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Circadian Locomotor Rhythms in the Cricket, *Gryllodes sigillatus* I. Localization of the Pacemaker and the Photoreceptor

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ABSTRACT—Circadian locomotor rhythm and its underlying mechanism were investigated in the cricket, *Gryllodes sigillatus*. Adult male crickets showed a nocturnal locomotor rhythm peaking early in the dark phase of a light to dark cycle. This rhythm persisted under constant darkness (DD) with a free-running period averaging 23.1 ± 0.3 hr. Although constant bright light made most animals arrhythmic, about 40% of the animals showed free-running rhythms with a period longer than 24 hr under constant dim light condition. On transfer to DD, all arrhythmic animals restored the locomotor rhythm. Bilateral optic nerve severance resulted in free-running of the rhythm even under light-dark cycles. The free-running period of the optic nerve severed animals was significantly longer than sham operated crickets in DD, suggesting that the compound eye plays some role in determining the free-running period. Removal of bilateral lamina-medulla portion of the optic lobe abolished the rhythm under DD. These results demonstrate that the photoreceptor for entrainment is the compound eye and the optic lobe is indispensable for persistence of the rhythm. However, 75% and 54% of the optic lobeless animals showed aberrant rhythms with a period very close to 24 hr under light and temperature cycles, respectively, suggesting that there are neural and/or humoral mechanisms for the aberrant rhythms outside of the optic lobe. Since ocelli removal did not affect the photoperiodically induced rhythm, it is likely that the photoreception for the rhythm is performed through an extraretinal photoreceptor.

INTRODUCTION

The circadian organization has been extensively studied in exopterygote insects. The optic lobe is now believed to be the locus of the circadian pacemaker in some species. In *Leucophaea maderae* and *Gryllus bimaculatus* the pacemaker tissue has been localized not only by the surgical lesion experiments but also recording the neural activity originating in the optic lobe kept *in vitro* culture conditions (Colwell and Page, 1990; Tomioka and Chiba, 1992). However, the species of which physiological organization of the circadian system has been studied are still rather limited. Even in those species, there are some discrepancies especially in the locus of the circadian pacemaker. In the cockroach *Leucophaea maderae* (Page, 1978) and the beetle *Anthia sexguttata* (Fleissner, 1982) the pacemaker is speculated in the lobula region of the optic lobe. However, it is believed in the lamina-medulla area in only two orthopteran insects, the cricket, *Gryllus bimaculatus* (Tomioka and Chiba, 1984) and the New Zealand weta *Hemideina thoracica* (Waddel *et al.*, 1990). To understand the circadian organization in insects it would be important to adopt a comparative approach to this issue.

In the present experiment, we used the cricket *Gryllodes sigillatus*, which occurs in the field only in summer and indoors during the rest of the year. They prefer dark places and usually aggregate to form a cluster under the shelter during the daytime, while crickets so far used for circadian rhythm studies were limited to rather solitary species occurring in the field. We characterized the circadian locomotor rhythm of *G. sigillatus* in various lighting conditions and localized the constituents of their circadian system, such as circadian pacemaker and photoreceptors, by surgical lesioning experiments. We found that the photoreceptors for entrainment and the pacemaker generating the rhythm are located in the compound eye and the lamina-medulla part of the optic lobe, respectively, like in *G. bimaculatus* and *H. thoracica* (Tomioka and Chiba, 1984; Waddel *et al.*, 1990). Lobeless crickets, however, exhibited rhythms in response to light and temperature cycles, suggesting that there is a secondary system responsible for these rhythms.

MATERIALS AND METHODS

Animals

Mature adult male crickets (*Gryllodes sigillatus*) were used. They were collected in Mie Prefecture and have been kept in laboratory for more than 140 generations. They were maintained under the standard environmental condition: at a constant temperature of 25 ± 0.5°C and a cycle of 12 hr light to 12 hr dark [LD12:12. L:06:00-18:00, Japanese standard time (JST)]. They were fed laboratory chow and water.

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Activity recording

Locomotor activity of individual crickets was monitored in an activity chamber, a transparent plastic box (9.5 × 6 × 2.5 cm). The chamber was mounted on an activity monitor board. On this, each chamber was flanked by a matched infrared emitter-detector (Takenaka Electronic Industrial Co., F3), allowing the locomotor activity to be measured by monitoring the events in which a cricket broke the infrared beam. An electrical signal, caused at each event, was collected by computer (NEC, PC9801), which summed the signals every 6 min; the total count of every 6 min was stored on a floppy diskette. The quantitative data were later analyzed with computer. Experimental animals were housed individually in the chamber after one of the forewings was removed to prevent any sound communication among individuals. The activity chambers were placed in an environment controlled room. Light-dark cycles were given by an electronic timer (Omron, H3CA) connected to a cool white fluorescent lamp. The light intensity within actographs varied with proximity within the environment-controlled room to the lamp and ranged from 75-540 lux at animal's level. In the dim light experiments, it was lowered to 1.5-8.1 lux by shading the lamp. Temperature was monitored continuously inside one of the activity chambers by a recording thermometer. There was no detectable daily temperature cycle associated with the light cycles.

Temperature cycles were given by an incubator that could be programmed to provide an approximately square-wave temperature cycle by switching between two thermostats. The transitions both from "low" to "high" temperature and from "high" to "low" temperature took about 10 min to complete.

Data analysis

Event records of locomotor activity were double plotted in a conventional manner by computer with a resolution of 6 min. Whether or not a rhythm was present and the free-running periods were objectively determined by the chi-square periodogram (Sokolove and Bushell, 1978). If peaks of the periodogram appeared above the 0.05 confidence level, the animals were designated as rhythmic. Values are shown as mean ± SD. In some experiments, we determined the phase of the rhythm on the first day of free-running by extrapolating the line fit to the activity onsets. Mean phases were estimated using circular statistics and whether or not phases of a group of animals were significantly clustered near a particular time of day was determined by the Rayleigh test (Batschelet, 1981).

