lower part of Fig. 6 shows the last 2 of the 3 days of recordings of the same animal stimulated under blue light. An evident circadian profile and lower amplitude ultradian cycles is seen. This is confirmed by the power spectrum analysis on the right, in which the main peak has a value of 23 hr and there is a low peak of 9.2 hr.

When the crayfish has reached an age of 4–8 weeks, it can respond to color red even though the ERG amplitude to this color is lower than when blue or white flashes of the same intensity are applied. Long-term recordings, however, show that there is greater circadian rhythm organization with red than with any one of the other two colors. In a typical experiment (Fig. 7) the last 2 of 4 days of recording are shown for a 30-day-old animal initially stimulated with white light (upper left chart) and the power spectrum analysis is also shown (upper right chart). Under these conditions, the poor organization of the circadian rhythm and the relative high amplitude of the ultradian rhythms are evident. This is corroborated by the power spectrum analysis values showing a 19.5 circadian peak, a 10.7 hr ultradian peak, and a first infradian peak of 111.1 hr, which is clearly due to the trend of the 4 days of recordings (the first 2 days are

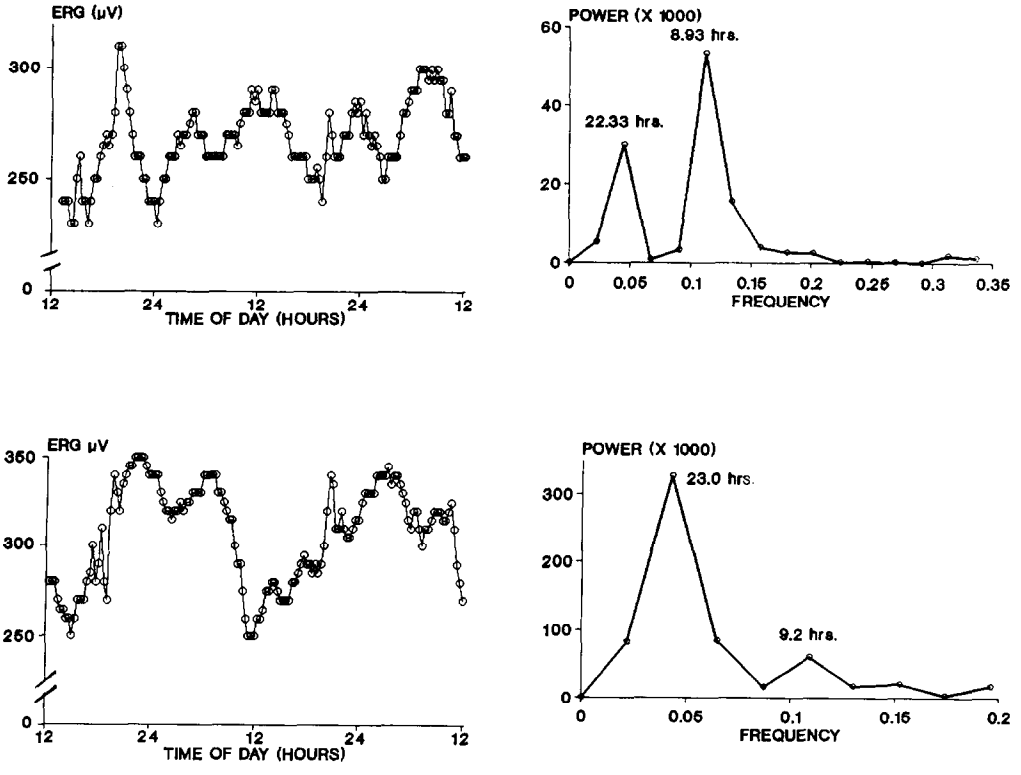

Fig. 6. Left: the upper chart shows ERG amplitude ultradian rhythm obtained from a 14-day-old crayfish stimulated with a white light. The lower chart shows ERG amplitude circadian rhythm obtained from the same animal when stimulated with a blue light. Right: power spectrum analysis computed from the raw data plotted in the left side of this figure (see text for further explanation). The horizontal axis is frequency and the vertical axis is the power spectrum estimate.

