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Analysis of coupling between optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus*

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Abstract. The coupling mechanism between weakly coupled two optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus* was investigated by recording the locomotor activity, under light-dark cycles with various lengths, after the optic nerve was unilaterally severed. The activity rhythm split into two components under the light cycles different from 24 h: one was readily entrained to the light cycle and the other only loosely entrained or freeran. Additional removal of the optic lobe on the intact side resulted in a loss of the entrained component and that on the blinded side caused the reverse effect, indicating that the entrained component was driven by the pacemaker on the intact side and the other by the one on the blinded side. The synchronization between the two components was achieved only in light cycles with a limited length between 23 and 25 h. Without this range, the desynchronization of the components occurred. In the split rhythm, the phase-dependent modulation of the period of freerunning component and the mutual suppression of locomotor activity during the subjective day phase were clearly observed. The suppression was also evident in the lights-on peak that was the masking effect of light. The light cycle with dim light significantly reduced the ratio of animals with the pacemaker coupling as well as the magnitude of the period modulation. These results suggest (1) that the mutual coupling is achieved only when the difference in the periods between the two pacemakers is within an allowable range, (2) that the photic information is also involved in the mechanism of mutual coupling, and (3) that the suppression of activity occurs at the regulatory center for locomotion.

Key words: Circadian rhythm – Cricket – Locomotor activity – Optic lobe – Pacemaker coupling

Introduction

It is well known that in many cases the circadian system is composed of two pacemakers which reside in bilaterally paired structures in the central nervous system such as the optic lobes of several insects (Nishiitsutsuji-Uwo and Pittendrigh 1968; Loher 1972; Page 1981; Fleissner 1982; Wiedenmann 1983; Waddel et al. 1990; Tomioka and Chiba 1992), the eyes of several opisthobranch mollusks (Jacklet 1969; Eskin and Harcombe 1977; Block and Roberts 1981), and the suprachiasmatic nuclei of rodents (Inouye and Kawamura 1982). In several cases, the two component pacemakers reportedly keep a steady phase relationship with each other to maintain a stable physiological or behavioral temporal structure through some coupling mechanism (Page 1981; Wiedenmann 1983; Roberts and Block 1985; Meijer and Rietveld 1989; Tomioka et al. 1991). To understand the circadian timekeeping, elucidation of the coupling mechanism between the component pacemakers is required.

The cricket provides a good model system for investigating the mutual coupling mechanism. This is because the two circadian pacemakers reside in well separated paired structures, i.e. the optic lobes, of the central nervous system, and each optic lobe pacemaker receives a photic input necessary for entrainment from the ipsilateral compound eye (Loher 1972; Tomioka and Chiba 1984). Taking these advantages in structural characteristics, Wiedenmann (1983) revealed that, in *Teleogryllus commodus*, the two optic lobe pacemakers only weakly coupled by his elegant experiment in which the locomotor activity of unilaterally blinded cricket was recorded under constant light. With the same technique, we have further demonstrated that, in *Gryllus bimaculatus*, the mutual coupling between the two optic lobe pacemakers includes the mutual phase-dependent modulation of freerunning period (Tomioka et al. 1991).

In the experiment reported here, the coupling mechanism was further investigated by recording the locomotor activity, under various light cycles, after the optic nerve was unilaterally severed. The results show that the mutu-

Abbreviations: CT, circadian time; DD, constant darkness; LL, constant light; LD, light to dark cycle; T, length of light to dark cycle; τ, freerunning period

al coupling can be achieved only within a limited range of the pacemaker's period, and that the photic information is also involved in the mechanism of mutual coupling.

Materials and methods

Experimental animals. Adult male crickets *(Gryllus bimaculatus)* reared in the laboratory were used throughout this study. They were kept under controlled conditions of temperature at 26±0.5 °C with a 12 h light to 12 h dark cycle (LD 12:12, light: 06:00–18:00, Japanese standard time). They were fed laboratory chow and water.

Activity recording. Activity was monitored as described previously (Tomioka et al. 1991). Briefly, animals were individually kept in an activity chamber of a transparent plastic box with a rocking substratum whose rocking, caused by a moving insect, was sensed by a magnetic reed switch placed on the bottom of the box. Before hausing the animal into the chamber, either of the forewings was removed to prevent any sound communication between individuals. A signal from the switch was collected by a computer, which summed the signals every 6 min; the total count of every 6 min was stored on a minifloppy diskette. The quantitative data were later analyzed with a computer. The activity chambers were placed in an environment-controlled room in which the temperature was kept constant at 27°±0.5 °C. The light was furnished by a cool-white fluorescent lamp connected to a timer. The light intensity within actographs varied with proximity within the environment-controlled room to the lamp and ranged from 250–400 lux at the animal's level. In the dim light experiment, it was lowered to 10–40 lux by shading the lamp.

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 knife, and the eye was prised open with forceps to expose the lamina-medulla-retina complex. The optic nerves were then cut. The optic stalk, which is a long nerve trunk between the medulla and the lobula, was cut when the lamina-medulla complex was removed. After placing a small square piece of plastic 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, and the wound was sealed with a small amount of melted wax. In the sham operation, the same procedure was carried out except cutting the optic nerve. At the end of the experiment, the head was carefully dissected to verify the success of the surgery under a dissecting microscope.