Surgery

Surgical lesions were performed on animals anesthetized with CO_2. The surgical procedure for the optic nerve severance or optic lobe removal was as follows. A cricket was placed on a specially designed platform to immobilize its head. The cuticle around the compound eye was cut with a fine razor, and the lamina-medulla-retina complex was exposed. Then, the optic nerve alone was cut or both the optic nerve and the optic stalk, which connects the medulla and the lobula neuropils, were cut when the optic lobe was removed. After placing a piece of thin aluminum film between the retina and the cut end of the nervous tissue to prevent the regeneration of the nervous connection, the eye capsule was replaced in its original position. The ocelli were removed after cutting the cuticle around them followed by the ocellar nerve severance. The wound was healed soon with a clotting of blood.

Histology

At the end of experiments, animals with the surgical operation were subjected to histological examination. The animals with lamina-medulla-retina complex lesioned were fixed in alcoholic Bouin's fixative for more than 24 hr, and were dissected to verify the success of the surgical lesions under a dissecting microscope. The brain was dissected out, dehydrated in ethanol, embedded in paraffin, and 15 μm horizontal sections were made with a microtome. The sections were stained with the conventional hämatoxylin-eosin yellow procedure.

RESULTS

Locomotor activity rhythm of intact animals

The locomotor activity of 102 intact animals was assayed under LD12:12 either with bright light of 75-540 lux (n=73) or dim light 1.5-8.1 lux (n=29). They showed a clear nocturnal rhythm (Fig. 1), but some of them showed weak activity that started several hours before the lights-off. The activity commonly peaked just after the lights-off under both bright light and dim light conditions (Figs. 1 and 2). Of the animals that were initially kept in bright LD 26 and 47 were transferred to DD and constant bright light (bright LL), respectively, and 27

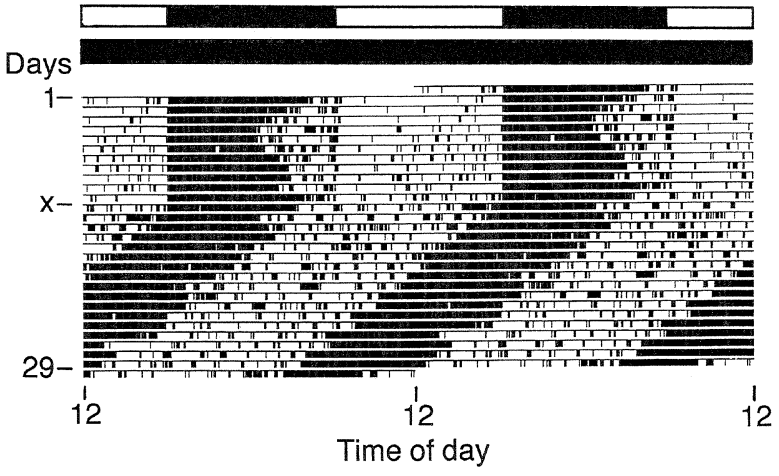


Fig. 1. Locomotor activity rhythm of a cricket kept under bright LD12:12 and transferred to DD at 18:00 on day 12. The cricket was nocturnally active but with sporadic activity during the light phase. In DD, the rhythm ran free with a period shorter than 24 hr. White and black bars indicate the light (white) and dark (black) phases. For further explanations see text.

of the animals that were assayed in dim LD to constant dim light (dim LL).

Under DD, all of the 26 animals showed a free-running rhythm with a period of 23.1 ± 0.3 hr as exemplified in Fig. 1. In dim LL, 10 of the 27 tested animals were rhythmic from the beginning of the dim LL, but, during prolonged dim LL, 3 of them became arrhythmic and 4 initially arrhythmic animals became rhythmic. The free-running period of most rhythmic animals gradually changed to be shortened during the 28 days of dim LL (example, Fig. 2A): the average free-running period for the first 10 days was 26.7 ± 2.3 hr (n=10), being significantly shortened to 24.4 ± 1.7 hr for the last 8 days of dim LL ($P<0.01$, t-test). The remaining 16 crickets were arrhythmic (example, Fig. 2B). After 28 days of dim LL, they were transferred to DD at JST 18:00. As a result, all of them showed free-running rhythms with a period of 23.2 ± 0.3 hr. The time of the activity onset at the first day of DD was estimated by extrapolating the activity onset under DD. As shown in Fig. 3A, the phases of animals that had been rhythmic were dispersed over the 24 hr ($p>0.174$, Rayleigh-test), whereas those of animals that had been arrhythmic were significantly concentrated around 18 hr after the LL/DD transition ($p<0.001$, Rayleigh-test) (Fig. 3B).

On the other hand, under bright LL condition, except one animal, in which a very faint rhythm of 29.1 hr was detected by the chi-square periodogram, the animals became arrhythmic with their activity evenly dispersed over the 24 hr period (example, Fig. 4). Seventeen of the arrhythmic animals were transferred to DD after 18 days of constant light. The LL/DD transfer was performed at JST 12:00. After 16 days of DD 11 of the animals were again exposed to constant light, then transferred to DD at JST 00:00 on day 51. In DD they restored the free-running rhythm. The average free-running period (23.1 ± 0.7 hr, n=17) in DD after the first LL was slightly shorter than that after the 2nd LL (23.5 ± 0.9 hr, n=11). Extrapolated phases of the rhythm on the first day of free-running concentrated around 18 to 21 hr after the LL/DD transition ($P<0.001$ and $P<0.015$ for the first and the second trials, respectively, Rayleigh-test) (Fig. 3C and D).

