Circadian Period Modulation and Masking Effects Induced by Repetitive Light Pulses in Locomotor Rhythms of the Cricket, *Gryllus bimaculatus*

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**ABSTRACT**—Effects of 15 min light pulses given at various intervals (every 1, 2, 4, 6, 8 and 12 hr) under constant darkness on the locomotor rhythm were investigated in the adult male cricket, *Gryllus bimaculatus*. A single pulse per 24 hr induced period modulation in a circadian phase dependent manner, yielding a period modulation curve (PMC): the 15 min light pulse lengthened the period in the early subjective night (CT11-16) and shortened it during the late subjective night to the early subjective day (CT20-5). Frequent light pulses modulated the freerunning period of the rhythm dependent on the interval of the pulses: when compared with the freerunning period in DD (23.74 ± 0.03 hr) the period was significantly shorter in intervals of 2 and 4 hr, but lengthened when the interval was 1 and 12 hr. Frequent light pulses also resulted in entrainment of the rhythm to run with the period of 24 hr and the ratio of the entrained animals varied from 12% to 72% depending on the interval of the light pulses. The period modulation and the entrainment by the repetitive light pulses could be interpreted according to the PMC. In about 15% of animals, the light pulses induced a rhythm dissociation, suggesting that the bilaterally paired circadian pacemakers have their own sensitivity to the entraining photic information. The light pulse caused a masking effect, i.e., an intense burst of activity. The magnitude of the light induced responses was dependent on the circadian phase. The strongest masking effect was observed in the subjective night. The phase of the prominent period modulation and of the marked masking effects well coincides with the previously reported sensitive phase of the photoreceptive system.

**INTRODUCTION**

Light is important for circadian time keeping. The most important role is to synchronize the circadian rhythm to the environmental cycle. The mechanism of photic entrainment has been profoundly studied in *Drosophila pseudoobscura* and other organisms by means of single light pulse exposure of the circadian rhythms freerunning in constant darkness (Pittendrigh, 1981a, b; Pittendrigh and Daan, 1976). Timing of the light pulse determines the magnitude and the direction of the phase shifts of the rhythm: the phase advances or delays when a light pulse is given at the subjective night, while such a shift is not usually induced in the subjective day (Johnson, 1990). A plot of the direction and magnitude of these responses as a function of the time yields the phase response curve (PRC), which is useful for the evaluation of the effects of light on the rhythm.

Continuous light is thought to change freerunning period continuously, yielding longer periods in nocturnal vertebrates and invertebrates (Aschoff, 1979). Although this continuous effect of light is hypothesized to be predictable on the basis of the PRC caused by discrete light pulses (Pittendrigh, 1981b), there is a paucity of experimental studies concerning this issue.

In the present study, a novel experimental protocol was employed to analyze the continuous action of light on the rhythm. We examined how repetitive light pulses given at various intervals, which would be intermediate between discrete and continuous light, modulate the freerunning period of locomotor rhythms in the cricket.

In addition to changes in the circadian rhythmicity, light pulses often induce changes in the activity of animals of which magnitude is often dependent on the phase of the rhythm (Bolbery and Huston, 1974; Gander and Moore-Ede, 1983). This effect of light is termed "masking effect" (Aschoff, 1960). Recent electrophysiological studies have revealed that visual elements, such as retinal photoreceptor cells and visual interneurons in the optic lobe, have a circadian rhythm in their sensitivity to light in insects, which is higher during the subjective night (Koehler and Fleissner, 1978; Tomioka and Chiba, 1982; Wills et al., 1985; Tomioka et al., 1993; Bult and Mastebroek, 1993). The repetitive light pulses are suitable for analyzing the relationship between the circadian rhythms of...
the photoreceptive system and the light induced masking activity.

MATERIALS AND METHODS

Animals

All experiments were performed with intact, adult male crickets, *Gryllus bimaculatus*, obtained from our laboratory colonies, which were maintained at 25 ± 0.5°C in a 12 hr light and 12 hr dark cycle (LD 12:12, light: 06:00–18:00, Japanese Standard Time, JST). To prevent any interindividual sound communication, one forewing was cut off just before the activity recording was started.