Data analysis. Event records for locomotor activity were double plotted by computer with a resolution of 6 min. In crickets with split rhythms, the onset of both components was always much sharper than the offset, and hence only the onset was used to calculate freerunning periods (τ) and phase. The onset was defined as CT 12 for each component separately in the splitting situation. A 12 circadian hour period following the activity onset was defined as the subjective night, and the rest as the subjective day. The daily τ was defined as the period between the two consecutive onsets. The average τ was estimated by calculating a regression line through daily activity onsets of 10 or more days of data. Deviation from this estimated average τ was determined as daily fluctuation of τ, expressed with a + or − sign for relative lengthening or shortening, respectively. When the two rhythmic components in the split state kept stable synchronous movement, the phase angle difference between the two split components was estimated as the time difference between the onsets of the components. The + sign was used when the loosely entrained component phase led the readily entrained one.

Results

Locomotor rhythm of animals with the optic nerve unilaterally severed under various LDs

Our previous experiment revealed that unilaterally blinded animals exhibited a rhythm splitting under constant light but could be readily entrained to LD12:12 (Tomioka et al. 1991). The fact could be interpreted by the following two possibilities. One was that the coupling between the bilaterally paired optic lobe circadian pacemakers was maintained only when the difference between their freerunning periods was small enough. The second possibility was that photic information somehow involved in the coupling mechanism. To test the likelihood of these possibilities, we examined the entrainability of unilaterally blinded animals in various light cycles, i.e. LD10.5:10.5 (T=21 h), 11:11 (T=22 h), 11.5:11.5 (T=23 h), 12:12 (T=24 h), 12.5:12.5 (T=25 h), 13:13 (T=26 h) and 13.5:13.5 (T=27 h).

In a total of 91 unilaterally blinded animals with the optic nerve unilaterally severed, locomotor activity was recorded under various light cycles. Forty-six of them were operated on the right side, and the remaining on the left side. Since similar activity patterns were yielded irrespective of the side of the operation, the data were pooled. The data for LD12:12 include 6 animals reported in the previous paper (Tomioka et al. 1991). The majority of them exhibited two rhythmic components: one component was readily entrained to the given T and the other either phase locked on the light cycle with certain phase angle difference (Fig. 1C) or freeran with a marked fluctuation in its τ (Fig. 1A, B, D and F). Theoretically, these rhythmic components may be due to three different sources: an intact pacemaker, a blinded pacemaker and a direct response to the light cycle (masking effect). We first examined whether any of the rhythmic components reflects the masking effect in 9 unilaterally blinded animals. The animals were transferred to constant darkness (DD) after the two components were exhibited by exposing to LD11:11 for several cycles. A representative result is shown in Fig. 2. Both the freerunning and the entrained components persisted in DD, coming closer to each other day by day to refuse into a single component. The result obtained in this particular animal was clearly reproduced in other 8 animals, indicating that the two components are not due to the masking effect but driven by endogenous pacemakers. The rhythm with the two rhythmic components will be thus referred to as the rhythm splitting.

To examine which component is driven by which pacemaker, we performed an additional unilateral optic lobe removal on 15 of the unilaterally blinded animals with the split rhythm. The animals were exposed to LD13:13 for several cycles after unilaterally blinded. Figure 3 shows two examples of the result. Removal of the optic lobe on the intact side resulted in an immediate loss of the entrained component (Fig. 3A), whereas the operation on the blinded side abolished the freerunning component (Fig. 3B). Exactly the same result was obtained in the other 13 animals. The result clearly indicates