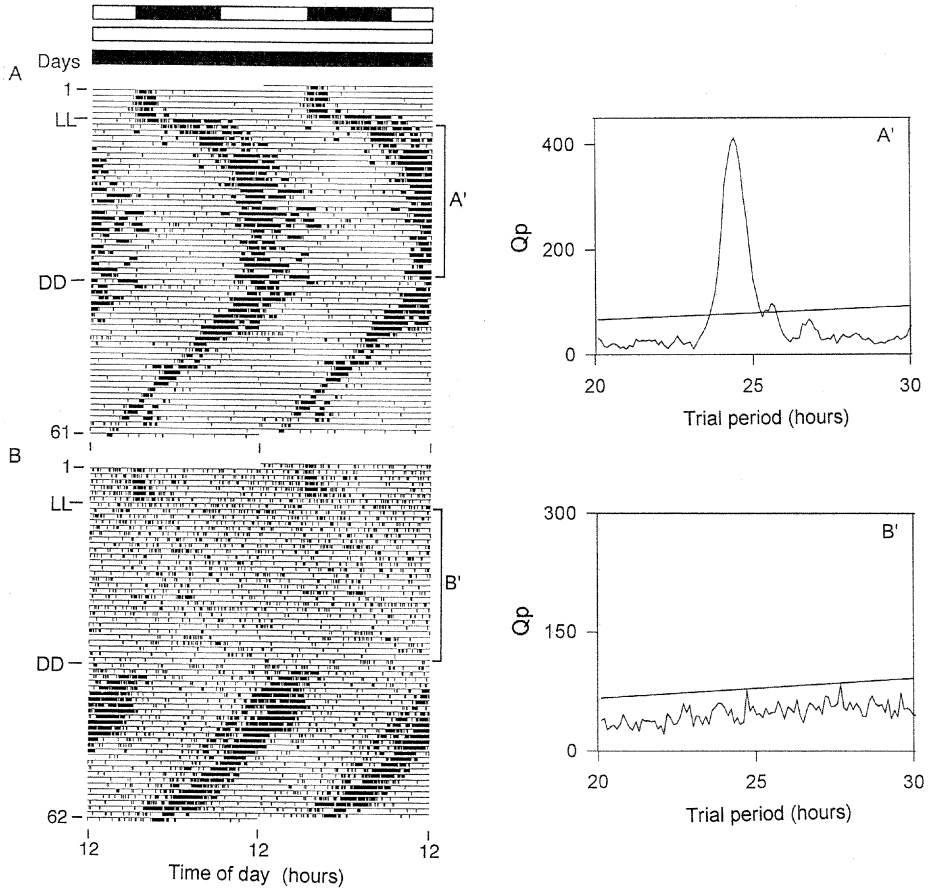


Fig. 2. Two activity records of animals under dim LL followed by DD. The animals were held in dim LD12:12 for the first 5 or 6 days, transferred to dim LL at 6:00, then to DD at 18:00 on day 34 or 35. Locomotor rhythm either persisted in dim LL with a period longer than 24 hr (**A**) or disappeared (**B**). In DD both animals showed a clear rhythm free-running with a period shorter than 24 hr. Right panels show results of chi-square periodogram analysis. **A'** and **B'** correspond to the periods **A'** and **B'** indicated in the actograms. For further explanations see Fig. 1 and text.

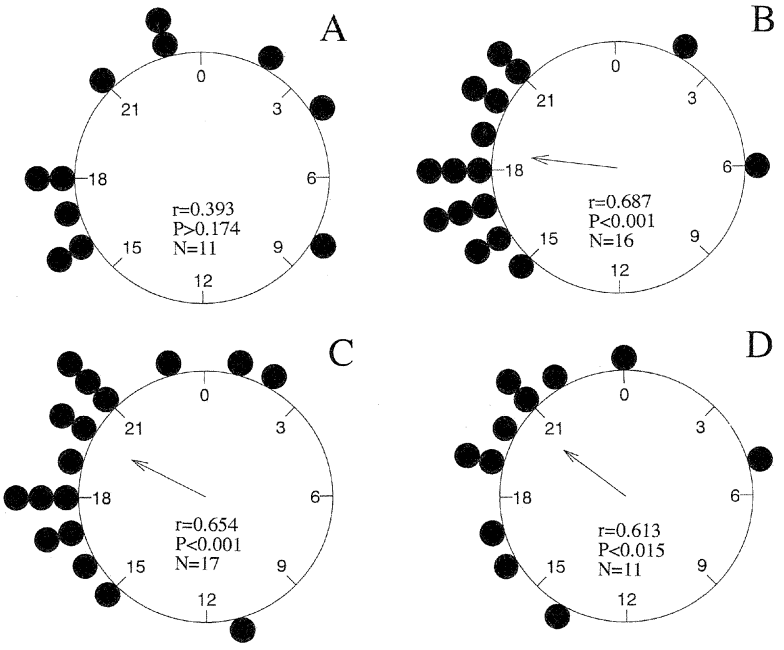


Fig. 3. Phases of the free-running rhythm of the first day after LL/DD transition. Each open circle represents a 24 hr clock face. (**A**, **B**) Results of rhythmic (**A**) and arrhythmic (**B**) crickets transferred from dim LL to DD. (**C**, **D**) Phases of rhythms after the first (**C**) and the second (**D**) transfer from bright LL to DD. Small filled circles show locomotor activity phases of individual crickets. Time 0 corresponds to LL/DD transition. The average phase of each population is indicated by the orientation of the arrow at the center of the face. The length of the arrow reflects the degree of synchrony in the population and corresponds to the r-values as described (Batschelet, 1981). The significance of the phase was calculated from the r-values by the Rayleigh test (Batschelet, 1981).

