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# Splitting in a Circadian Activity Rhythm: The Expression of Bilaterally Paired Oscillators*

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**Summary.** Circadian stridulation rhythms of the male Australian field cricket *Teleogryllus commodus* (Walker) recorded in continuous light (LL) and constant temperature sometimes split spontaneously or after a pulse of low temperature into two activity components per cycle. Both components exhibited different free-running periods longer than 24 h, referring to separate oscillatory control mechanisms. One of the components disappeared when an optic lobe was removed.

Two activity components per circadian cycle with different periods were also found after the ommatidial nerves between retina and optic lobe had been cut on one side during the last larval instar. In LL, one period was then longer, the other shorter than 24 h; these periods correspond to the free-running rhythms of intact crickets in LL and constant darkness (DD) (Loher 1972), and indicate that they can be simultaneously expressed in the same cricket. The two underlying pacemakers are only weakly coupled, they jointly or alternatingly influence the overt rhythm causing a spectrum of different patterns. Additional removal of the optic lobe on the intact side eliminated the LL-component (>24 h) while the DD-component (<24 h) continued. Removal of the de-afferented optic lobe had the reverse effect. Each component of the split activity rhythm can therefore be attributed to either side of the brain. The circadian control mechanism of the calling song in *T. commodus* is interpreted as being composed of two bilaterally organized subunits.

## Introduction

Recurring changes in physiological states during the 24-h day are found in most living organisms, and led to the concept of 'the physiological clock' (Bünning 1958). This is an oscillatory mechanism which free-runs under constant laboratory conditions with an inherent period different from 24 h. Its ability to become entrained to external zeitgebers produces synchrony with environmental fluctuations during the 24-h day.

The actual mechanisms of this self-sustained pacemaker (Pittendrigh and Bruce 1957) are still unknown. One major concern in circadian rhythm research during the last decade has been the organization of the oscillatory systems in unicellular and in multicellular organisms (Pittendrigh 1974). Are all circadian fluctuations in an organism controlled by one 'master clock'? Or are they driven by several non-hierarchically organized pacemakers which may interact, but which are independent from a superior central timer? Are either of these possible mechanisms, single or compound oscillators?

Although there is evidence for a unitary circadian pacemaker in some cases (e.g. *Gonyaulax,* McMurray and Hastings 1972, but see also Krasnow et al. 1980), circadian control mechanisms are frequently described as a multi-oscillator system. One major argument favoring this hypothesis, at least in multicellular organisms, is internal desynchronization, which has been found in rhythms recorded simultaneously from one or several physiological functions within the same organ-

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[*] Dedicated to Professor Erwin Bünning, my "Doktor-Großvater", on the occasion of his 75th birthday

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*Abbreviations: DD* continuous darkness; *LD* light-dark cycle; *LL* continuous light; *LTP* pulse of low temperature; $\tau$ free-running period ism (data summarized by Winfree 1980). The occurrence of individual oscillatory characteristics such as different free-running periods or phase shifting behaviors in these rhythms is inconsistent with the hypothesis of a single pacemaker.

A special case of internal desynchronization is 'splitting'. This phenomenon appears in records of single physiological functions and is distinguished by two components per circadian cycle, each showing a different free-running period. The two components may eventually lock on to each other. Preferred states of stable phase angle differences between them are either 0° or 180°. Splitting is generally considered evidence for mutually coupled pacemakers underlying a normally unimodal rhythm, and has been described mainly for activity patterns in vertebrates (Pittendrigh 1960, 1974; Pittendrigh and Daan 1976; Hoffmann 1969, 1971; Gwinner 1974; Underwood 1977). In insects, splitting has been reported thus far for cockroaches (Pittendrigh 1974; Wiedenmann 1977, 1980), and for crickets (Rence 1976).