Activity recording

The animals were kept individually in an activity chamber with rocking substratum whose movement caused by a moving animal was sensed by a magnetic reed-switch connected to a computer for data storage. The signals of the movement were summed every 6 min. Food and water were available *ad libitum* and the temperature was kept constant at 25 ± 0.5°C. Light dark cycles and light pulses were given by a 15 W cool white fluorescent lamp controlled by an electric timer. The light intensity at the chamber was about 250 lux. To examine effects of 15 min light pulses given at intervals of either 1, 2, 4, 6, 8, 12 or 24 hr under constant darkness on the locomotor activity rhythm, animals were exposed to the light pulses after several days of LD12:12.

Data analysis

Event records of individuals were double plotted in a conventional manner by computer with a resolution of 6 min. The onset of activity was always much sharper than the offset, and hence only the onset was used to estimate freerunning period and phase. The onset was defined as circadian time 12. A 12 circadian hour period following the activity onset was defined as the subjective night and the rest as the subjective day. The daily freerunning period was defined as the period between the two consecutive onsets. The freerunning period of individuals was determined by averaging the daily freerunning periods. In steady state entrainment, the phase angle between the rhythm and the light cycle was determined as the time difference between the light-on nearest to the activity onset and the vertical line eye-fitted through the activity onsets. If the onset preceded light-on, the value was expressed as positive value, whereas it expressed as negative value when the onset was later than the light-on. The activity induced by a light pulse was determined as follows: The activity, which was a number of rocking of the substratum caused by an animal, was counted for 30 min before and after the light-on and the former value was subtracted from the latter. Values are expressed as means ± SEM.

Statistical analysis

The difference between mean values was statistically analyzed with two-tailed student t-test. The correlation between two parameters was analyzed with the Pearson’s correlation coefficient. Significance was considered at *P* < 0.05.

RESULTS

Activity rhythms in constant darkness

We assayed the locomotor rhythm in constant darkness (DD) with 34 animals. As shown in Fig. 1, most animals showed a locomotor rhythm freerunning with a period shorter than 24 hr (Fig. 1), while 4 of them showed a rhythm with a period slightly longer than 24 hr. The average freerunning period was 23.74 ± 0.03 hr.

Freerunning period modulation by a single 15 min light pulse given every 24 hr

We first analyzed the effect of a 15 min light pulse per 24 hr on the freerunning period of the rhythm in 64 crickets. The light pulse was given at either 12:00, 20:00 or 00:00 (JST).

Representative examples of activity records are shown in Fig. 2. All the animals assayed showed a circadian rhythm in this condition, and the rhythm either freeran or synchronized to the cyclically given light pulses (Fig. 2B, E-group). Animals showing freerunning rhythms were further classified into three

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**Fig. 1.** Locomotor activity of a cricket, *Gryllus bimaculatus*, transferred to constant darkness (DD) after several days of LD12:12. White and black bars indicate light (white) and dark (black) portions, respectively.
Fig. 2. Locomotor rhythms of crickets, Gryllus bimaculatus, transferred to constant darkness with a single 15 min light pulse given every 24 hr at 12:00 (A) or 20:00 (B-D) on the day indicated by LP. The rhythms synchronized to the light cycle (B), freeran with a period shorter (A) or longer (C) than 24 hr, or split into two components (D). Note that the light pulses induced an intense burst of activity in a phase dependent manner. For further explanations see Fig. 1 and text.
groups, i.e., animals with a period shorter than 24 hr (Fig. 2A, S-group), with a period longer than 24 hr (Fig. 2C, L-group), and with two freerunning components (Fig. 2D, T-group). As shown in Fig. 3A, the ratio of animals of the four groups varied dependent on the time at which the light was given. Animals of the S-group most frequently occurred when the pulse was given at JST 00:00, while animals of the E-group mostly observed when the pulse was given at JST 12:00. In case of entrainment, the rhythm initially ran with a period longer or shorter than 24 hr and eventually synchronized to the cycle when the onset reached at an appropriate phase angle relationship to the light-on. As shown in Table 1, the phase angle between the light-on and the activity onset was $+0.44 \pm 0.22$ hr in steady state entrainment. In the freerunning rhythm, the period changed clearly dependent on the circadian time at which the light pulse was given (Fig. 2A).