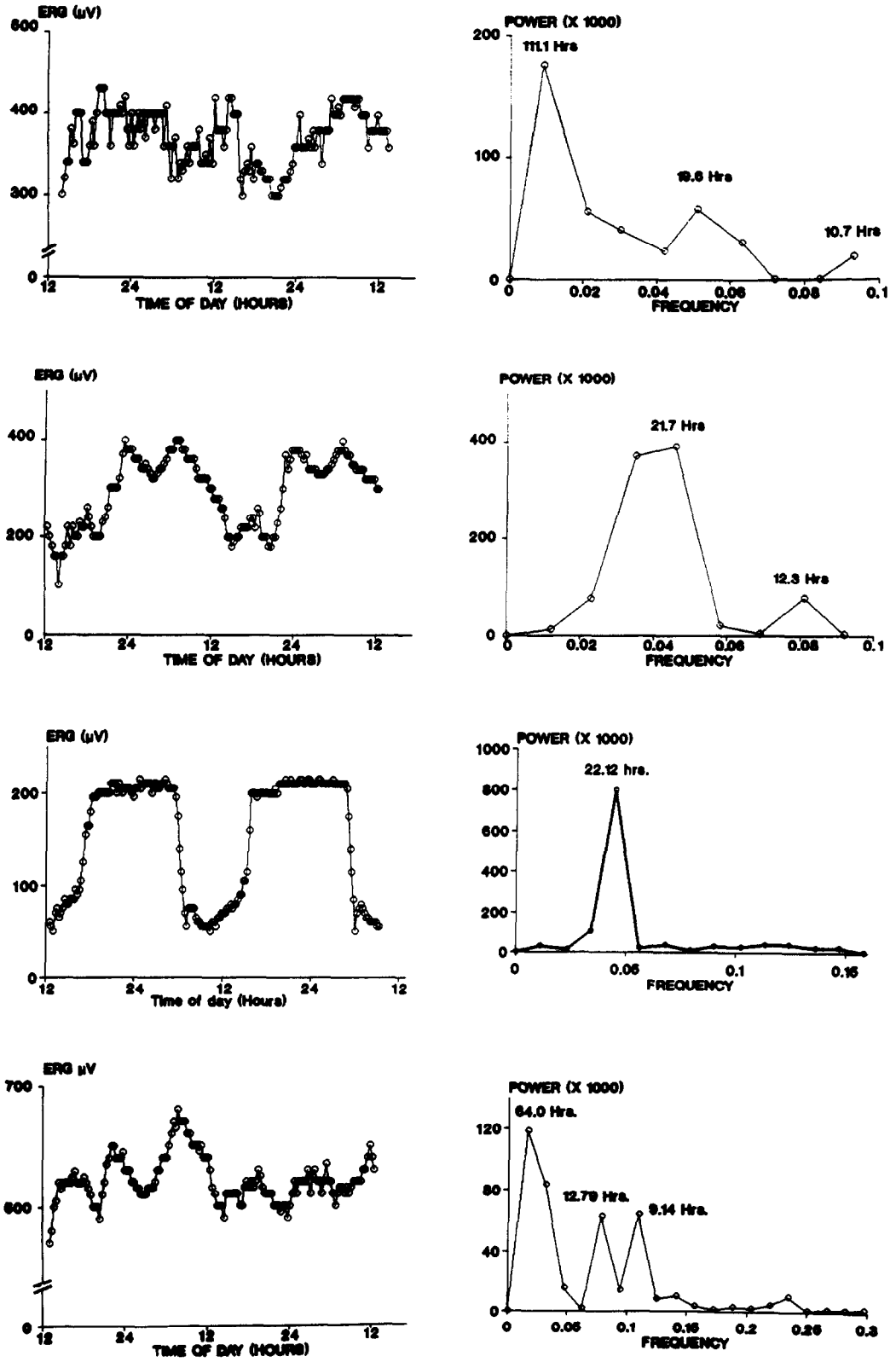

Fig. 7. Left: the first chart shows ERG amplitude rhythm obtained from a 30-day-old crayfish stimulated with a white light, second chart with a blue light, third chart with a red light and fourth chart with a white light again. Note the evident circadian rhythm when the animal is stimulated with a red light, even though there is a low voltage in the ERG. Right: power spectrum analysis computed from the raw data plotted in the left of this figure (see text for further explanation). The horizontal axis is frequency and the vertical axis is the power spectrum estimate.