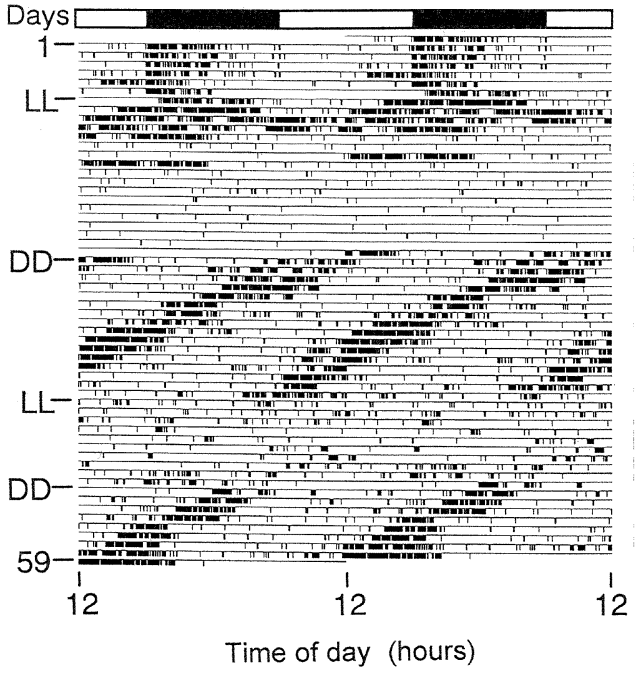


Fig. 4. Locomotor activity of a cricket alternatively exposed to bright LL and DD. The cricket was first held in bright LD12:12 for the first 6 days then transferred to LL at 6:00, to DD at 12:00 on day 25, LL at 12:00 on day 41, then to DD at 0:00 on day 51.

Gross anatomy of the brain

The anatomical structure of the brain is similar to that of other orthopteran insects. The protocerebrum can be roughly divided into two constituents, i.e., the cerebral lobe and the optic lobe. The optic lobe consists of three neuropils: lamina, medulla and lobula from distal to proximal.

Effects of bilateral optic nerve severance on the locomotor rhythm

In crickets and cockroaches, the compound eyes have been thought for a long time to be the only circadian photoreceptor, since disconnection of the optic lobe from the retina results in free-running of the locomotor rhythm (Loher, 1972; Nishiitsutsuji-Uwo and Pittendrigh, 1968b; Page, 1978; Tomioka and Chiba, 1984).

To examine whether this is also true in our cricket, we assayed the locomotor activity of 12 animals whose optic nerves were bilaterally severed under bright LD12:12 for more than 20 days. After severing the optic nerves, the operated animals exhibited a free-running rhythm with the period of 23.6 $\pm$ 0.2 hr even under the LD (example, Fig. 5A). The rhythm often started to run free several hours later than the projected lights-off. This may be explained as the phase shift caused by the severance of the optic nerve. The free-running period was slightly but significantly longer than that of intact animals kept in DD ($p<0.01$, t-test). Five animals with the sham operation were perfectly entrained to the LD throughout the recording period (example, Fig. 5B).

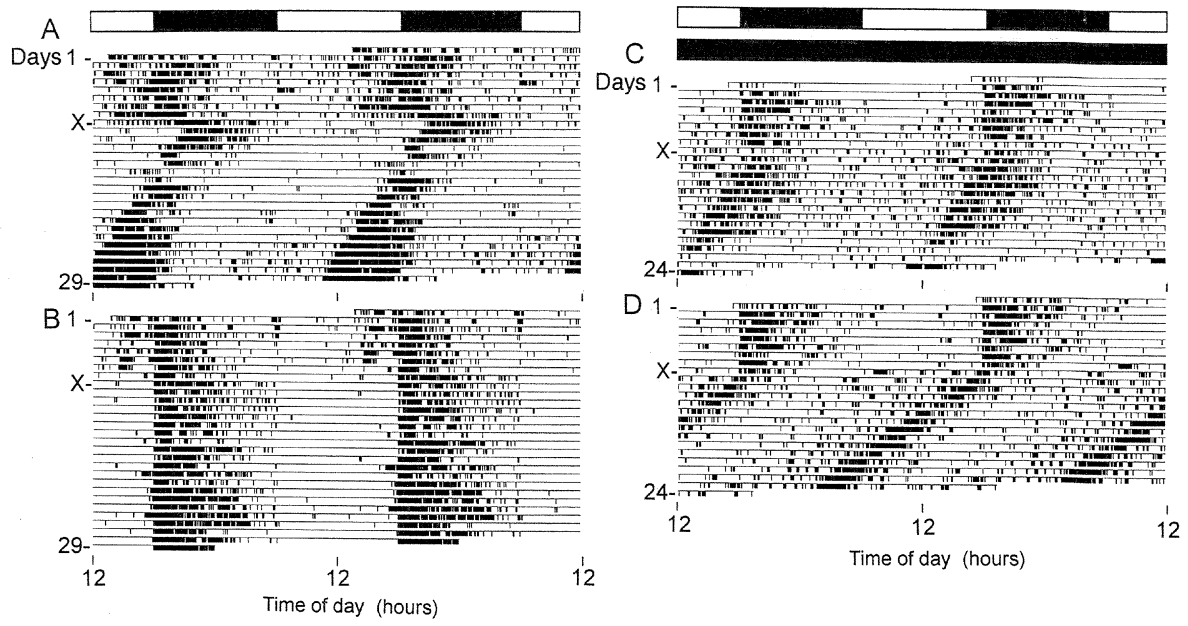


Fig. 5. Locomotor activity rhythms of the animals with optic nerves bilaterally severed (**A**, **C**) or receiving sham operation (**B**, **D**). The animals were either transferred to DD (**C**, **D**) immediately after the operation (X) or kept under bright LD12:12 throughout the record (**A**, **B**). In both conditions, the rhythm of the optic nerve severed animals ran free with a period shorter than 24 hr like intact animals kept in DD. For further explanations see text and Fig. 1.

