Journal of Photoscience (2002), Vol. 9(2), pp. 13–16

Photoreception for Photoperiodism and Circadian Rhythms in the Blow Fly

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A comparison of the functional components underlying photoperiodism and circadian rhythmicity in the same species is an interesting issue in the context of unravelling clock mechanisms. In the present study, covering or surgical removal of the compound eyes was performed to localize photoreceptors for photoperiodism to control reproductive diapause and for entrainment of circadian locomotor rhythms in the blow fly *Protophormia terraenovae*. Intact flies showed a long-day photoperiodic response. When the compound eyes were covered by silver paint, diapause incidence increased under diapause-averting conditions of a long-day photoperiod and constant light, as if flies were kept under constant darkness. Covering of a medial region of the head capsule or solvent painting of the compound eyes gave no significant effects. When the compound eyes were removed, flies did not distinguish the photoperiod, whereas removal of antennal lobes or ocelli did not affect the photoperiodism. Intact flies showed a freerunning rhythm under constant darkness. The rhythm entrained to light-dark (LD) cycles with light of high and low intensity. When the compound eyes and ocelli were surgically removed, the rhythm entrained to LD cycles with light of high intensity but freeran under LD cycles with light of low intensity. The results suggest the retinal pathways are involved in photoperiodism and that flies use both retinal and extraretinal pathways for rhythm entrainment. Under dim light-LD cycles, the retinal pathways mainly mediate rhythm entrainment. Retinal photoreceptors seem to be used both for photoperiodism and entrainment of the rhythm.

Key words: compound eyes, circadian rhythm, extraretinal photoreceptors, photoperiodism, *Protophormia terraenovae*

INTRODUCTION

The period of day or night length in the daily cycle is an important cue to know seasons of a year. Many organisms use photoperiod for photoperiodism and entrainment of circadian rhythms. Both are biological timing systems controlling development and various kinds of behaviors. Photoperiod is received by certain photoreceptors to entrain circadian clocks, which drive motor programs controlling behaviors. In photoperiodism there could be three steps after photoreception. The first one, a time measurement system, discriminates light or dark of the daily cycle, and measures duration of each phase presumably using the circadian clock. The second one, a counter system, involves summation of the information for successive days to a point at which endocrinological switches occur. The third one is an endocrine system controlling developmental phenomena.

In insects, although genetic elements constituting the circadian clock system have been unveiled, practically nothing is known about concrete physiological mechanisms involved in photoperiodism. With interests in functional components underlying photoperiodism, we examined the photoreceptors for the photoperiod and made a comparison in photoreception between the two biological timing systems, i.e., photoperiodism and rhythm entrainment. For the comparison it is important to use species that are sensitive to photoperiod for photoperiodism and rhythm entrainment at the same developmental stage. We used female adults of the blow fly *Protophormia terraenovae*. This species show a long-day photoperiodic response to control reproductive diapause. Under long-day conditions flies reproduce and under short-day conditions they enter diapause. They also show a clear locomotor rhythm with the activity in the photophase.

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MATERIALS AND METHODS

Female adults were used from a laboratory culture of the blow fly *Protophormia terraenovae* (Diptera: Calliphoridae) that reared under LD 18:6 at 25°C.

Photoperiodism. Larvae and pupae were kept under LD 18:6 at 25°C. After emergence adults were individually kept under LD 18:6, LD 12:12, constant light (LL) with the intensity of the light, 1.4 x 10^{-3} W/m^2 (low) or 1.4 W/m^2 (high) in the photophase, or constant darkness (DD). Females were fed with sucrose and water, and for the last three days additionally with a piece of beef liver. Painting or surgical operation of the compound eyes (CE) was carried out on the day of adult emergence (day 0) and flies were dissected on day 13 to examine the ovarian development. Flies without yolk deposition in the oocytes were judged to be in diapause.

Activity recording. Locomotor activity was measured at 20°C in terms of the number of times that the fly interrupted the infrared beam. On day 3 or 4, painting or surgical removal of the CE was carried out. After the treatments, the activity was recorded under LD 12:12 for 9 or 10 days and subsequently under LD 13:13, or LD 11:11 for 9 or 10 days. Light of low or high intensity described in the above was used in the photophase. Rhythmicity was evaluated by chi-square periodogram and visual inspection.