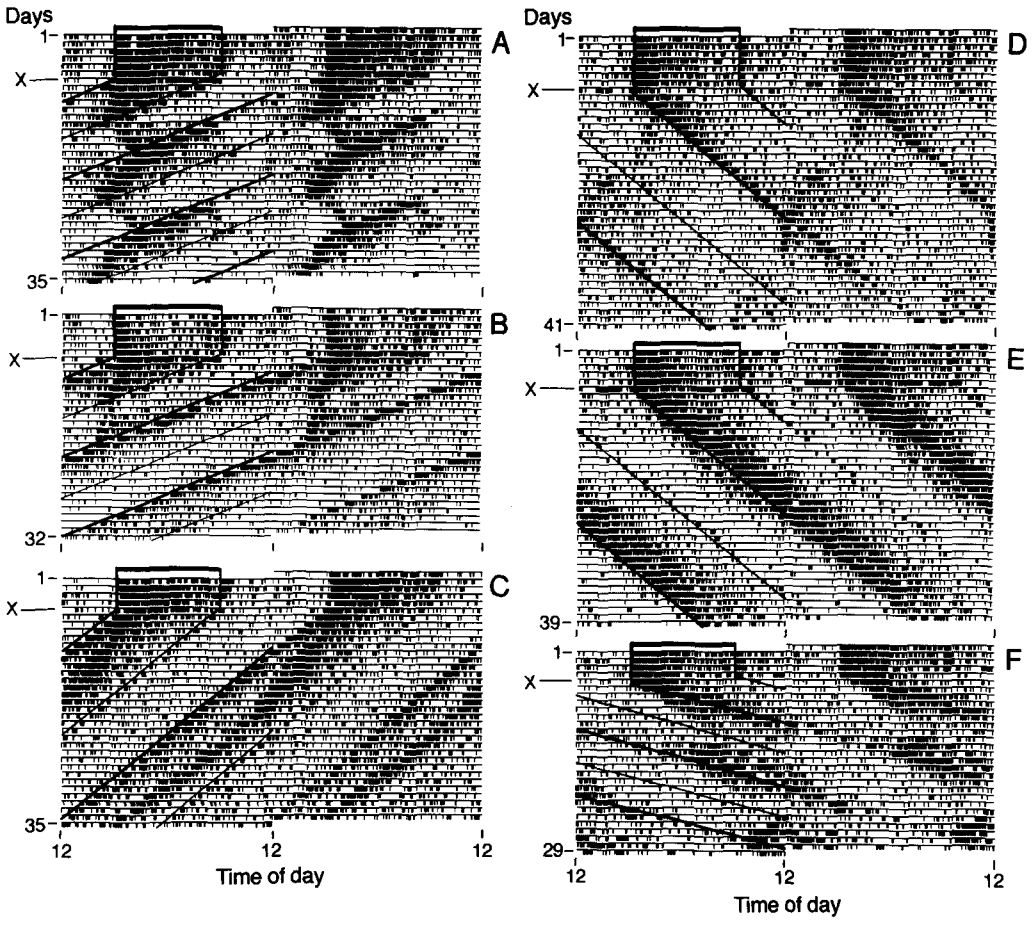

Fig. 1A–F. Six examples of activity rhythms from animals that received unilateral optic nerve severance on the day of transfer to different LDs (*X*). Animals were held in LD12:12 for the first several days. The given LD was LD11:11 (**A, B**), LD11.5:11.5 (**C**), LD12.5:12.5 (**D, E**) or LD13.5:13.5 (**F**). Lights-off and -on are indicated on the left panel by thick and thin lines, respectively. In **A, B, D,** and **F** the desynchronization of two rhythmic components occurred on transfer to the new LD: one entrained to the LD while the other one freeran with an apparent relative coordination. In **B**, the two components merged into a single one at the crossing. In **C** and **E** two rhythmic components appeared on transfer to the new LD, but they ran with the same freerunning period to keep a steady phase angle relationship after several transient cycles

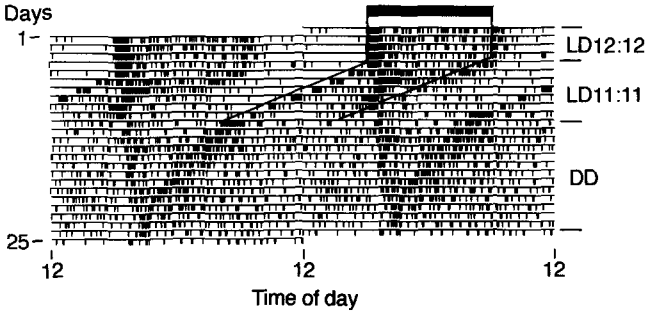

Fig. 2. Activity record of a unilateral optic nerve severed animal. Light regimes are indicated on the right of the actogram. *Black bar* indicates the dark phase. The animal was held in LD12:12 for the first 4 days then transferred into LD11:11 followed by DD. The operation was performed on the day of transfer to LD11:11. Note that the two split components appeared in LD11:11 persisted in DD, merging into a single component

that the entrained and the freerunning components are controlled by the pacemaker on the intact and the blinded side, respectively.

In the remaining 67 unilaterally blinded animals, activity was recorded in various Ts for at least 3 weeks. Twenty-two of them had the two components clearly entrained to the given LD: One component was readily entrained to the light cycle with its onset at the L/D transition while the other was only loosely entrained with certain phase angle difference relative to the L/D transition (hence, relative to the onset of the readily entrained component) (for example, Fig. 1C and E). This state will be referred to as the mutual coupling state. The occurrence of the mutual coupling was restricted between T = 23 h and 25 h (Fig. 4A). The loosely entrained component often initially ran with τ slightly different from the T until reaching the stable phase angle relationship with the entrained component. The phase angle difference between the onsets of the two components was the function of T: the freerunning component phase lagged the entrained one in case of Ts less than 24 h but phase led under Ts longer than 24 h (Fig. 4B). The rhythm in the coupling state was always unimodal in T = 24 h (see Tomioka et al. 1991).