To examine a possibility that the light-dark cycle somehow lengthened the free-running period, locomotor activity was recorded under DD after the same operation in additional 6 animals and after the sham operation in 9 animals. Although, again, the average free-running period (23.6 ± 0.4 hr) of the animals with the optic nerve severed was longer than that of the sham operated animals (23.1 ± 0.1 hr) ($p<0.01$, t-test, Fig. 5C, D). These facts suggest that the circadian photoreception for the entrainment is performed by the compound eyes, and the neural connection between the optic lobe and the compound eye plays some role in determining the free-running period even in DD.

Effects of bilateral optic lobe removal on the locomotor rhythm

In cockroaches and crickets, both the left and the right optic lobes are believed to contain the circadian pacemaker. This statement based not only on the fact that bilateral optic tracts transection or optic lobe removal abolishes activity rhythms (Loher, 1972; Nishiitsutsuji-Uwo and Pittendrigh, 1968a; Roberts, 1974; Tomioka and Chiba, 1984), but also on the fact that the rhythm of the electrical neural activity persists in the isolated optic lobe (Colwell and Page, 1990; Tomioka and Chiba, 1992).

To reveal the role of the optic lobe in the locomotor rhythm of *G. sigillatus*, we assayed the locomotor activity of 27 animals with optic lamina-medulla portion bilaterally removed. After the locomotor activity was recorded under the light dark cycle for several days, the operation was carried out. The success of the operation was confirmed by postmortem histological examination; in most cases, the lobula area was substantially shriveled. Fifteen of them were transferred to DD immediately after the operation. In visual inspection, all of them immediately became arrhythmic, showing activity dispersed over 24 hr period (Fig. 6A). However, the chi-square periodogram analysis revealed that only one animal showed a very faint ultradian rhythm with a period of 18.1 hr.

The remaining 12 were kept under LD after the operation. In 3 of them, this operation totally abolished the circadian rhythm like in the animal kept in DD. However, the remaining 9 animals showed an aberrant rhythm in which activity dispersed over the 24 hr but tended to peak in the light phase to early night phase (example, Fig. 6B). This rhythm neither perfectly synchronized to the light cycle nor ran free, but the chi-square periodogram analysis detected a rhythmic component with an average period of 23.9 ± 0.3 hr. The period was significantly longer than that of the intact animals kept in DD ($p<0.001$, t-test) or of the animals with optic nerves cut bilaterally and kept in light cycles ($p<0.001$, t-test). To examine a possibility that the light cycle induces the aberrant rhythm, the 9 animals with the aberrant rhythm were transferred to DD. In DD, they immediately became arrhythmic (Fig. 6B).

To examine whether the ocelli were involved in the aberrant rhythm, we assayed locomotor activity, for at least 22 days under LD, of 14 animals receiving removal of three ocelli in addition to the bilateral removal of the lamina-medulla complex. Ten of them showed an aberrant rhythm peaking from late day to early night. Sham operation of ocelli removal was performed in 12 animals and a half of them also showed the aberrant rhythm. The daily activity profiles for the operated animals showing the aberrant rhythm and for the sham oper-

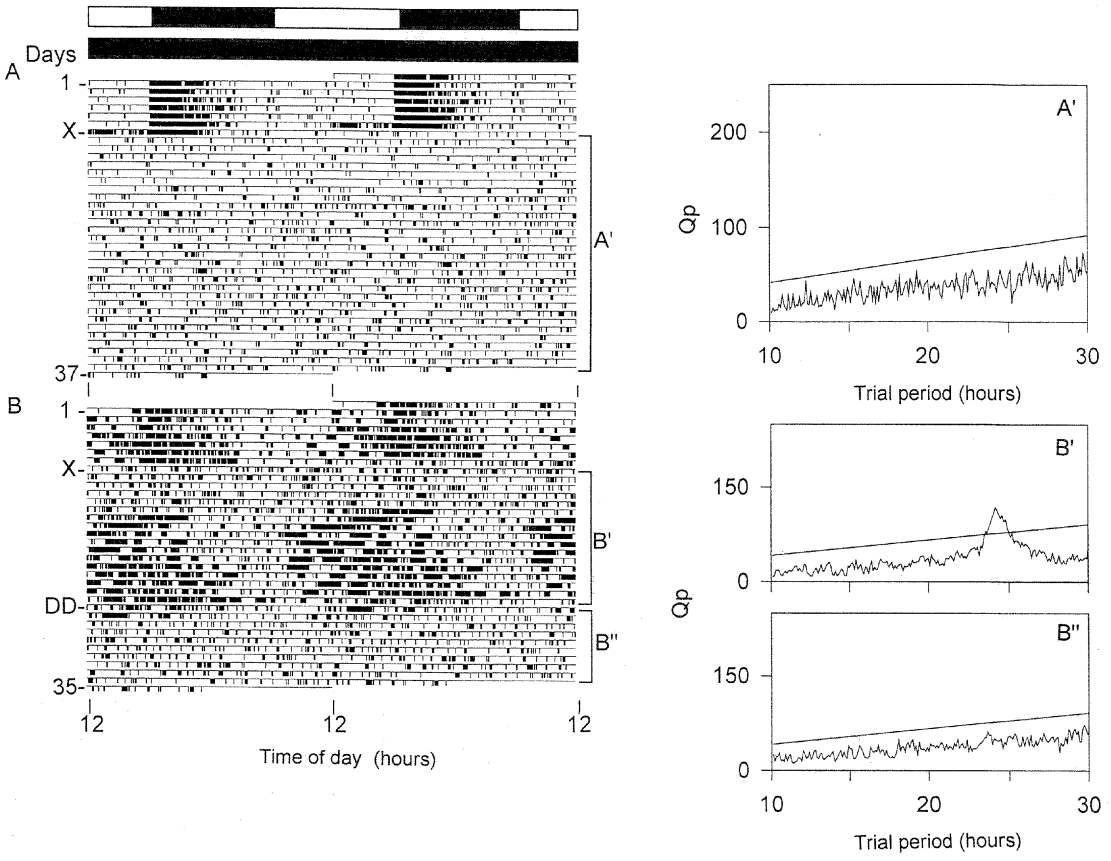
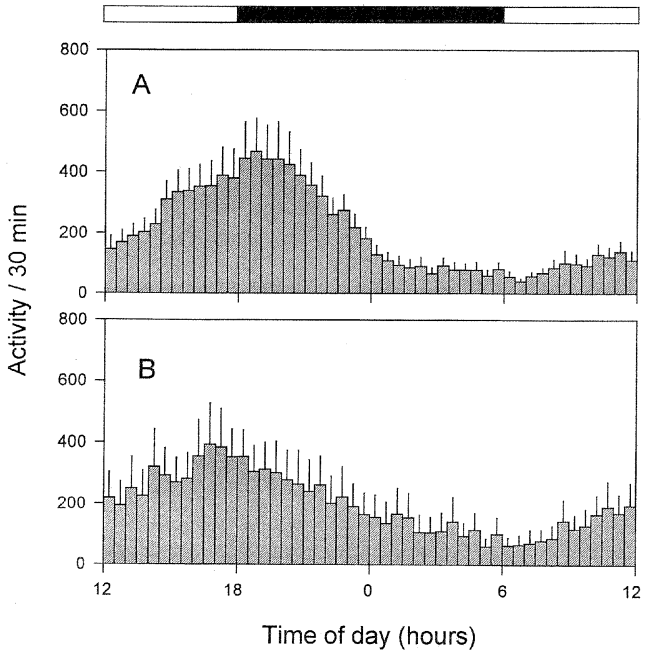