Temporal calling song patterns of the cricket *Teleogryllus commodus* are well-known circadian rhythms (Loher 1972; Sokolove and Loher 1975; Rence 1976). The free-running periods in continuous light (LL) are longer, in constant darkness (DD) shorter than 24 h; disconnection of the ommatidial nerves on both sides between optic lobe and compound eye results in a DD-rhythm (i.e. a free-running rhythm with a period characteristic of that in DD in intact animals) which is independent of external light regimes, and destruction of both optic lobes leads to arrhythmic activity patterns. The circadian rhythm continues, however, if only one lobe is removed.

This paper deals with splitting in the circadian calling song rhythm of *T. commodus* following various experimental manipulations. The properties of split activity patterns are reduced to characteristics of a pair of unimodal rhythms, indicating the redundant structure of the underlying pacemaker. They are traced back to a bilateral organization, and it is shown that the individual optic lobes participate in the control of the circadian rhythm.

## Materials and Methods

Splitting was investigated in the circadian stridulation rhythm of the Australian field cricket *Teleogryllus commodus* (Walker). Unless noted otherwise, crickets were reared as described by Loher (1972), and singing of individual adult males was recorded by techniques similar to those of Loher and Wiedenmann (1981). The insects were exposed to light-dark cycles (LD) 12:12 h (L about 50 lx) for up to 4 weeks following the imaginal moult, and subsequently kept under continuous light (LL, about 50 lx). Temperature was constant throughout the experiments (27±1 °C). Pulses of low temperature (LTP, 2±1 °C, 2 h) were presented by placing the cricket in a pre-cooled glass vial which was transferred to an illuminated refrigerator (light intensity about 50 lx). Control animals were subjected to the same handling except that the glass vial remained at the experimental temperature throughout. Up to four pulses were given to the same cricket; the time interval between two such perturbations was at least 14 days.

Splitting of circadian patterns under LL into two components with different periods, one longer, the other shorter than 24 h, was induced surgically. Crickets used in these experiments were collected from the cultures during the seventh larval instar, kept individually in glass vials, and fed daily with dog chow and lettuce. LD and temperature matched the conditions provided later, during the first part of the experiment (LD 12:12, 27±1 °C). Two to four days after the penultimate moult, the ommatidial nerves of one compound eye were cut between retina and optic lobe. The ommatidia were also destroyed to prevent axonal regeneration. In another group of crickets the entire optic lobe was removed in the last larval instar. The operations lasted maximally for 10 min and were carried out under $CO_2$-narcosis. Following the last moult – 10 to 14 days later – the adult crickets exhibited a closed head capsule instead of a compound eye. Individuals with residual ommatidia were excluded from the experiments.

Before the adult crickets were transferred to the experimental boxes they were deafened by cutting off the front tibiae in order to reduce acoustical interference. The method used for recording the stridulation was similar to that of Loher and Wiedenmann (1981), except for a modified round cage with two concentric walls. The external one was padded with white rubber foam for sound deadening; inside, a conic wall of translucent plastic directed the chirping sound from any site of the cage to a crystal microphone which was attached above the narrow end. Light reached the cricket through the gaze-lid of the cage and indirectly through the rubber foam. Light programs and temperature corresponded to those of the other group (see above).

Free-running periods in the activity pattern were measured by means of a foil stencil placed on the actogram. Overall slopes in the onset and end of activity (if possible at least 10 consecutive days) were compared with lines on the stencil which represented periods in steps of 0.1 or 0.2 h. The process of measuring was repeated independently several times. The results differed by maximally ±0.1 h.

In eleven crickets that were blinded on one side as described before, one optic lobe was additionally removed during the last part of the experiment. Techniques were as described by Loher (1972). Shamoperations were accomplished by opening the head capsule only. All operations were checked at the end of an experiment. The brains were fixed in Bouin, embedded in paraffin, and 10 µm-sections were examined after Azan staining.