not shown). In the second chart of Fig. 7, 2 days of recordings of the same animal stimulated with blue light are shown on the right. It is evident that the circadian profile has become clearer and even though ultradian rhythms have not disappeared completely, their amplitude has been reduced. The last two facts can be seen even more clearly on the power spectrum analysis on the right, which shows the peak of greatest statistical significance, 21.7, and an ultradian peak of 12.3 hr. The test stimulus for the same animal was then shifted to a red light (third chart in Fig. 7). The raw data chart shows how the circadian rhythm is organized, in spite of much lower ERG amplitude values than in the two previous conditions (200–50 μV). This chart also shows the substantial regularity of the circadian cycles and the virtual disappearance of high frequency cycles. These facts are confirmed by the corresponding power spectrum analysis showing only one significant peak at 22.1 hr. When the animal was stimulated again with white light (in spite of the fact that it is 10 days older than at the beginning of the recording), there seemed to be a regression in the circadian rhythm; again, high frequency oscillations superimposed on circadian oscillations were seen (Fig. 7, lower left). The corresponding power spectrum analysis (lower right hand side) also shows that in addition to a 64 hr period (due to the trend of the data) there are ultradian periods of 12.7 and 9.1.

DISCUSSION

The results shown in this paper confirm the findings by Fanjul-Moles and Fuentes-Pardo (1988) regarding the asymmetrical development of spectral sensitivity during ontogeny in the crayfish. In fact, while crayfish younger than 4 weeks show a clear response to blue, they also show a total lack of response to red. A response to red is shown only after 4 weeks of age. Furthermore, at this age the ERG voltage attains values of almost 800 μV with a blue light and in no case exceeds 200 μV with a red light. This is evidence that the photoreceptors involved in the detection of long wavelengths (600–700 nm) have a relatively late development.

Crayfish respond to blue from the age at which white light stimulation produces, in long-term recordings, a regular activity in which ultradian cycles prevail and there are hardly any evident circadian cycles (Fanjul-Moles *et al.*, 1987). The presence of ultradian rhythms may show a lack of coupling between the crayfish eyestalk oscillators due to insufficient maturation of the synchronization mechanisms during this stage of development (Fuentes-Pardo *et al.*, in press). Therefore, the fact that blue light stimulation induces a long-term response pattern in which there are reduced ultradian cycles and well-defined circadian cycles (Figs 6 and 7) showing relatively stable parameters (Figs 1–3) supports the interpretation that blue photoreceptors are true circadian photoreceptors, i.e. structures involved in the rhythm synchronization process. Synchronizing effects have been found in plants such as *Acetabularia* (Schmid, 1986) and invertebrates such as *Pectinophora gossypiella* and *Drosophila pseudoobscura* (Bruce and Minis, 1969; Frank and Zimmerman, 1969). In spite of the ERG low voltage obtained with a red light, crayfish of this age show a clear circadian rhythm as a response to light. Furthermore, circadian parameters show great stability (Figs 1–3) and the shift from white to red light results in the complete disappearance of high frequency cycles that appear with a white light. Consequently, well-defined circadian cycles are established (Fig. 7).

In addition, these facts show evidence of long wave photoreceptor involvement (which is even more powerful than short wave) as a synchronizer of ERG circadian rhythm. Synchronizing effects involving red light similar to the ones discussed here have been reported for invertebrate and vertebrate species (Ehret, 1960; Hastings and Sweeny, 1960; Takahashi *et al.*, 1984). The fact that ERG amplitude during ontogenic development is always lower with red light than with blue or white light while the circadian system's characteristics are more precisely defined at this wavelength (600–700 nm) leads to an interpretation based on greater gain by the clock reset system with a long wavelength. Similar differences between the retina's spectral sensitivity and the spectral sensivity of the circadian clock's mechanisms have been proposed in *Periplaneta americana* (Mote and Black, 1981).