Fig. 6. Two examples of locomotor activity of the animals with optic lamina-medulla portion bilaterally removed. (**A**) The animal was transferred to DD at 18:00 on the day of the operation (X). No rhythmic component was detected by the chi square periodogram. (**B**) The animal was held in LD12:12 for 20 days after the operation (X) and then transferred to DD. Statistically significant rhythm was detected by the periodogram in bright LD12:12 but the rhythm immediately disappeared in the ensuing DD. **A'**, **B'** and **B"** correspond to the periods **A'**, **B'** and **B"** indicated in the actograms. For further explanations see text and Fig. 1.



ated animals are shown in Fig. 7. The average period of the aberrant rhythm was 24.0 ± 0.5 hr and 24.0 ± 0.2 hr for the ocelli removed and for the ocelli sham operated animals, respectively.

In additional 4 animals, we have partially removed the medulla area in addition to the complete removal of the contralateral lamina-medulla portion. Post-mortem histological examination revealed that the medulla area was removed by 70-90% and the lobula area was substantially shriveled. They were placed in DD after the operation and their locomotor activity was recorded for more than 25 days. Two animals showed a very faint rhythm with free-running period of 19.6 hr and 18.5 hr (example, Fig. 8A), whereas the remaining two were arrhythmic (example, Fig. 8B).

Effects of temperature cycles on locomotor rhythm

We assayed the locomotor activity of 4 sham operated and 13 optic lobe removed crickets for 14 days under tem-

Fig. 7. Average daily profiles of locomotor activity of lobeless animals kept under bright LD12:12. (**A**) Crickets receiving bilateral optic lobe and ocelli removal (n=10). (**B**) Crickets receiving bilateral optic lobe removal and sham operation of ocelli removal (n=6). In both groups, activity peaked around lights-off. Vertical bars indicate SEM.