Figure 3B shows a period modulation curve (PMC) obtained from animals freerunning in the light cycle. The average freerunning period was 23.83 ± 0.11 hr. When compared to the freerunning period in DD (23.74 ± 0.03 hr), it is estimated that the 15 min light pulse lengthened the period in the early subjective night (CT11-16) and shortened it during the late subjective night to early subjective day (CT20-5). The shape of the curve was quite similar to that of the phase response curve for a single 3 hr light pulse (Okada et al., 1991).

**Table 1. Phase angle between light-on and activity onset in steady state entrainment of the locomotor rhythm of the cricket, Gryllus bimaculatus, in various lighting conditions.**

<table>
<thead>
<tr>
<th>Intervals between light pulses (hr)</th>
<th>N</th>
<th>Phase angle (mean ± SEM hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>+0.32 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>+0.84 ± 0.11</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>+1.07 ± 0.08</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>+0.66 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>+0.62 ± 0.02</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td>+0.63 ± 0.08</td>
</tr>
<tr>
<td>24</td>
<td>14</td>
<td>+0.31 ± 0.22</td>
</tr>
</tbody>
</table>

N, number of animals used.

**Effects of frequent 15 min light pulses on locomotor activity**

With 88 adult male crickets, we next examined the effects of 15 min light pulses, given at intervals of 1, 2, 4, 6, 8 or 12 hr, on freerunning rhythms. The animals were first held in LD 12:12 for several days, then transferred to constant darkness with 15 min light pulses. Figure 4 shows representative results. All the animals assayed showed a circadian rhythm in those conditions, and the rhythm either freeran or synchronized to the light cycle to show a 24 hr rhythm.

The ratio of the animals entrained to the cycle (E-group) was dependent on the interval: more animals were entrained when the interval was longer (Fig. 5A). The animals of this group were entrained to 24 hr and none showed frequency multiplication. In most cases, the rhythm initially freeran and synchronized to the light cycle when the activity onset reached at the appropriate phase angle relationship to the light pulse. The phase angle between the activity onset and the nearest light-on was always positive and fell between +0.3 and +1.1 hr (Table 1).

Animals showing freerunning rhythms were again classified into three groups, i.e., animals with a period shorter than 24 hr (S-group, Fig. 4A), with a period longer than 24 hr (L-group, Fig. 4C), and with two freerunning components (T-group, Fig. 4D).

Animals of the T-group showed a unimodal rhythm at the beginning of the short light pulses, but the rhythm suddenly split into two components (Fig. 4D). The two rhythms mostly freeran with different periods but in some cases one of them seemed to synchronize to the light cycle. The T-group animals were excluded from further analysis of the freerunning period. The rhythm dissociation occurred most frequently at intervals of 1 and 8 hr, but there was no significant correlation between the occurrence and the intervals between light pulses ($r = 0.28, p > 0.05$). To examine whether the two rhythmic components were endogenous, 6 animals with rhythm disso-

![Fig. 3.](image-url)
Fig. 4. Locomotor rhythms of crickets, Gryllus bimaculatus, transferred to constant darkness with 15 min light pulses at an interval of either 4 hr (A), 8 hr (B) or 1 hr (C, D) on the day indicated by LP. The rhythms synchronized to the light cycle (B), freeran with a period shorter (A) or longer (C) than 24 hr, or split into two components (D). In D, the animal was transferred to DD on day 25, and the two rhythmic components clearly persisted even in DD. Note that the light pulses induced an intense burst of activity in a phase dependent manner. For further explanations see Fig. 1 and text.
Fig. 5. (A) Ratio of crickets, Gryllus bimaculatus, with rhythms either entrained to 24 hr (E), freerunning with periods shorter (S) or longer (L) than 24 hr, or split into two rhythmic components (T). (B) Dependency of the period of locomotor rhythms of the cricket, Gryllus bimaculatus, on intervals between light pulses. A horizontal line shows the freerunning period in DD (23.74 hr). *P < 0.05, **P < 0.01 in comparison with the period in DD (t-test). Vertical bars indicate SEM. Numbers indicate the number of animals used.