The remaining 45 animals exhibited a desynchronization of the two rhythmic components on change in T (for example, Fig. 1A, B, D and F). The component driven by the blinded pacemaker freeran with a marked fluctuation in τ. In one animal from T = 22 h, however, the split activity merged into a single peak, at a criss-crossing, to be entrained to the LD (Fig. 1B). There were some important points worth noting in the activity rhythm with the desynchronization. First, activity level fluctuates with the relative position of the two components: It was higher when they were in phase and lower when out of phase as has been reported for the splitting under constant light (Wiedenmann 1983; Tomioka et al. 1991). The

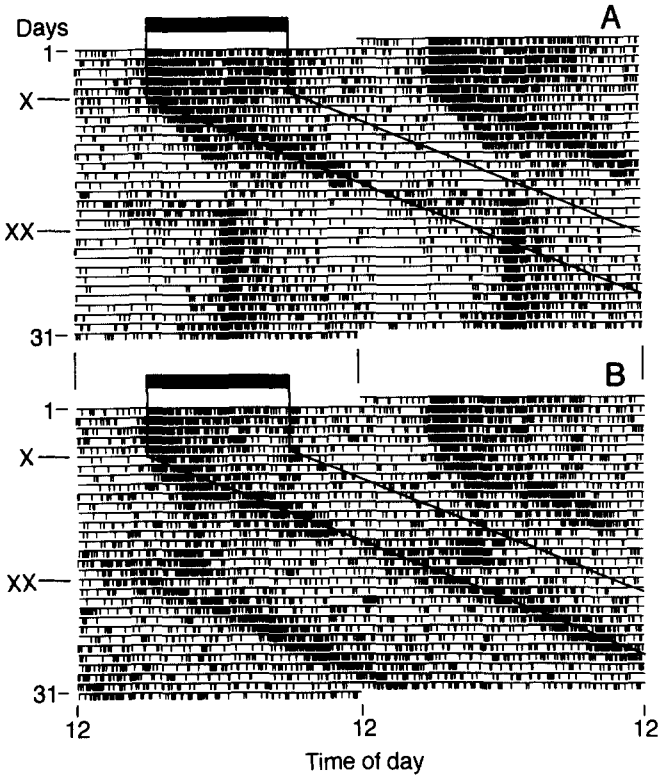

Fig. 3A, B. Two examples of activity rhythms from animals that received unilateral optic nerve severance on the day of transfer from LD12:12 to LD13:13 (*X*). Both animals showed activity rhythm splitting into two components in LD13:13. In **A**, immediately after optic lobe removal on the intact side (*XX*), an entrained component disappeared, and the freerunning component became obvious. In **B**, a freerunning component disappeared immediately after removal of the blinded optic lobe. For further explanations see Fig. 2

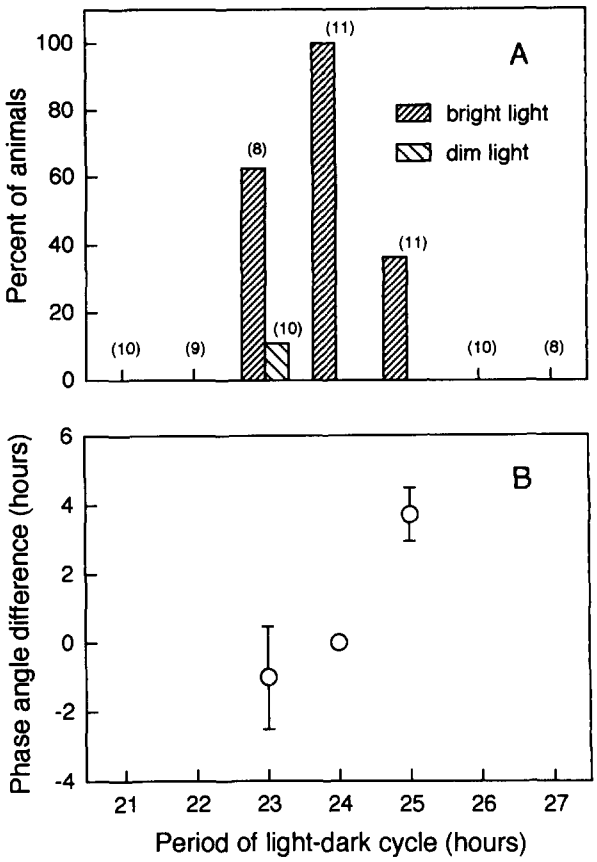

Fig. 4A, B. Percent animals showing pacemaker coupling (**A**) and phase angle difference between the two rhythmic components (**B**) plotted as a function of the length of given LDs. **A** The coupling occurred only between T=23 and 25 h. In dim LD the occurrence of the coupling significantly reduced. The *number* given at the *top of each bar* indicates the number of animals examined. **B** The phase angle difference between onsets of the two components was also a function of the T. Each point represents a mean (±SE) of animals given in **A**

lights-on peak was also markedly reduced when it occurred during the subjective day of the freerunning component (Fig. 1D). Second is the change in τ of the freerunning component. The average τ of the component, which was restricted between 22.5 and 25.5 h, appeared to correlate with that of the entrained component ($r=0.789$, $P<0.001$) (Fig. 5A). This correlation was more evident for the first 10 day period of the new LDs ($r=0.933$, $P<0.001$) (Fig. 5B). However, the τ of the freerunning component changed according to the phase angle relationship to the entrained component. Figure 6 shows the change in τ of the freerunning component as a function of the circadian time (CT) at which the onset of the entrained component occurred. Although the shape of the modulation curve slightly differed among experiments with different Ts, a common feature was that τ was lengthened when the onset of the entrained component fell during the subjective night (CT 12–22) but was relatively shortened during the subjective day (CT 06–12).

