J. exp. Biol. (1979), 79, 163–168
With 2 figures
Printed in Great Britain

BILATERAL EFFECTS ON RETINAL SHIELDING PIGMENTS DURING MONOCULAR PHOTIC STIMULATION IN THE CRAYFISH, *PROCAMBARUS*

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(*Received* 20 *June* 1978)

SUMMARY

Bilateral light adaptation of distal retinal shielding pigments was observed a few minutes after 60 min of sustained photic stimulation (2·69 Cd/m^2) of a single eyestalk of the dark-adapted crayfish. Pigment migration was observed in intact animals as a diminution of eye glow area and electroretinogram amplitude, and was confirmed in sectioned material. The effect upon the contralateral eye was abolished by surgical bisection of the cerebral ganglion. It is suggested that the mechanism involved in the bilateral effect may be responsible for the mutual entrainment of ERG circadian rhythms of both eyes.

INTRODUCTION

In decapod crustaceans, a variety of evidence supports the concept that distal (d) and proximal (p) retinal shielding pigments (RSP) behave as effectors independent of one another in response to light or darkness. Early observations of the pigment cells have suggested that there is hormonal control of the dRSP and not the pRSP (Kleinholz, 1936). Since these effectors modulate retinal sensitivity (Crozier & Wolf, 1939; Aréchiga, Fuentes-Pardo & Barrera-Mera, 1974), the activation of retinal photoreceptors is apparently the first step in the light-adaptation photomotor response of RSP. Like the vertebrate pupillary photomotor reflex, this change is proportional to the amount of light applied to the eye. Thus far, the heterolateral component of this phenomenon, corresponding to the vertebrate consensual reflex, has been poorly understood in these animals. Early observations on the RSP photomotor response of decapod crustaceans (Parker, 1897; Von Frisch, 1908; Castle, 1927; Bennitt, 1932*a*), which were intended to determine the existence of such reciprocal influences between left and right eyes, are far from conclusive. We have recently reported (Barrera-Mera & Abasta, 1978) that after photic stimulation of either eye in *Procambarus bouvieri*, bilateral diminution of retinal sensitivity occurred with a latency of 15–25 min. Furthermore, the strong tendency for the circadian rhythms of the electroretinogram (ERG) to maintain the same period and a constant phase relation (Barrera-Mera, 1978) suggests a close reciprocal influence between the two eyes. These considerations led us to postulate that unilateral light-stimulation activates the neuroendocrine

system in both eyestalks and thus modulates the retinal sensitivity consensually. Such modulation could depend on mobilization of dRSP by the action of light-adaptation hormone (Kleinholz, 1936; Josefsson & Kleinholz, 1964; Fernlund, 1971) liberated from the sinus gland. The present experiments were designed to learn the effects upon RSP position of sustained photic stimulation of one eye or the other by recording the ERG and measuring the eye glow area (EGA) of intact preparations, and measuring RSP distribution in sectioned eyes. Experiments were carried out during the rest phase of the ERG circadian cycle because during this period a major depression of ERG voltage is seen after unilateral light-stimulation (ULS) of the eyestalk (Barrera-Mera & Abasta, 1978).

METHODS

The experiments were performed upon 105 specimens (8–12 g body weight) of *Procambarus bouvieri* (Ortmann) between 10.00 a.m. and 3.00 p.m. (i.e. during the rest phase of the ERG circadian rhythm). A cork glued to the posterior carapace of the animal was fixed by a clamp as described by Aréchiga *et al.* (1974). The eyes were also fixed to the carapace by means of acrylic cement. Each animal remained in a small black cage, half full of water, which was kept inside a sound proof box at 21 ± 1 °C in complete darkness, interrupted only by the periodic photic stimuli applied to obtain the ERG responses. The test light-stimuli of 3 s duration (1·34 cd/m^2) were given during 2–3 days every 2·5 min, and sustained ULS to the contralateral eye was applied during the rest phase of the ERG circadian rhythm. ERG responses were continuously recorded by means of metal microelectrodes; signals were amplified by a Tektronix 122 amplifier and recorded on a Physiograph (Narco Bio-Systems).

Both test light-stimuli and sustained photic stimulation (2·69 cd/m^2) were applied with a small 222 Philips flashlight bulb (0·25 A; 2·2 V). The stimuli were applied as a point source to the corneal surface by means of a glass tube attached to the lamp. The glass tube was painted black on the outside and tapered to about 800–600 μm diameter at the point of exit of the light. Surgical bisection of cerebral ganglia was as described earlier (Barrera-Mera, 1976).

To characterize migration of RSP brought about by ULS, the exact positions of both pigmentary effectors were obtained by use of the technique of Parker (1897). After the animals were killed in hot water at 80 °C, the eyes were left for 24 h in 5% aqueous Formalin, and then frozen unstained sections (70 μm) were made.

Pigmentary migrations were characterized in terms of RSP indices calculated by a modification of the De Bruin & Crisp (1957) technique. The dRSP index (DPI) was obtained by measuring the corneal thickness (c) plus the width of the dRSP layer (d) and dividing by two times the distance of the external margin of dRSP to the basal membrane (r). The pRSP index (PPI) is calculated from the formula $PPI = (2b/p)/r$, where b is the distance of migration of pRSP from the basal membrane, and p is the thickness of pRSP beneath the basal membrane.