## Results

### *'Splitting' Occurring Spontaneously or After Pulses of Low Temperature*

97 intact male crickets of the species *Teleogryllus commodus* were recorded over 3 years. A gradual split of the circadian stridulation rhythm into two components with different periods, both longer

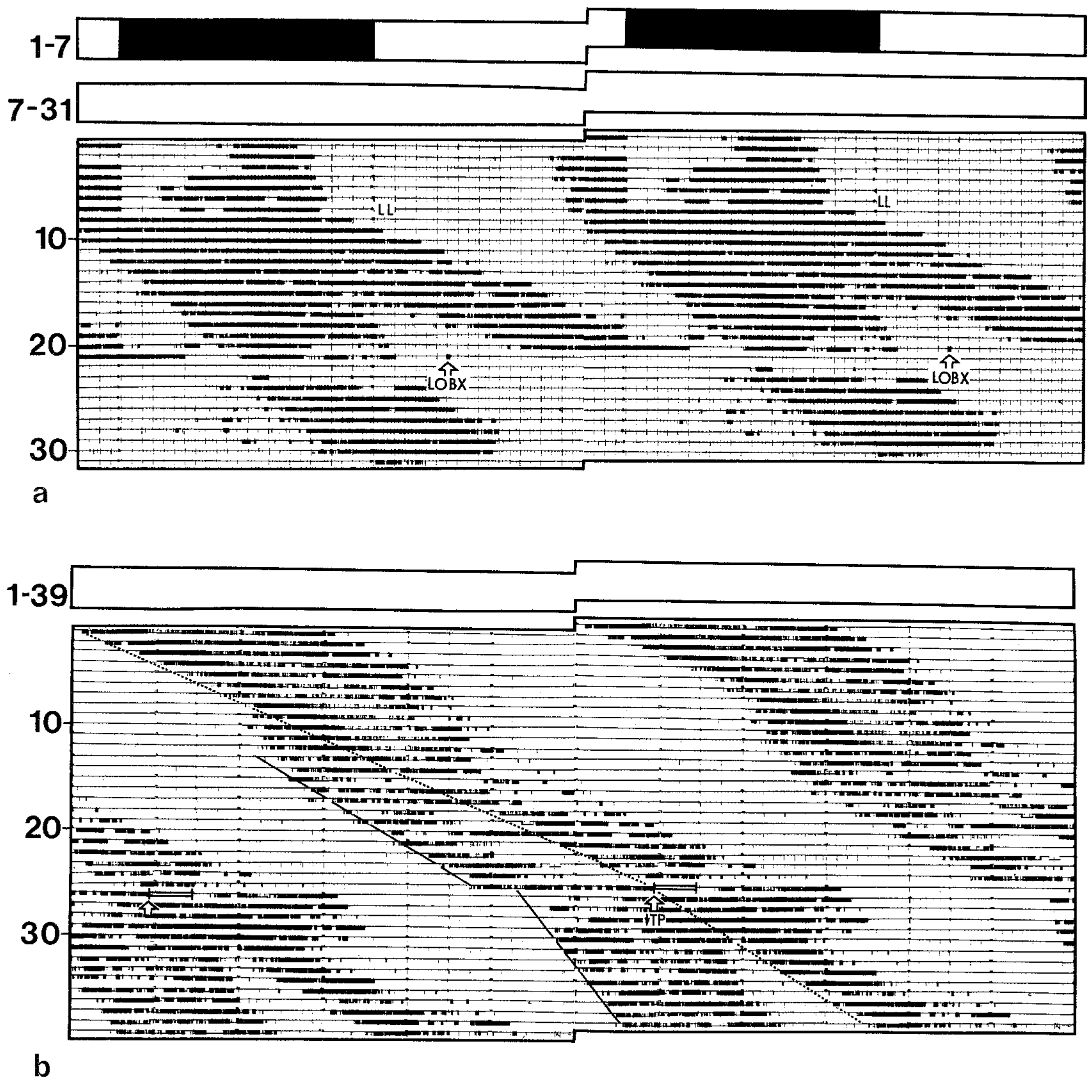

**Fig. 1a, b.** Stridulation patterns of male *Teleogryllus commodus* which split into two activity components per circadian cycle, revealing different periods ($\tau_1$, $\tau_2$) both longer than 24 h. Light regimes given are indicated above double-plotted actogram. *Abscissa:* twice the time of the day; *ordinate:* days of experiment. **a** Entrainment in light-dark cycle (LD) 12:12 (days 1–7), and a free-running rhythm in subsequent LL (days 7–31); temperature was constant throughout (27 ± 1 °C). 'Spontaneous' splitting ($\tau_1 = 24.5$ h, $\tau_2 = 25.4$ h) occurred without any obvious external trigger 10 days after the LD/LL-transition. One activity component disappeared following monolobectomy on day 21 (LOBX). **b** Free-running rhythm under continuous light (LL) during days 1–39; constant temperature (27 ± 1 °C) was interrupted by exposure to a 2-h pulse of low temperature (1 ± 0.5 °C) on day 26 (↓TP). Splitting is evident 5 days later ($\tau_1 = 24.5$ h, *continuous line*, $\tau_2 = 24.8$ h, *dotted line*). The pattern before the pulse can be interpreted as being composed of two components as well, which is accentuated by a continuous ($\tau = 24.8$ h) and a dotted line ($\tau = 25.1$ h)

than 24 h (Figs. 1a, b) was found in 13% of all cases. In LL these 13 insects showed a unimodal activity pattern for up to 32 days before a separation into two components occurred. The difference between the two free-running periods averaged 0.7 h.

In 3 out of these 13 crickets no obvious external trigger for the split in the rhythm is known. Figure 1a shows one of these 'spontaneous' cases in which the separation occurred 2 to 10 days after the LD/LL transition.

For 10 additional crickets splitting appeared between one and ten days after exposure to a 2-h pulse of low temperature (LTP, Fig. 1b). This refers to about 20% of the 52 LTP-experiments conducted with 37 intact crickets. The proportion of insects exhibiting split rhythms following LTP-treatment (10/37) is significantly greater than those showing spontaneous splitting (3/53) ($\chi^2$-test, 1%-level). Only unimodal calling song patterns were found in 7 intact animals that were exposed to a total of 15 control experiments without cooling. Cold exposure therefore increases the probability of splitting in circadian rhythms of *T. commodus*, although the mechanism is still unknown. The occurrence of splitting after cold pulses was not phase related in crickets, unlike the situation in the cockroach *Leucophaea* (Wiedenmann 1977).