Locomotor rhythm of animals with optic nerve unilaterally severed in dim LD

The difference in the shape of the period modulation curves among the different Ts suggests involvement of photic information in the mechanism of mutual coupling. To examine this possibility, locomotor rhythm of 10 unilaterally blinded animals was recorded under the LD11.5:11.5 (T=23 h) with lowered light intensity (10–40 lux). Figure 7 exemplifies two of the records. In contrast to the bright light T=23 h experiment, 9 animals (some 90%) showed a desynchronization on transfer to the dim light LD T=23 h (Fig. 4A); one component was readily entrained to the LD, while the other freeran with the average τ of 23.92±0.20 (SD) h. With an exception (Fig. 7B), the freerunning component merged into the entrained one at the criss-crossing, however (Fig. 7A). The merged rhythm never split again. During the desynchronization the phase-dependent fluctuation in τ of the freerunning component and in the activity level of both components were evident. However, the amplitude of the freerunning period modulation curve was smaller than that in the bright light experiment (Fig. 8).

Entrainment in sham operated animals

Entrainability of a total of 33 animals with their optic nerve unilaterally sham operated was examined under

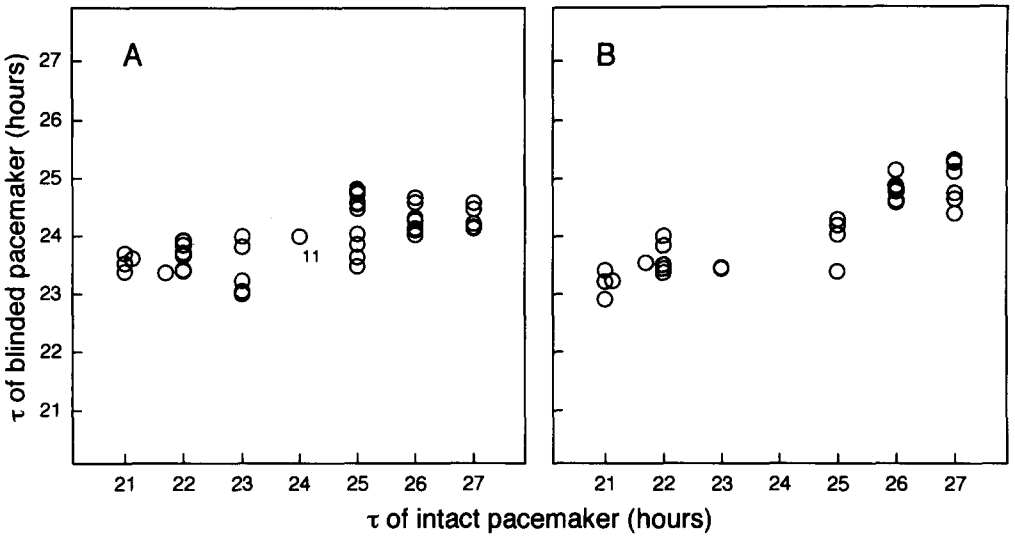

Fig. 5A, B. Relationship between average freerunning periods of the blinded and intact pacemakers for the whole recording period (**A**) or for the first 10 day period (**B**). Each *open circle* represents datum for an individual animal. The values were the same in animals kept in T = 24 h ($N = 11$). In **B** the data for the animals showing mutual pacemaker coupling were omitted

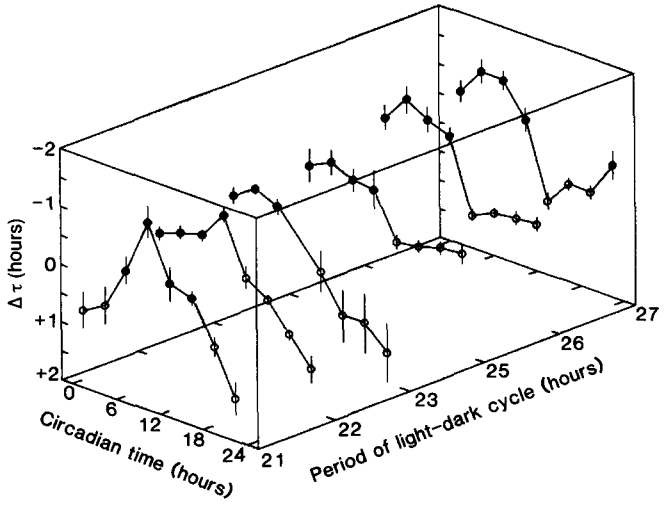

Fig. 6. Change of the freerunning period of the freerunning component plotted as a function of its circadian time at which the onset of the entrained component occurred. *Ordinate*, difference from the mean freerunning period. Data from 3 to 10 animals were pooled. Each *point* represents the mean (± SE) calculated by collapsing the data in 3-h bins. *Open* and *filled circles* indicate positive and negative values, respectively. Note that although the form is somewhat different among the curves, the period was lengthened when the entrained component occurred during the subjective night but was relatively shortened during middle of the subjective day