The involvement of the distal and proximal accessory pigments and of the reflector pigment (tapetum) as light retinal sensitivity modulators in crayfish has been well established (Fuentes-Pardo and García, 1979; Goldsmith, 1978; Kong and Goldsmith, 1977; Bryceson, 1986). It is known that distal retinal pigment granules, as well as their regulating system, appear relatively late in development (Hafner *et al.*, 1982). The proximal retinal pigment emerges from the first stages of development (Hafner *et al.*, 1982) and is independent of the neuroendocrine regulation since its migration seems to be regulated by 5-hydroxytryptamine (Aréchiga *et al.*, 1990). It is also known that this pigment migrates even in the isolated eye (Olivo and Larsen, 1978; Frixione *et al.*, 1979), that its maximum spectral sensitivity coincides with that of visual photoreceptors (600 nm) (Olivo and Chrismer, 1979), and that it is closely related to the reflector pigment containing pterines, substances which are considered to be photosensitive to short wavelength (blue) radiation for which tapetum shows maximum absorbency (Ghidalia, 1985). Therefore, as proximal and distal pigments appear at different times on the course of ontogeny in the crayfish, it may be assumed that the asymmetric development of spectral sensitivity in this species is based on both the appearance of a minimum of two sets of photoreceptors and the involvement of two different retinal sensitivity modulation systems at two different times during ontogeny. It is evident that these retinal spectral sensitivity modulation systems could also be involved in the circadian function of photoreceptors, which in spite of having been stimulated with the same light intensity would detect a different amount of light depending on the wavelength. This would translate into different information received by the nervous centers related to the rhythms synchronization mechanisms.

Consequently, these results suggest the presence of two systems of circadian photoreceptors. One of

these systems has an early development (detecting color blue) and the other one has a late development (detecting color red). As the projection sites for both systems are different (Nässel, 1977) it is possible to sustain that at least two independent systems are involved in the synchronization of the ERG circadian rhythm: a short wavelength system involving an eminently neural regulation and a long wave detection system involving the endocrine system, and especially, the sinus gland.

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REFERENCES

Aréchiga H., Bañuelos E., Frixione E., Picones A. and Rodríguez-Sosa L. (1990) Modulation of the crayfish retinal sensitivity by 5-hydroxytryptamine. *J. exp. Biol.* **150,** 123–141.

Bruce V. G. and Minis H. D. (1969) Circadian clock action spectrum in a photoperiodic moth. *Science* **163,** 583–585.

Bryceson K. P. (1986) Diurnal changes in photoreceptor sensitivity in a reflecting superposition eye. *J. comp. Physiol.* **158A,** 573–582.

Ehret C. F. (1960) Action spectra and nucleic acid metabolism in circadian rhythms at the cellular level. *Cold Spring Harbour Symp. Quant. Biol.* **25,** 149–158.

Enright J. T. (1981) Methodology. In *Handbook of Behavioral Neurobiology*, Vol. 4, *Biological Rhythms* (Edited by Aschoff J.), pp. 21–39. Plenum Press, New York.

Fanjul-Moles M. L. and Fuentes-Pardo B. (1988) Spectral sensitivity in the course of the ontogeny of the crayfish *Procambarus clarkii*. *Comp. Biochem. Physiol.* **91A,** 61–66.

Fanjul-Moles M. L., Moreno-Sáenz E., Villalobos-Hiriart N. and Fuentes-Pardo B. (1987) Circadian rhythm in the course of the ontogeny in crayfish. *Comp. Biochem. Physiol.* **88A,** 213–219.

Frank D. K. and Zimmerman W. F. (1969) Action spectra for phase shifts of a circadian rhythm in *Drosophila*. *Science* **163,** 688–689.