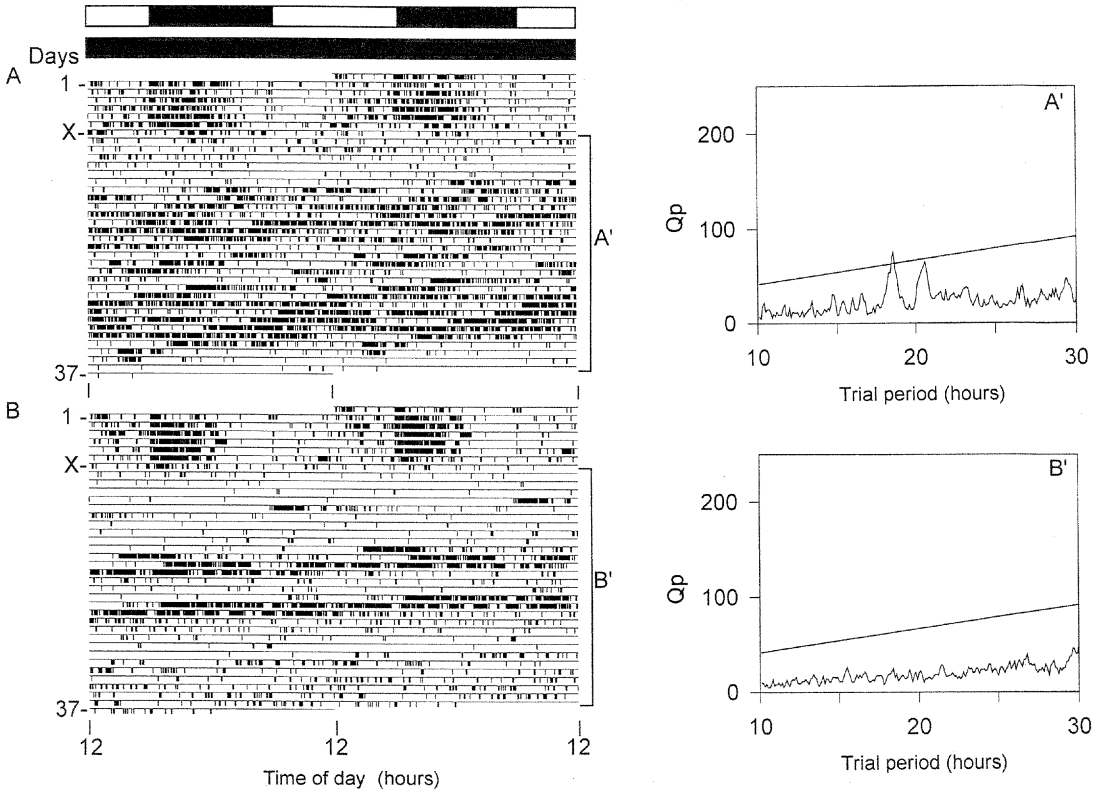


Fig. 8. Two examples of locomotor activity of the animals of which medulla portion on one side was partially removed and the contralateral lamina-medulla complex was completely removed. They were transferred to DD at 18:00 on the day of the operation (X). A very faint but significant rhythm was detected by the chi square periodogram analysis (right panel) in **A**, but not in **B**. **A'** and **B'** correspond to the periods **A'** and **B'** indicated in the actograms. For further explanations see text and Fig. 1.

perature cycles with 12 hr 25°C to 12 hr 28°C. Sham operated crickets showed a clear rhythm free-running with a period shorter than 24 hr (23.6 ± 0.1 hr, n=4) as though they were in constant temperature (Fig. 9A). Of the operated crickets, 6 were arrhythmic, whereas the remaining 7 showed a rhythm peaking in the thermophase and the activity rather uniformly distributed during the thermophase (Fig. 9B, C). The average period calculated by the chi-square periodogram was 23.8 ± 0.3 hr (n=7). Three of the sham operated and 6 of the rhythmic lobeless crickets were then transferred to a temperature cycle of 12 hr 25°C:12 hr 26°C. The sham operated animals still freeran with the period of 23.3 ± 0.4 hr (n=3). Most of the lobeless animals, however, turned to be arrhythmic; only two of them showed a faintly rhythmic activity similarly peaking during the thermophase. The pattern of activity distribution in the rhythmic lobeless animals was quite different from that observed in the light dark cycle where the activity concentrated from the late light phase to the early dark phase (Fig. 9D, E).

DISCUSSION

Circadian locomotor rhythm

Adult male crickets, *Gryllodes sigillatus*, showed a clear nocturnal rhythm peaking in the early night phase. The rhythm persisted under DD and, in some animals, under dim LL (Figs. 1 and 2), suggesting that it is generated by an endogenous mechanism. The period depended on the light intensity, being shorter in DD than in constant light. This is consistent with the generalization by Aschoff (1979) that the period in DD is shorter than in constant light in arthropods. During the prolonged dim LL, the free-running period of the rhythmic animals gradually shortened (Fig. 2A). This may be explained as a reduction of light sensitivity somewhere in the photic entrainment pathway and/or effects of aging. However, in DD the period was rather stable and constant, suggesting the latter to be unlikely.

In bright LL and dim LL, 98% and 60% of animals were arrhythmic, respectively, with activity dispersed over the 24 hr. The cause of the arrhythmicity in constant light is still not well understood (Aschoff, 1979). Nevertheless, all constant light arrhythmicity so far studied shares a common characteristic: When an arrhythmic system is transferred to darkness, rhythmicity is promptly restored, and its phase, relative to the preceding LL/DD transition, implies that the pacemaker motion began from a phase at the beginning of the subjective night (CT 12 or close to it) (Pittendrigh, 1981). In this cricket the activity onsets were significantly concentrated around 19-21 hr after the LL/DD transition in three different trials (Fig. 3). This finding might be interpreted as that the preceding con-