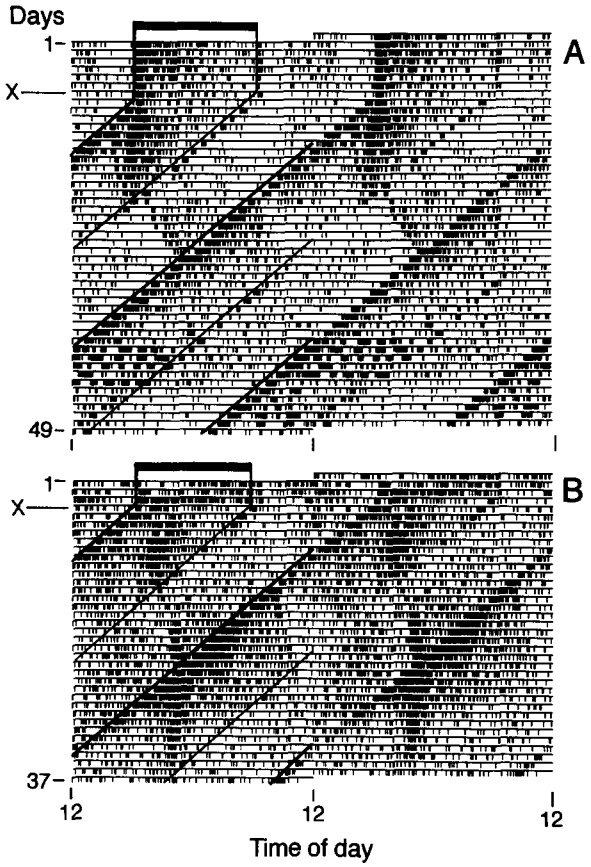

Fig. 7A, B. Two examples of activity rhythms from animals that received unilateral optic nerve severance on the day of transfer from LD12:12 to LD11.5:11.5 with dim light (*X*). On transfer to the new LD, activity rhythm split into two components: one entrained to the given LD and the other freeran with a relative coordination. In **A** the two components merged into a single one at the crossing. In both cases, the activity level and the freerunning period changed as a function of the mutual phase relationship. For further explanations see text and Fig. 1

various LDs, i.e. LD10.5:10.5 (T = 21 h), 11:11 (T = 22 h), 11.5:11.5 (T = 23 h), 12:12 (T = 24 h), 12.5:12.5 (T = 25 h), 13:13 (T = 26 h) and 13.5:13.5 (T = 27 h). Four to 5 animals had their locomotor activity measured under each lighting condition. Except in T = 21 h, animals were readily entrained to the given light cycles, showing stable unimodal nocturnal rhythms sometimes with a brief activity peak at lights-on (Fig. 9B, C). Under T = 21 h, about half of the animals had their locomotor rhythm not synchronizing to the light cycle but freerunning with a relative coordination in which the τ fluctuated as a function of phase angle relationship with the

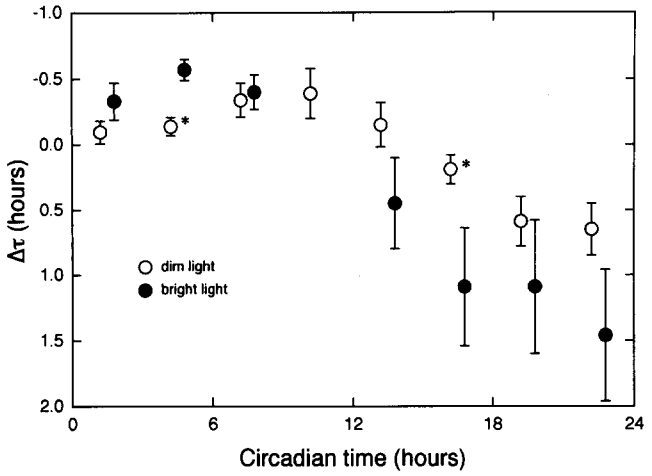

Fig. 8. Period modulation curves for LD11.5:11.5 with bright light *(filled circles)* or dim light *(open circles)*. Data from 3 (bright light) or 9 animals (dim light) were pooled. Each point represents a mean (±SE) calculated by collapsing the data in 3-h bins. Although the shapes are similar to each other, the amplitude is smaller in the dim LD11.5:11.5. * $P<0.01$ (two tailed *t*-test, compared with data from the bright light experiment). For further explanations see text and Fig. 6