Following the removal of one optic lobe in the last larval instar adult males of *T. commodus* never showed splitting in their circadian calling song rhythm. This included 15 insects with undisturbed rhythms, as well as 20 crickets exposed to a total of 27 LTP-experiments.

In six of the ten intact crickets that exhibited splitting following an LTP, activity patterns were unequivocally unimodal before the cold treatment. The activity of the other four cases could be interpreted as being already composed of two components before the pulse, although at all times only one free-running period prevailed (Fig. 1b). In 7 of the 10 crickets both periods were different from the original rhythm: in 4 insects one was longer, the other shorter, while in 3 crickets both were longer. In the remaining three actograms a shorter period in addition to the persisting original rhythm was found.

Crickets with a split rhythm showed a considerable change in their activity pattern after removal of either optic lobe (Fig. 1a, day 21). Only one component per circadian cycle reappeared following the period of postoperative shock, whereas the other disappeared ($n=2$).

### *'Splitting' Induced by Surgery*

The disadvantage of not being able to predict the rare event of spontaneous or cold-induced splitting was by-passed by using a surgical technique. Disconnecting the ommatidial nerves between either compound eye and its associated optic lobe, two to four days after the penultimate moult, proved to be a good tool for further investigation of split rhythms. In LD, 17 of 25 crickets subjected to such lesioning exhibited a stridulation pattern similar to unoperated insects, i.e., the rhythms were entrained and most of the activity occurred during dark (Fig. 2a, days 1–14; Fig. 2c, d, days 1–15). An example of eight exceptional actograms (about 30%) is presented in Fig. 2b. In LD these crickets showed a free-running rhythm shorter than 24 h as well as entrained activity. Independent of this variability in LD unilaterally blinded crickets usually produced a period shorter than 24 h ($\tau_s$) under LL in addition to a free-running rhythm longer than 24 h ($\tau_1$). The above mentioned rhythms in LD which were shorter than 24 h persisted in LL as $\tau_s$-component (Fig. 2b). The spectrum of different patterns is illustrated by examples given in Fig. 2a–d. The variability in the appearance of