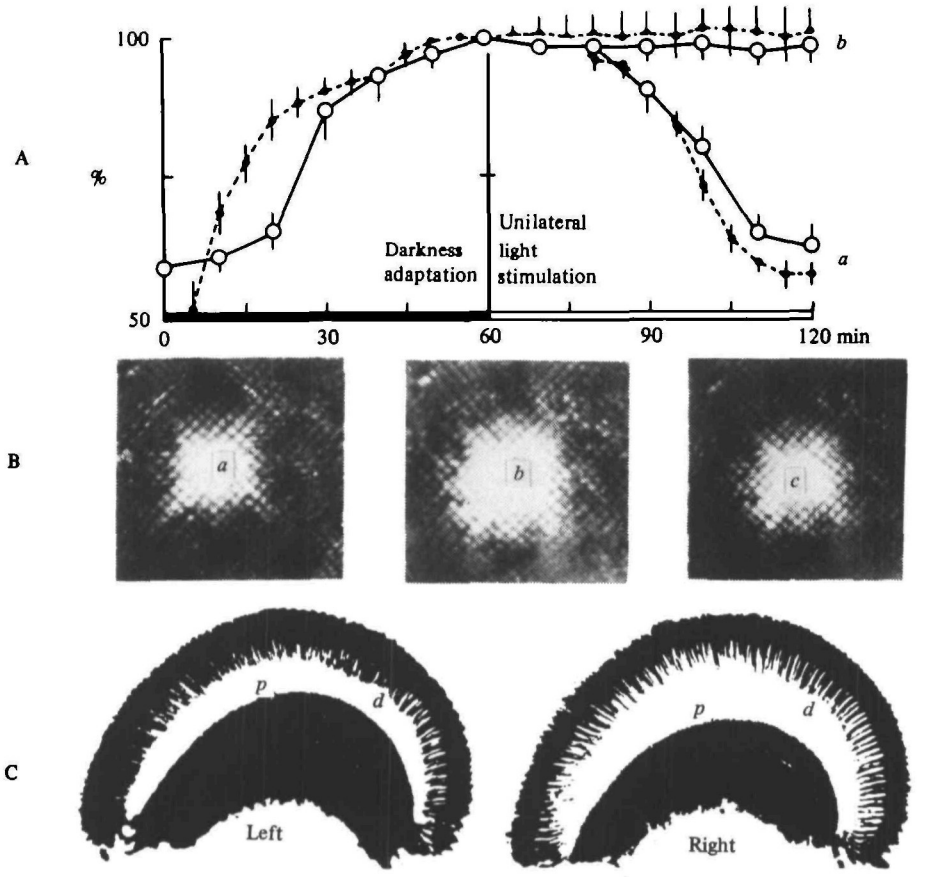

Fig. 1. (A) Effects of 60 min of unilateral light-stimulation (ULS) upon the electroretinogram (ERG) (●) and the eye-glow area (EGA) (○) after 60 min of dark-adaptation. ERG and EGA depression (A, *a*) are not seen in split-brain animals (A, *b*). (B) Changes in EGA size. From left to right: (*a*) light-adapted by the application of light (2·69 cd/m²), for 60 min, (*b*) dark-adapted for 60 min, (*c*) light-adapted by ULS for 60 min. (C) Light-adaptation of distal (*d*) retinal shielding pigment is symmetrical in both eyes of the intact animal in response to photic stimulation of the left eye. In contrast, proximal (*p*) shielding pigment in right eye remains as for the dark-adapted state. The pRSP of the right eye is in the position for a partially light-adapted eye due to the particular time in the circadian rhythm.

RESULTS

Light-adaptation (2·69 cd/m²) of both eyes for 60 min in intact animals was followed by a dark period of 60 min. Subsequent ULS produced a clear diminution of both ERG and EGA (Fig. 1 A, *a*). This response had a relatively long latency (15–25 min) and also showed a slow decrement with time. With return of the original illumination, there was an increase in ERG and EGA with a time course similar to that of the decrease during ULS. Surgical bisection of the cerebral ganglion usually resulted in no change of ERG or EGA during the ULS (Fig. 1 A, upper trace *b*). However, in 6 of the 25 operated animals the ERG increased a small amount immediately after ULS.