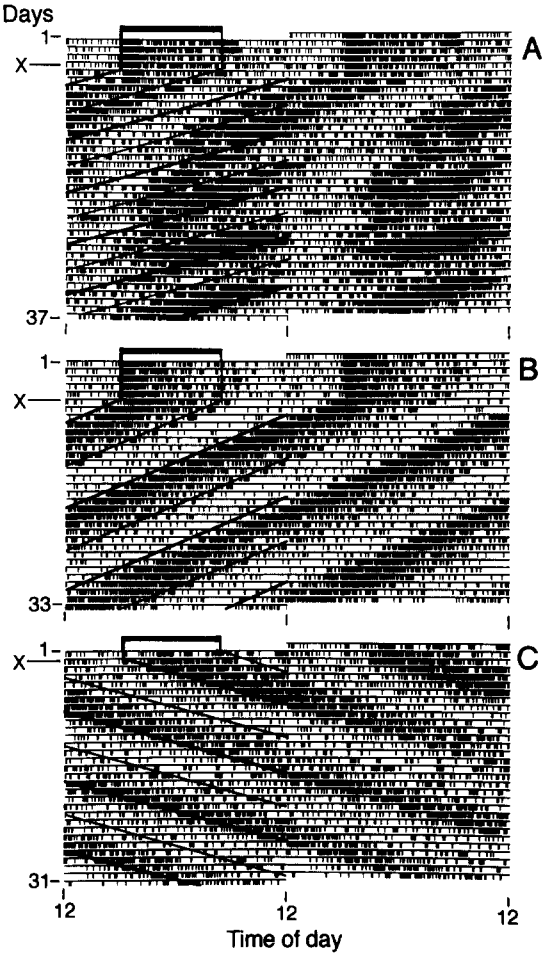

Fig. 9A–C. Three examples of activity rhythms from sham operated animals kept under LD10.5:10.5 (**A**), LD11:11 (**B**) or LD13.5:13.5 (**C**). The animals were held in LD12:12 for the first several days, then transferred to the different LDs after the sham operation (*X*). The lights-off and -on are indicated on the left panel by *thick* and *thin lines*, respectively. **A** In LD10.5:10.5 the rhythm freeran with an apparent relative coordination. **B** and **C** Animals were readily entrained to LD11:11 (**B**) or LD13.5:13.5 (**C**)

light cycle (Figs. 9A, 10). The period lengthened when the light onset fell during the subjective day, while it relatively shortened if the light onset occurred during the late subjective night. In some cases, an intense activity peak occurred at lights-on (Fig. 9A). The intensity of the lights-on peak was clearly dependent on the phase at which the lights-on occurred, indicating that the peak was a phase-dependent masking effect of light.

Discussion

A circadian system composed of two bilaterally paired pacemakers

Transfer of crickets to LDs with Ts different from 24 h after unilateral optic nerve severance resulted in a splitting of the activity rhythm into two rhythmic components: one readily entrained to the LD and the other only loosely entrained or freeran with a marked relative coordination. The results of unilateral optic lobe removal clearly demonstrated that the entrained component is attributable to the optic lobe pacemaker on the intact side and the other to the one on the blinded side.

In the two-oscillator hypothesis of Pittendrigh and Daan (1976), two split components are thought to reflect the action of two functionally separate oscillators. Light intensity has differential effects on the periods of the two oscillators one of which is assumed to control the initial or evening activity peak and to be synchronized by dusk, while the other is assumed to control the morning peak and to be synchronized by dawn. Under unsplit conditions, the two oscillators are mutually coupled with the evening oscillator phase-leading the morning one by a few hours. The qualitative intuitions of Pittendrigh and Daan model have received mathematical foundation (Daan and Berde 1978). The two oscillator hypothesis

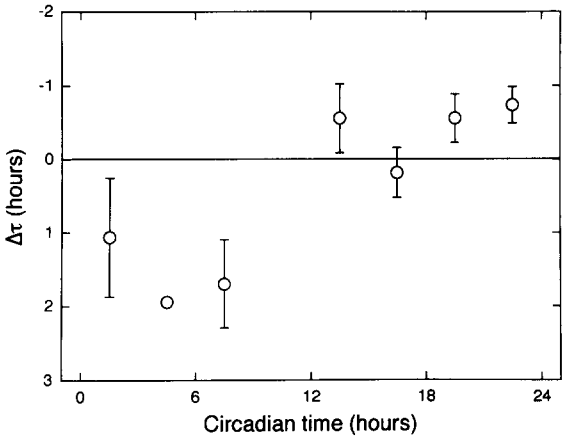

Fig. 10. Change of the freerunning period during the external relative coordination in LD10.5:10.5 plotted as a function of circadian time at which the lights-on occurred. *Ordinate*, difference from the mean freerunning period. Data were obtained from the animal whose actogram is shown in Fig. 9A. Each point represents the mean (±SE) calculated by collapsing the data in 3-h bins. Note that the period was lengthened when the lights-on occurred during the subjective day but was shortened during the subjective night

has been found consistent in the cricket *Teleogryllus commodus,* where the early-evening and the before-dawn components behave as though controlled by two separate oscillators (Wiedenmann and Loher 1984).

However, the results of this and previous studies with *G. bimaculatus* (Tomioka et al. 1991) contrast with the two-oscillator model in that the two split components driven by the optic lobe pacemakers appear to overlap completely in the unsplit state in this cricket. It is likely that the two optic lobe circadian pacemakers have exactly the same functional properties, both with respect to their response to light and with respect to their control over activity, since the rhythm splitting and the changes in τ and in activity level were equally induced by cutting optic nerves on either side. Although the intact pacemaker appeared to dominate formally over the blinded pacemaker, since only the τ of the blinded pacemaker greatly varied with the relative position to the intact one, this dominance of the intact pacemaker was probably due to its output waveform modified by given LDs. This statement is based on the fact that under LD the circadian rhythm of optic lobe efferent electrical activity has large peaks at D/L and often at L/D transitions, none of which appears in DD (Tomioka and Chiba 1986, 1992). Although the blinded pacemaker may also have similar effect on τ of the intact pacemaker as has been suggested in the previous study (Tomioka et al. 1991), its effect may be canceled by LD as a powerful zeitgeber.