Treatments of the CE and histological autopsy. Methods for covering, surgical operations and histological examination are described in [1] and [2]. For covering silver paint was used. In the CE removal experiment, for examination of photoperiodism the CE were bilaterally removed and for examination of the rhythm entrainment the CE and all ocelli were deprived.

RESULTS AND DISCUSSION

In intact flies diapause incidences were lower under LD 18:6 and LL than those under 12:12 and DD. Flies discriminated photoperiod with light of both low and high intensity. Therefore, in the covering experiment we used light of low intensity to prevent the light from coming through the paint. Figure 1A shows effects of covering of the CE under diapause-preventing conditions of LL and LD 18:6. When the CE were bilaterally covered, diapause incidence increased as if they were under DD. Covering of the medial region of the head and painting with a clear solvent of the CE were not effective. When the CE were bilaterally removed, all the flies developed their ovaries both under LD 18:6 and under LD 12:12. Figure 1B shows the effects of different kinds of surgical operation on the diapause incidence under LD 12:12 with light of high intensity. In contrast with the CE removal, diapause incidences in removal of the ocelli, removal of unilateral antennal lobe, or sham operation were not different from the intact group.

When bilateral CE were covered or removed, flies lost the sensitivity to photoperiod. The results indicate that *P. terraenovae* perceives photoperiod through the CE. However, these two treatments gave a contrast in the reproductive status: The covering induced diapause, whereas the removal induced ovarian development. Absence of the photoperiodic receptor might be physiologically different from perception of constant darkness through the photoperiodic receptor.

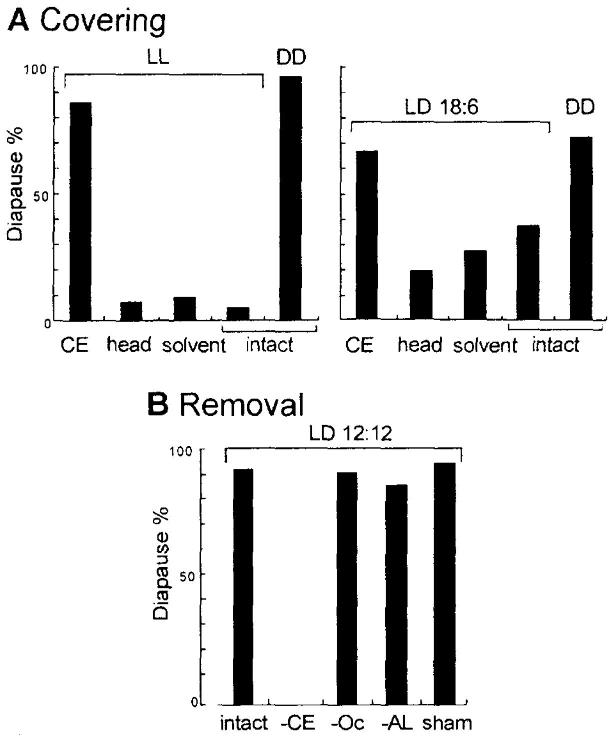

Figure 1. Effects of covering (**A**) and removal (**B**) of compound eyes on diapause incidence in *Protophormia terraenovae*. **A**. Flies were kept under LL or LD 18:6 with light of low intensity, 1.4 x 10^{-3} W/m^2. Bilateral compound eyes (*CE*) or a medial region of the head capsule (*head*) were painted with pasted silver. Bilateral compound eyes were painted with a clear solvent (*solvent*). Results of intact flies under DD are also shown. n=26-64. **B**. Flies were kept under LD 12:12 with light of high intensity, 1.4 W/m^2. Bilateral compound eyes (*-CE*), all ocelli (*-Oc*), unilateral antennal lobe (*-AL*) were surgically removed. *sham*, sham operation. n=17-21. (Altered from [1])