Frixione E., Aréchiga H. and Tsutsumi V. (1979) Photomechanical migration of pigment granules along the retinula cell of the crayfish. *J. Neurobiol.* **10,** 573–590.

Fuentes-Pardo B. and García M. C. (1979) Effect of light deprivation on the neurohumoral activity of the visual system of the crayfish. *Comp. Biochem. Physiol.* **64A,** 649–555.

Fuentes-Pardo B., Fanjul-Moles M. L. and Moreno-Sáenz E. (in press) Effect of a single pulse light on the ERG rhythm during the course of the crayfish ontogeny. *J. interdisc. Cycle Res.*

Ghidalia W. (1985) Structural and biological aspects of pigments. In *The Biology of Crustacea* (Edited by Bliss D. E.), Vol. 9, pp. 302–375. Academic Press, New York.

Goldsmith T. H. (1978) The effects of screening pigments on the spectral sensitivity of some crustacea with scotopic (superposition) eyes. *Vision Res.* **18,** 475–482.

Hafner G. S., Tokarski T. and Hammond-Soltis G. (1982) Development of crayfish retina: a light and electron microscopic study. *J. Morph.* **173,** 101–118.

Hastings W. L. and Sweeney B. M. (1960) The action spectrum for shifting the phase of the rhythm of luminescence in *Gonyaulax polyedra*. *J. gen. Physiol.* **43,** 697–705.

Hoffmann K. (1981) Photoperiodism in vertebrates. In *Handbook of Behavioral Neurobiology*, Vol. 4, *Viological Rhythms* (Edited by Aschoff J.), pp. 449–474. Plenum Press, New York.

Joshi D. and Chandrashekaran M. K. (1985) Spectral sensitivity of the photoreceptors responsible for phase shifting the circadian rhythm of activity on the bat *Hipposideros spheoris*. *J. comp. Physiol.* **156A,** 189–198.

Kong K. L. and Goldsmith T. H. (1977) Photosensitivity of retinular in white eye crayfish (*Procambarus clarkii*). *J. comp. Physiol.* **44,** 1089–1102.

Mote L. M. and Black R. K. (1981) Action spectrum and threshold sensitivity and entrainment of circadian running activity in the cockroach *Periplaneta americana*. *Photochem. Photobiol.* **34,** 257–265.

Nässel D. R. (1977) Types and arrangement of neurons in the crayfish optic lamina. *Cell Tissue Res.* **179,** 45–75.

Nuboer J. F., Nuys W. M. van and Steenberger J. C. van (1983) Colour changes in a light regimen as synchronizers of circadian activity. *J. comp. Physiol.* **151,** 359–366.

Olivo R. F. and Chrismer K. L. (1979) Spectral sensitivity of screening-pigment migration in retinular cells of the crayfish *Procambarus*. *Vision Res.* **20,** 385–389.

Olivo R. F. and Larsen M. E. (1978) Brief exposure to light initiates screening-pigments migration in retinular cells of the crayfish *Procambarus*. *J. comp. Physiol.* **125,** 91–96.

Page T. L. (1981) Neural and endocrine control of circadian rhythmicity in invertebrates. In *Handbook of Behavioral Neurobiology*, Vol. 4, *Biological Rhythms* (Edited by Aschoff J.), pp. 95–124. Plenum Press, New York.

Page T. L. (1982) Extraretinal photoreceptors in entrainment and photoperiodism in invertebrates. *Experientia* **38,** 1007–1013.

Sánchez J. A. and Fuentes-Pardo B. (1977) Circadian rhythm in the amplitude of electroretinogram in the isolated eyestalk of the crayfish. *J. comp. Biochem. Physiol.* **91A,** 61–66.

Schmid R. (1986) Twofold effect of blue light on a circadian rhythm in *Acetabularia*. *J. interdisc. Cycle Res.* **17,** 99–107.

Takahashi J. S., De Coursey P., Bauman L. and Menaker M. (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* **308,** 186–188.