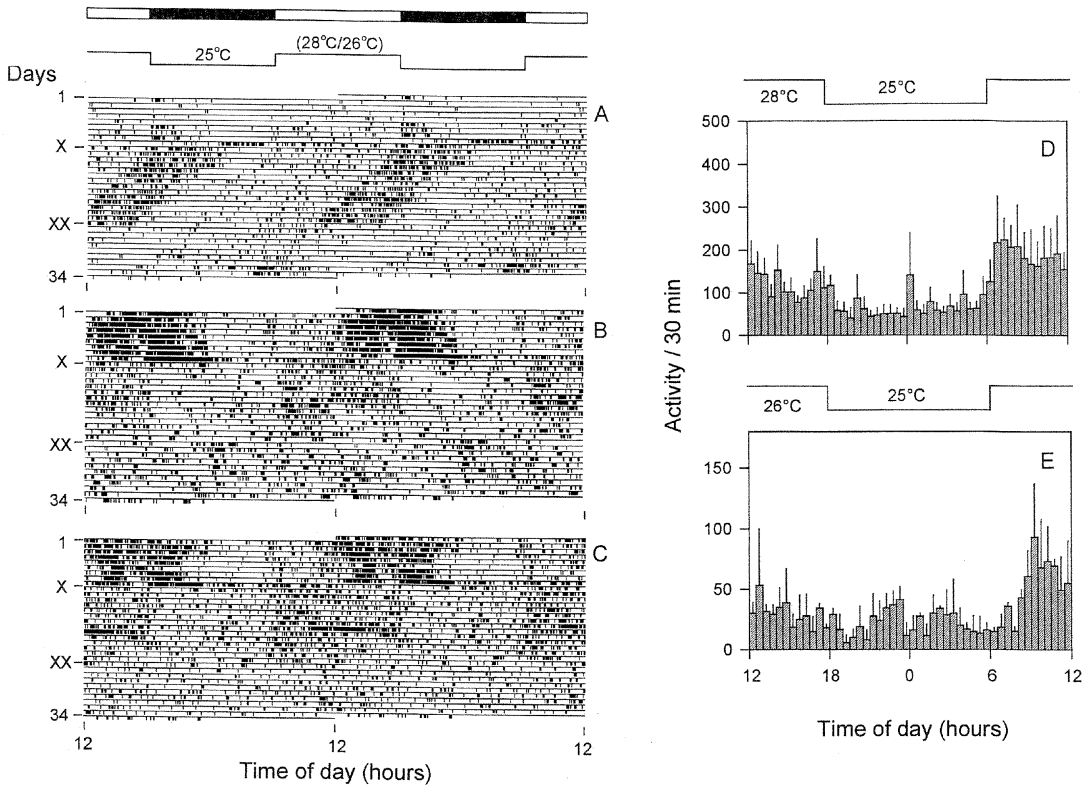


Fig. 9. Locomotor activity of crickets with sham operation (**A**) and with their optic lobes bilaterally removed (**B**, **C**). The crickets were held in bright LD12:12 at 25°C for the first 9 days. They then received the operation and were transferred to DD with temperature cycle of 12 hr 25°C: 12 hr 28°C on the day indicated by X. They were further transferred to 12 hr 25°C: 12 hr 26°C on 25 th day (XX). Sham-operated animal freeran in the temperature cycles. Most lobeless crickets were rhythmic under the temperature cycle with an amplitude of 3°C, while in the cycle with an amplitude of 1°C the rhythm persisted only faintly (**C**) or immediately disappeared (**B**). (**D**, **E**) Average daily profiles of locomotor activity of rhythmic lobeless animals under 12 hr 25°C:12 hr 28°C (**D**) or 12 hr 25°C:12 hr 26°C (**E**) (n=7 and 2, respectively). Vertical bars indicate SEM.

stant light stopped the pacemaker also around the late subjective day to the early subjective night. There is an alternative explanation that the arrhythmicity could be induced by dissociation of several constituent oscillators as suggested for cockroaches and house sparrows (Page, 1985; Takahashi and Menaker, 1982). However, this hypothesis appears to be not applicable to this cricket species since the phase of the animals that had been rhythmic did not concentrate on a particular phase: if the dissociated moving oscillators are reset to a particular phase, then the rhythm free-running in constant light should also be reset by the LL/DD transition.

Photoreceptor for photic entrainment

In cockroaches and crickets, the compound eyes have been thought to contain photoreceptors involved in normal entrainment to light cycles (Nishiitsutsuji-Uwo and Pittendrigh, 1968b; Loher, 1972; Page, 1978; Tomioka and Chiba, 1984). The results reported here show that the compound eyes are the principal and probably the only effective pathway for entrainment of the circadian pacemaker also in *Gryllodes sigillatus*. Interestingly, however, the free-running period of the bilaterally blinded animals was significantly longer than that of the sham operated animals even in DD (Fig. 5). This suggests that information from the compound eye that is independent of light and transmitted through the optic nerve somehow accelerates the movement of the pacemaker, since it has been shown that photoreceptors have spontaneous electrical noise, dark bumps, even in the dark (Devoe, 1985), and that post-synaptic lamina neurons have prominent dark noise which seems to be synaptic in origin (Laughlin, 1973).

Optic lobe as the locus of the circadian pacemaker

The results described here further confirmed the conclusion drawn from earlier works in cockroaches and other cricket species showing that the optic lobe is indispensable for manifestation of the circadian locomotor rhythm (Nishiitsutsuji-Uwo and Pittendrigh, 1968a; Loher, 1972; Sokolove and Loher, 1975; Tomioka and Chiba, 1984). Bilateral removal of the optic lamina-medulla portions abolished the locomotor rhythm (Fig. 6) and the removal of a large part of the medulla neuropil area resulted in manifestation of a very faint rhythm with short free-running period or in complete loss of the rhythm (Fig. 8). These facts suggest that the medulla portion of the optic lobe is the locus of the circadian pacemaker.

In the present experiment, however, a large portion of animals exhibited rhythms both under light and temperature

cycles even after complete removal of the optic lamina-medulla portions (Figs. 6, 7 and 9). The photoperiodically induced rhythm and the thermoperiodically induced rhythm were different in several points. The photoperiodically induced rhythm peaked in the late light phase to early dark phase, and did not clearly synchronize to light cycles (Fig. 6B). In the temperature cycles, however, activity synchronized to the cycle, peaking in the thermophase and rather uniformly distributing in the phase (Fig. 9B-E). These differences suggest that light and temperature induces the rhythms through different mechanisms. Similar photoperiodically induced rhythms have been observed in optic lobeless *G. bimaculatus* but only when the nervous connection between the retina and the cut end of the optic stalk was regenerated (Tomioka and Chiba, 1989). Therefore, this is the first report for the photoperiodically induced rhythm in the lobeless crickets without neural connection between compound eye and the brain. Removal of three ocelli had no effect on expression of the rhythm (Fig. 7). Although we cannot totally exclude a possibility that some unknown factor secreted from the compound eye is involved in the rhythm, it is most likely that the photoreception for the photoperiodic induction of the rhythm is performed by an extraretinal photoreceptor(s) probably located in the brain.

We did not carefully examine the endogeniety of the rhythmicity in the present experiment. There is an explanation that the rhythms of lobeless animals are not simply exogenous but an expression of a secondary oscillator(s) synchronizing to the light or temperature cycle. This hypothesis comes from the evidence that some hemimetabolous insects have such secondary oscillator(s) outside of the optic lobe; some of them are entrainable to light cycles but only when nervous connections between the retina and the central brain are regenerated (Tomioka and Chiba, 1989) and others to temperature cycles (Rence and Loher, 1975; Page, 1985). Further careful experiments are required to clarify this issue.

Our present results demonstrated that the driven system of the *Gryllodes* circadian system is so susceptible to temperature and light cycles that under these cyclical environments the lobeless crickets exhibit the aberrant rhythms. However, this susceptibility of driven system is overcome by the optic lobe circadian pacemaker to sustain a basic nocturnal rhythm even under temperature cycles. This may have biological significance in that temperature and light in their natural habitat often change abruptly in association with human activity.

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