In intact females, the freerunning period of the locomotor activity rhythm under DD was about 25.0 h. The rhythm entrained to LD cycles with activity restricted to the photophase. Figure 2A shows effects of covering of the CE on entrainment of the activity rhythm. Painting the CE with a clear solvent had no effects on the activity pattern. When the CE were covered, most flies did not entrain but freeran under light of both low and high intensity. The covering of the CE prevented the rhythm from entraining to the LD cycle and produced freerunning rhythm. Figure 2B shows effects of removal of the CE. All of the sham-operated flies entrained to the LD cycles. After removal of the CE, however, most flies showed entrainment to LD cycles and no flies exhibited freerunning with light of high intensity. With light of low intensity, most flies showed freerunning rhythm and no flies showed entrainment. Entrainment after removal of the CE indicates the presence of extraretinal photoreceptors and their participation in rhythm entrainment. However, under LD cycles with light of low intensity, the

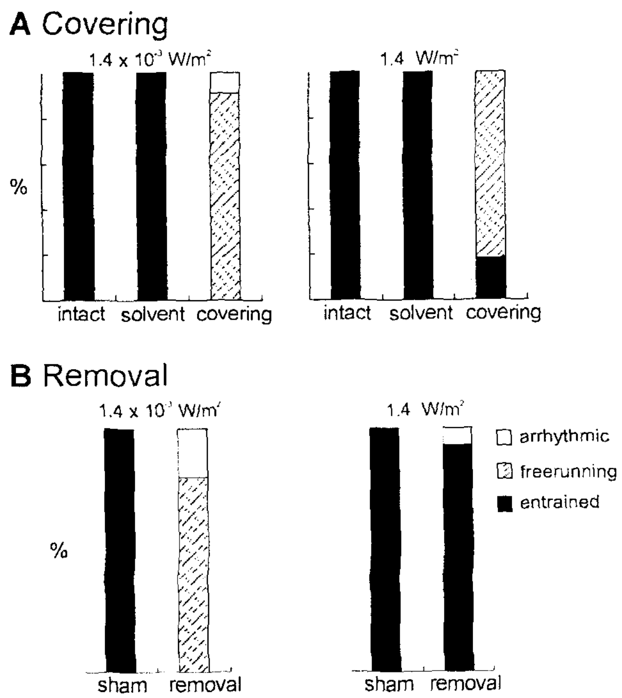

Figure 2. Effects of covering (**A**) and removal (**B**) of the compound eyes on entrainment of circadian rhythm in *Protophormia terraenovae*. Entrainability of the locomotor activity rhythm to LD cycles was determined. Two kinds of the intensity of the light, 1.4 x 10^{-3} W/m^2 (left) and 1.4 W/m^2 (right) were used in the photophase. **A**. Bilateral compound eyes were painted with pasted silver (*covering*) or with a clear solvent as a control (*solvent*). n=3-11. **B**. Bilateral compound eyes were surgically removed (*removal*) or sham-operated (*sham*). n=4-20. (Altered from [2])