An allowable range of mutual coupling

The results of the present study demonstrated that the coupling between the bilaterally paired pacemakers can be established only with Ts between 23 and 25 h. The finding confirms our previous proposition that the coupling could be maintained when the difference in τ between the two pacemakers is small enough (Tomioka et al. 1991). We could thus estimate that the allowable range for mutual coupling may be within 24 ± 1.0 h, since τ_{DD} of the optic lobe pacemaker is close to 24 h (Tomioka and Chiba 1982). However, even under $T = 23$ and 25 h, a considerable number of animals had a desynchronization between the pacemakers (Fig. 4A). Possible explanations for this are that the period difference in these animals was out of this range, and/or that the coupling force in these animals was weaker than those with coupled pacemakers.

At the periphery of the allowable range, the coupled two pacemakers showed a marked phase separation with uppermost value of 6 h (see Fig. 4B). The range of phase separation was far smaller than that known for the splitting in mammalian species where the two rhythms settle down in a nearly 180° phase-separation even when entrained (Boulos and Morin 1986).

Possible mechanism of mutual coupling

The coupling mechanism between the bilaterally paired pacemakers is still largely unknown. The mutual coupling necessarily involves phase-shifting (Daan and Berde 1978). The phase-shifting force of the pacemaker, which is reflected in the period modulation curve (Fig. 6), is far weak relative to that of light (see Okada et al. 1991), but apparently sufficient to keep synchronous movement in intact animals without external entrainment cues. Similar weak coupling between the pacemakers has been reported in the mollusk *Bulla gouldiana* (Page and Nalovic 1992) and the insects *Blaps gigas* (Koehler and Fleissner 1978), and *Teleogryllus commodus* (Wiedenmann 1983). The phase-shifting mechanism which may be commonly shared in these animals is the most challenging issue to be addressed.

The results of the present study provide some evidence showing the involvement of photic information in the coupling mechanism. Both the number of animals showing the mutual coupling and the amplitude of the period modulation curve were significantly reduced with lowering the light intensity of given LDs (Figs. 4A and 8), suggesting that the coupling force depends on the light intensity. That the change in the shape of the period modulation curve was dependent on T (Fig. 6) also suggests the involvement of the light in the coupling mechanism. These results even confirm the assumption that light intensity influences on the coupling strength (amplitude) of the pacemakers (Daan and Berde 1978).

It is reasonable to assume that the light affects the contralateral pacemaker through a neural pathway, since the photic information is conveyed to the contralateral optic lobe by neurons interconnecting bilateral medullae (Tomioka 1992). By comparing the period modulation curve obtained from an external relative coordination (Fig. 10) with those caused by the pacemaker interaction (Fig. 6), it is presumed that the light and the pacemaker interaction shift the pacemaker in the same direction, since the lights-on occurred at 180° (12 h in CT) out of phase relative to the activity onset. It is thus likely that the light and the pacemaker cooperate to regulate the phase of the contralateral pacemaker. Therefore, there seem to exist three separate pathways to regulate the phase or movement of the circadian pacemaker, i.e. two for photic information from the ipsilateral and the contralateral compound eye, and the temporal information from the contralateral pacemaker. An experiment is in progress to identify these pathways.

Activity modulation

It has been reported that the activity is additively increased when the pacemakers are in phase in the insect *Leucophaea maderae* (Page 1983), *T. commodus* (Wiedenmann 1983) as well as mammalian species (for example, *Tupaia belangeri,* Meijer et al. 1990). In addition to this activity increment, this cricket showed a marked activity suppression when the two pacemakers are out of phase. The suppression was observed in activity driven by the blinded pacemaker as well as the intact pacemaker, suggesting that it is principally caused not by a light cycle but through the mutual interaction of the pacemakers. The suppression appeared strongest when the activity occurred in the subjective day of the partner pacemaker (Fig. 1, also see Fig. 6 in Tomioka et al. 1991). The

suppression has been reported for the diurnal tree shrews *(T. belangeri)* where it is strongest at the end of the active phase (the early subjective night) (Meijer et al. 1990). Thus, it may be possible that the suppression of activity influences during the rest phase of the activity rhythm both in nocturnal and in diurnal animals to keep a stable nocturnality or diurnality, respectively.

Although the mechanism of the suppression is still unknown, the suppressing process probably strikes on the regulation center for the locomotor activity, since the suppression was also observed in the masking effect of light occurring at lights-on (Fig. 1D). A question remaining to be answered is how the pacemaker regulates the locomotor activity. In cockroaches, Colwell and Page (1990) suggested that the optic lobe pacemaker inhibits the activity during the subjective day to induce a nocturnal locomotor rhythmicity. The idea is consistent with our data since the pacemaker suppresses the expression of activity during the subjective day. It is also consistent with the fact that the optic lobe removal resulted in a higher activity level (Tomioka and Chiba 1989). However, further critical study is needed to elucidate the down stream pathway controlling the locomotor activity.

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