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Pigment-dispersing factor induces phase shifts of circadian locomotor rhythm in the cricket *Gryllus bimaculatus*

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Pigment-dispersing factor (PDF) is an octadecapeptide distributed in the optic lobe and the brain in a variety of insect species. There are lines of evidence suggesting possible involvement of PDF in the insect circadian system. However, its physiological roles in the circadian time keeping mechanism have not been clearly defined. In this study, we have examined the phase shifting effects of *Gryllus*-PDF on the circadian locomotor rhythm in the cricket *Gryllus bimaculatus* of which circadian clock is located in the optic lobe. Phase shifts in the circadian activity rhythm were measured following microinjection of 22nl of vehicle (Ringer's solution) or 0.1mM PDF into the optic lobe through the compound eye at various circadian times. The results showed that PDF induced phase shifts of the circadian clock in a phase-dependent manner, suggesting that it may play a role as an input signal for the circadian clock.

Keywords: cricket, circadian rhythm, pigment dispersing factor, optic lobe

INTRODUCTION

The circadian clock regulates about 24 hr rhythmicity in various physiological functions such as locomotor activity, sensitivity of the visual system, hormonal secretion, etc. [1]. The circadian system consists of an input pathway for the synchronization with zeitgeber, the circadian clock, and an output pathway to overt rhythms. The output pathway through which the clock drives overt rhythms remains not clear. Recent studies in *Drosophila* yielded lines of evidence suggesting pigment-dispersing factor (PDF) to be one candidate of the output molecule of the clock [2]. PDF was expressed in the lateral neurons (LNs), which are believed to be the clock neurons. A large proportion of pdf^0 flies that lack PDF became arrhythmic after several days in constant darkness [2]. Ectopic expression of PDF near normal targets of lateral neurons resulted in arrhythmia [3]. However, there is a lack of knowledge for the physiological roles of PDF, except a report that PDF causes phase dependent phase shifts in circadian locomotor rhythms in the cockroach *Leucophaea maderae* [4].

In the crickets, *Gryllus bimaculatus*, the circadian clock is located in the optic lobe, where PDH is widely distributed [5]. The fact suggests that PDF would be involved in the circadian system also in this cricket. In this study, we examined the effects of administration of exogenous *Gryllus*-PDF on the locomotor rhythm of the cricket *G. bimaculatus*. We found that PDF induced phase shifts of the rhythm but the direction and amplitude of the shifts differed from those induced by light, temperature and serotonin.

MATERIALS AND METHODS

Adult male crickets from the laboratory colony were used. Behavioral analysis was performed in constant light (LL), at constant temperature 25 ± 0.5 °C. Experimentally blinded animals were prepared by surgical removal of the unilateral lamina-medulla complex under CO_2 anesthesia and by covering another compound eye with a piece of alminum foil painted with

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black coating compound. Locomotor activities of the animals were individually recorded by an activity chamber facilitated with rocking substratum whose movement caused by a moving animal was sensed by a magnetic reed switch. The switch was connected to a computer that summed a total number of movements every 6min and stored them on a hard disk.

Event records of individuals were double plotted by a computer and phases were determined by fitting a straight line through activity onsets. Phase shifts in the circadian activity rhythms were measured following microinjection of 22nl of vehicle (10% food dye in Ringer's solution) or 0.1mM of PDF into the optic lobe through the compound eye. The injection was made using nanoliter injector (WPI, A203XVY) mounted on a micromanipulator (Narishige, M-3333) at various circadian times. The resulted phase shifts were analyzed to quantify the amplitude and direction. At the end of the experiments, all experimental animals were dissected under the dissecting microscope to verify the success of the surgery and the inner site of injection.

RESULTS AND DISCUSSION

Microinjection of PDF through the compound eye on male adult crickets showed clear phase shifts (Fig.1). However, crickets administered with vehicle exhibited minimum amplitude of phase shifts in the circadian locomotor activity rhythm, regardless of injection time in the circadian cycle (Fig. 2). PDF administration induced advance phase shifts during the middle subjective day to the middle subjective night (CT 6 to CT 18) with a maximum advance of 1.46 $\pm$ 0.26 hr (mean $\pm$ SEM; $P < 0.001$, t-test compared with vehicle treated animals) at CT 18. Whereas it caused delay phase shifts during the late subjective night to the early subjective day (CT 20 to CT 4) with a maximum delay of -1.47 ± 0.33 hr ($P < 0.01$) at CT 0.