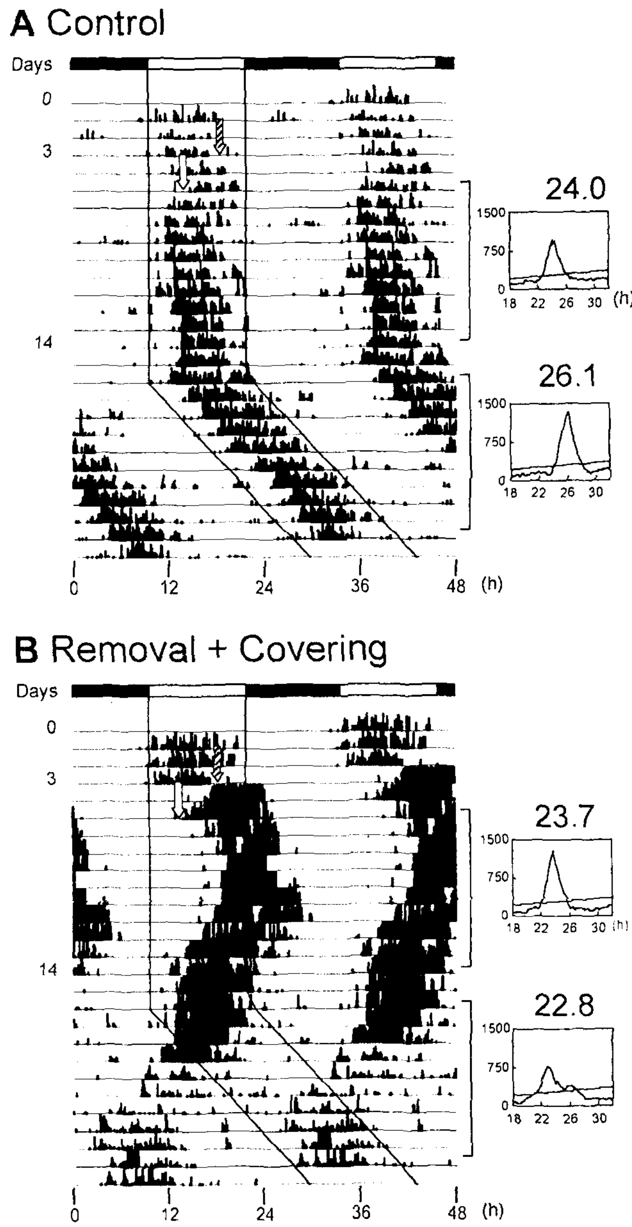

Figure 3. Effects of removal and subsequent covering of the compound eyes on the locomotor activity rhythm in *Protophormia terraenovae*. The intensity of the light in the photophase was 1.4 W/m^2 **A**. The compound eyes were sham-operated on day 3 (hatched arrow) and painted with a clear solvent on day 5 (open arrow). The rhythm completely entrained to LD cycles. **B**. The compound eyes were removed (hatched arrow) and painted with pasted silver and black synthetic resin (open arrow). The rhythm freeran. (From [2])

rhythm did not entrain but freeran. Therefore, in *P. terraenovae*, the CE must also participate in rhythm entrainment, in addition to the extraretinal photoreceptors. It seems that, with light of low intensity in the photophase, the extraretinal photoreceptors are not operative and the rhythm is entrained to LD cycles through the CE.

With light of high intensity, the rhythm entrained to LD cycles after removal of the CE but covering of the CE deprived entrainability. To solve this conflict another experiment was designed. The CE were first removed, the operated area was then covered and the entrainability to LD cycles with light of high intensity was examined. After this treatment, some flies showed freerunning activity rhythms (Fig. 3). Considering the fact that the rhythm entrained to LD cycles after removal of the CE, we infer that painting of the operated region prevents light from coming to the extraretinal photoreceptors. These results indicate that the extraretinal photoreceptors receive light passing principally through the CE. It appears that entrainment is mediated primarily by signals from the retinal photoreceptors when the intensity of the light is low.

Histological examination of the brain after removal of the CE for experiments on photoperiodism and rhythm entrainment showed that the retina and lamina were completely removed and the medulla and lobula complex remained. Therefore, we cannot exclude a possibility that the lamina or other nearby tissues to the retina might be involved in perception of the photoperiod.

In another blow fly *Calliphora vicina* (Diptera, Calliphoridae), adults remain sensitive to photoperiod to control maternal induction of larval diapause and entrainment of the circadian rhythm after removal of the optic lobe that connects the CE with the mid-brain. This means that extraretinal photoreceptors in the brain are operative both for photoperiodism and rhythm entrainment in *C. vicina* [3, 4]. Even in the same family of Calliphoridae, *P. terraenovae* and *C. vicina* use different systems for photoreception. It seems that depending on species retinal photoreceptors, extraretinal photoreceptors or both types of photoreceptors are operative. To clarify neural mechanisms underlying biological timing systems in *P. terraenovae*, pathways of the photoperiodic information from the CE to the time measurement system, and to the circadian clock should be identified as a next step.

REFERENCES

1. Shiga S. and H. Numata (1997) Induction of reproductive diapause via perception of photoperiod through the compound eyes in the adult blow fly, *Protophormia terraenovae*. *J. Comp. Physiol. A*. 181, 35-40.

2. Hamasaka Y., Y. Watari, T. Arai, H. Numata and S. Shiga (2001) Retinal and extraretinal pathways for entrainment of the circadian activity rhythm in the blow fly, *Protophormia terraenovae*. *J. Insect Physiol.* 47, 867-875.

3. Saunders D.S. and B. Cymborowski (1996) Removal of optic lobes of adult blow flies (*Calliphora vicina*) leaves photoperiodic induction of larval diapause intact. *J. Insect Physiol.* 42, 807-811.

4 Cymborowski B., R.D. Lewis and D.S. Saunders (1994)