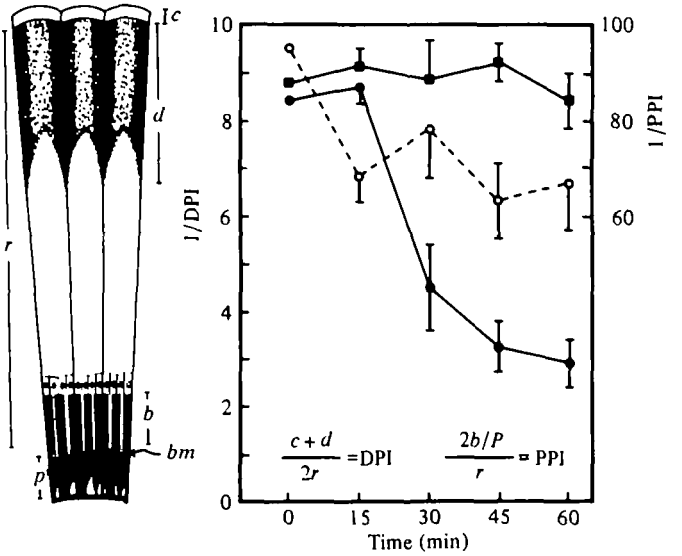

Fig. 2. Left illustration shows the distance from the external end of dRSP to the basal membrane (*r*), corneal lens thickness (*c*), and the proximal (*b*, *p*) and distal RSP (*d*) positions. These parameters were measured to calculate the distal (DPI) and proximal (PPI) pigmentary indices after heterolaterally induced light-adaptation. DPI values are indicated for intact (●) and for split-brain (■) animals, for contralateral eyes. PPI values (○) are from both intact and split-brain preparations since they did not differ. Vertical lines represent the standard deviation ($n = 8$). Notice the position of basal membrane (*bm*) between *b* and *p* proximal pigments.

Fig. 1(B) shows EGA in an intact light-adapted animal (*a*), after 60 min of dark-adaptation (*b*), and after 60 min of ULS (*c*).

In intact, previously dark-adapted animals, the application of light for 60 min to the left eye was followed by a migration of the dRSP toward its light-adapted position, to as equal degree in the stimulated and non-stimulated sides (Fig. 1C). In a dark-adapted eye, the dRSP layer is normally about one third as thick since it remains distally concentrated. As can also be seen in Fig. 1 C, the pRSP behaved in a strikingly different way; complete light-adaptation of pRSP (distal migration) was observed in the illuminated left eye, while the pRSP of the right eye remained in the dark-adapted position (proximal migration). In split-brain animals exposed to ULS, both d and p RSP moved to the light-adapted position only in the stimulated eye, the pigments in the contralateral eye remaining in the dark-adapted position.

Fig. 2 shows that ULS changed the RSP index of distal retinal shielding pigments in the contralateral eye before (●) but not after (■) the surgical bisection of the cerebral ganglion. Brain bisection had no effect on the action of ULS on the RSP index for proximal RSP, so values before and after bisection have been plotted together (○). During ULS there was usually no change in the proximal indices of the contralateral eye, but in a few intact preparations ($n = 5$) and split-brain ones ($n = 3$) a slight distal migration of the pRSP to a light-adapted position was observed after 15 min of ULS (Fig. 2).

DISCUSSION

Considerable independence between the distribution of retinal shielding pigments of left and right eyes of crustaceans has been reported by Parker (1897) and Castle (1927) when one eye was covered and the other exposed to light. However, under similar experimental conditions Von Frisch (1908) and Bennitt (1932*a*) observed a similar migration of pigments in both eyes. These observations are difficult to interpret, for reasons such as the relatively long duration of stimulation by light, the circadian influence on the position of RSP, the neural regulation of proximal RSP, and the possible spread of light to the opposite eye. The present measurements of RSP distribution, by two techniques, together with the electrophysiological evidence, support the previous proposal of a mutual modulatory influence in the crayfish visual system (Barrera-Mera & Abasta, 1978).

Since the EGA size, like the RSP indices, changes proportionally to the light intensity (Aréchiga *et al.* 1974) and also, during ULS, follows a temporal course similar to that of the ERG, we believe that most modulation of retinal sensitivity by ULS is achieved by RSP mobilization. The 15–25 min latency in the response is probably due to the diffusion time of the light-adapting hormone and the rate of movement of dRSP. Although other possibilities cannot be ruled out, we believe the heterolateral sustained response neurones located in the optic tract (Wiersma & Yamaguchi, 1966), could be the neural pathway conveying light information between the neurohaemal systems of the sinus glands of both eyestalks. The small amount of pRSP migration seen during ULS exposure in eight animals is probably due to a slight spread of light to the non-illuminated eye. This change could be mediated by a neural control exerted ipsilaterally by the activated retinular photoreceptors as described in other crustaceans (Ludolph, Pagnanelli & Mote, 1973) and also in the crayfish (Olivo & Larsen, 1978). Finally, the existence of a circadian rhythm in the position of both distal (Welsh, 1930; Fingerman & Lowe, 1957) and proximal pigments (Bennitt, 1932*b*) in the visual system of several decapod crustaceans and also in *Procambarus bouvieri* (Aréchiga, Fuentes & Barrera, 1973), complicates the role of RSP mobilization upon retinal sensitivity for both short and long periods of time (Barrera-Mera & Abasta, 1978). If the distal RSP position is indeed the most important modulatory influence upon retinal sensitivity in both sides during light-stimulation of one eye only, this bilateral influence may be the synchronizing process for the entrainment of the left and right ERG during circadian changes. This seems to be the first reported mechanism involved in the coupling of symmetrical circadian pacemakers of apparently identical hierarchical importance (Barrera-Mera, 1978).

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