The cricket circadian clock responded to the PDF injections in a time-dependent manner, suggesting that it may play an important role in phasing the circadian oscillator. Similar phase shifting effects have been reported in cockroaches [4], however its amplitude and direction of phase shifts are just opposite of cricket's results. It may be due to the following reasons: In this study, crickets received unilateral removal of the

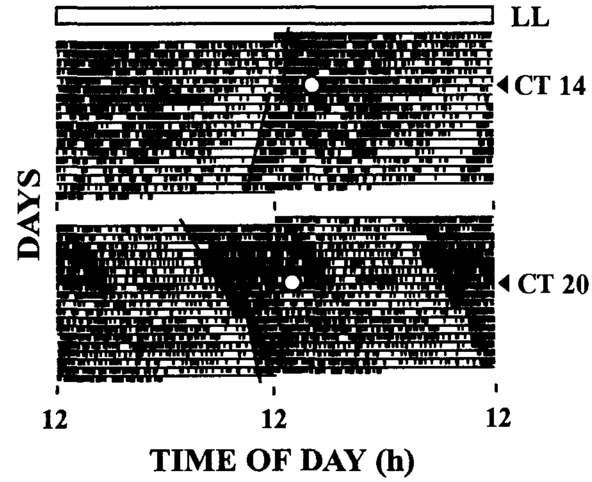

Fig. 1. Representative double plotted actograms illustrating advance phase shift at CT 14 and delay phase shift at CT 20 induced by a single administration of 22nl of PDF (0.1mM) through compound eye in the cricket *G. bimaculatus*. Open circles indicate the time at which PDF was administered. The white bar above the actograms indicates constant light. The abscissa represents time of day while ordinate represents the number of days.

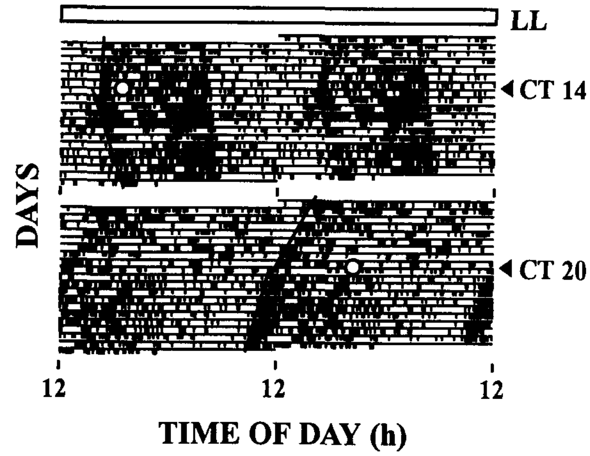

Fig. 2. Representative double plotted locomotor activity records of crickets, *G. bimaculatus* showing the minimum phase shifting effects of 22nl of vehicle at CT 14 and CT 20. Open circles indicate time of microinjection. The abscissa represents time of day while ordinate represents the number of days.

lamina-medulla compound eye complex, hence they had a single pacemaker. Therefore, the effects of PDF on locomotor rhythm may be considered to occur at the pacemaker. In cockroaches, however, the effects of PDF probably occurred through mutual coupling between two optic lobe pacemakers, because they are tightly coupled [6]. The second probable reason is that conspecific PDF was injected in this study, but in cockroaches, *Acheta domestica* PDF was used, which differed at two amino acid residues from the PDH of the cockroach *Periplaneta americana* [7]. The phase shifts induced by the *Gryllus*-PDF are mirror image of light induced phase shifts at various circadian times [8], suggesting that PDF and light are not sharing the common pathway to the circadian pacemaker.

In conclusion, this preliminary study reports first phase shifting effects of conspecific PDF on the circadian locomotor rhythm. This study as well as earlier reports suggests that PDF may be involved not only as an output component but also may act as an input signal candidate in the circadian clock of the cricket *G. bimaculatus*.

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