Fig. 6. Phase dependency of activity induced by light pulses given at intervals of 1 hr (A), 2 hr (B), 4 hr (C) or 6 hr (D) in the cricket, Gryllus bimaculatus. Note that the light induced activity is greater during the subjective night. Vertical bars indicate SEM.

Discussion

Modulation of freerunning period by light pulses

The present experiment revealed that the light pulse modulates the freerunning period in a phase dependent manner (Figs. 2, 4). In the period modulation curve (PMC) thus yielded, the period lengthening effect of light (CT11-16) is more prominent than the shortening (CT20-5), but the shortening
phase seems broader than lengthening phase. The phase during which the period is affected by light well corresponds to the phase during which the light induces major responses in the visual system (Tomioka and Chiba, 1982; Tomioka et al., 1993).

It was also revealed that frequent light pulses modulated the freerunning period dependent on the interval of the pulses. In intervals of 2 and 4 hr, the period was significantly shorter than that in DD, while it was significantly lengthened when the light was given every 1 or 12 hr. In 6 and 8 hr intervals the period was slightly longer than that in DD. The period modulation would be interpreted in terms of PMC. The more prominent lengthening portion of PMC makes the period longer when a few light pulses were given at longer intervals. Probably because of the broader shortening phase, the shorter pulse interval makes many pulses fall on the period shortening phase to cause the shortening of the freerunning period. In the shortest interval of 1 hr, however, light pulses strike the rhythm as reflected in PMC, resulting in the lengthening of the period. From the PMC, the net lengthening is 0.2 hr and the expected freerunning period is thus 23.94 hr, which is exactly the same value obtained for the interval of 1 hr.

We have discussed period modulation in terms of PMC. However, there is some possibility that the PMC might change in response to the light pulses. In the cockroach, Leucophaea maderae, the phase response curve (PRC) has a prominent phase delay portion (Wiedenmann, 1977). The freerunning period under 10 min LD cycle varies with ratio of LD: the period is not only lengthened but also shortened dependent on the LD ratio (Roberts, 1982). The unexpected shortening of the period has been interpreted as the result of change in PRC shape into one with a prominent advance portion, since it is known that the change in freerunning period is associated with the change in PRC shape (Pittendrigh and Daan, 1976). Our present results might be interpreted in a similar way.

In the experiment with a single light pulse per 24 hr, the ratio of animals entrained, freerunning with a shorter or longer period differed depending on the time when the light pulse was given. This difference may be also explained by the PMC. When the light pulse was given at JST 12:00, which would be expected to fall around CT6, the rhythm would freerun with a period about 23.7 hr until entrainment was achieved when the light initially fell around CT12. Similar explanation would be appropriate to the case where the light pulse was given at JST 0:00 (around CT18 on the first day of DD). Since, in this case, 18 hr advance would be needed to reach the steady state entrainment, most animal could not achieve the entrainment during the recording period. In the case where the light pulse was given at JST 20:00 (around CT14 on the first day of DD), the light pulse would cause the lengthening of the period to run with longer period. The rhythm would be expected to synchronize when light fell at the beginning of the subjective night. The animals continued to freerun with longer period may be explained that they have endogenously longer period or that the initial lengthening by the light have some after effects to lengthen the freerunning period.

**Entrainment of locomotor rhythms by light pulses**

The locomotor rhythm was sometimes entrained to the light cycles with 15 min light pulses. The ratio of entrained animals varied from 12% to 79% dependent on the interval between light pulses: the entrainment was most frequently achieved when light pulses were given every 12 and 24 hr. The phase angle between light-on and activity onset was +0.63 and +0.44 hr, respectively. The result could be interpreted in terms of PMC as in Drosophila pseudoobscura eclosion rhythms (Pittendrigh, 1981a, b). In the single pulse entrainment, the light pulse fell just after CT12, causing a lengthening of the freerunning period by about 0.3–0.5 hr to entrain the rhythm with a single phase delay. In 12 hr intervals, one pulse falls in the lengthening portion of the PMC to cause 0.3–0.5 hr period lengthening of the rhythm and the other in the period shortening portion to cause shortening by about 0.1–0.4 hr; the rhythm was thus entrained by the skeleton photoperiod with two pulses. Likewise, in other intervals, the entrainment would be interpreted in terms of PMC, although the prediction becomes more complicated with the increase of pulses.

**Masking effect of light**

One of the most striking evidence revealed in the present study is the phase dependent masking effect induced by light. It was greater during the subjective night than during the subjective day. This would be related to the circadian change in sensitivity of the visual system. The compound eye shows a clear circadian rhythm of the electroretinogram (ERG) in constant darkness, peaking in the subjective night (Tomioka and Chiba, 1982). The visual interneurons in the optic lobe also show a clear rhythm both in spontaneous discharge and in light induced responses, which are much greater during the subjective night (Tomioka et al., 1993). There is a close parallelism between the circadian change in the activity of visual interneurons (Fig. 1 in Tomioka et al., 1993) and that in the light induced activity (Figs. 4A, 6). Thus, it is likely that the circadian change in magnitude of the light induced activity reflects the circadian change in the sensitivity of the visual system. To further clarify this issue, it is deserved to examine the effects of some biogenic amines such as serotonin and octopamine, which are known to modulate the sensitivity of visual neurons (Tomioka et al., 1993; Kloppenburg and Erber, 1995), on the masking effect.

An important finding is that the amplitude of the light induced activity was a function of the light pulse interval. It was greatest when the interval was 4 hr. It could be related to the adaptation of the visual system. When the light pulse was given, the light-responding neurons fired at high frequency but the firing was soon reduced by an adaptation mechanism (Tomioka et al., 1994). After lights-off, the firing frequency is even less than that before the light pulse, and this hypo-sensitive phase continues more than 2 hr. This seems to be related to the smaller amplitude for 1 hr and 2 hr intervals. The reason that the amplitude at 6 hr interval was less than that for 4 hr is unclear. This issue deserves further study.
Dissociation of two rhythms

Eleven to 37% of animals showed a rhythm that split into two components when transferred to cycles of short light pulses. In most cases the two rhythmic components freeran with slightly different freerunning periods. The two components are both endogenous in origin, since they freeran in the ensuing DD. Since it has been shown that the locomotor rhythm of this cricket is driven by a pair of circadian pacemakers, one in each optic lobe of the protocerebrum (Tomioka and Chiba, 1992), the rhythm dissociation seems to show a dissociation of the two pacemakers. There are two possible explanations for the underlying mechanism. First, the two pacemakers have different sensitivity to light, which causes the change in period by different amount. Second, the short light cycle somehow affects the coupling between the pacemakers, since the light plays an important role in pacemaker coupling (Tomioka, 1993; Tomioka and Yukizane, 1997): The light information is conveyed by the dorso-caudal area of the compound eye and conveyed to the contralateral pacemaker to synchronize it with the ipsilateral pacemaker (Tomioka and Yukizane, 1997). Considering that the coupling is also disturbed by a step change in temperature (Ikeda and Tomioka, 1993), it is suggested that the bilateral pacemaker systems have their own sensitivity to an external Zeitgeber.

Similar rhythm dissociation induced by light pulses has been reported for ERG rhythms in beetles, Blaps gigas (Koehler and Fleissner, 1978). Thus, it seems rather a common feature that the two pacemakers tend to change their coupling states when confronted with the short light cycle.

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