**Fig. 2a–d.** Stridulation patterns of male *T. commodus* following unilateral intersection of the ommatidial nerves during the early last larval instar (16–26 days before day 1). Light programs (LD and subsequent LL) were as indicated above the double plotted actograms, the temperature was constant throughout (27±1 °C). *Abscissa:* twice the time of day; *ordinate:* days of experiment. **a** Entrainment during LD (days 1–14); in subsequent LL the rhythm splits into two uninterrupted components (days 14–41). One ($\tau_s$) is shorter, the other ($\tau_1$) is longer than 24 h. The free-running periods in the onsets of activity are $\tau_s=23.7$ h (days 19–23, and 34–41), and $\tau_1=25.6$ h (days 22–31). The end-slopes of activity exhibit periods of $\tau_s=23.6$ h (days 23–27, and 39–41) and $\tau_1=25.4$ (days 15–25, and 32–38). *Inset:* interpretation of this pattern (see Discussion). On day 41 the optic lobe of the intact side was removed (LOBX). Following a gap of any stridulatory activity only the $\tau_s$-component came back, yet, the $\tau_1$-rhythm disappeared completely. The onset slope of the remaining component refers to a period $\tau_s=23.9$ h. When extrapolated back it connects close to the activity onsets of the entrained pattern. **b** A clear-cut free-running rhythm ($\tau_s=23.3$ h) appeared already in LD (days 1–16), and continued in subsequent LL (days 16–56). Additional activity occurring during the dark of LD, as well as the phase-relation between preceding zeitgeber and the origin of the free-running $\tau_1$-rhythm in LL indicate that the second activity component was entrained during LD. In LL both components criss-cross exhibiting overall periods of $\tau_s=23.5$ h and $\tau_1=25.5$ h. **c** Entrainment in LD (days 1–15); in subsequent LL (days 15–52) a continuous band of activity drifts across the 24-h day exhibiting an overall periodicity longer than 24 h. The slopes in the onset and end of activity change and phase shift 'spontaneously'. Periods in onset refer to 26.5 h (days 15–22), 23.6 h (days 23–27), 25.5 h (days 27–32), and 26.3 h (days 44–52). The values for the end slope are 24.9 h (days 15–17), 24.7 h (days 18–32), 23.3 h (days 35–38), 25.6 h (days 38–44), 23.3 h (days 45–48), and 25.3 h (days 48–51). In addition to these changes in $\tau$ the end-slope was phase delayed three times: by 2.5 h (days 17/18), by about 11 h (days 34/35), and by about 8.5 h (days 44/45). The variability in the pattern reveals an obvious regularity which is consistent with a $\tau_1$-rhythm ($>24$ h) and an additional component with a period shorter than 24 h ($\tau_s$). The latter appeared in the pattern only at times when both overlap; it is pointed out by *dotted lines* referring to a period of 23.3 h. **d** Entrainment in LD (days 1–15); in subsequent LL only one activity component with a period longer than 24 h is apparent (days 15–39). The amount of activity per circadian cycle increases (days 15–17, 24–28, and 36–39) and decreases (around day 19 and day 31) regularly. This is the result of a quite stable end-slope with a period $\tau_1=25.6$ h and pertinent onsets of activity, that seem to oscillate around a parallel line to the end-slope. When the optic lobe of the intact side was removed (day 40, LOBX), the $\tau_1$-rhythm disappeared and, instead, a period shorter than 24 h ($\tau_s=23.6$ h) came up (days 44–59). Its extrapolation (*dotted lines*) leads back to the entrained rhythm (day 15), indicating its origin. A decrease of $\tau_1$ in the onsets and a temporal increase of the amount of activity per cycle, that exist around day 25 and 37, coincide with the overlap of $\tau_1$-activity and $\tau_s$-extrapolation (c.p. **c**)

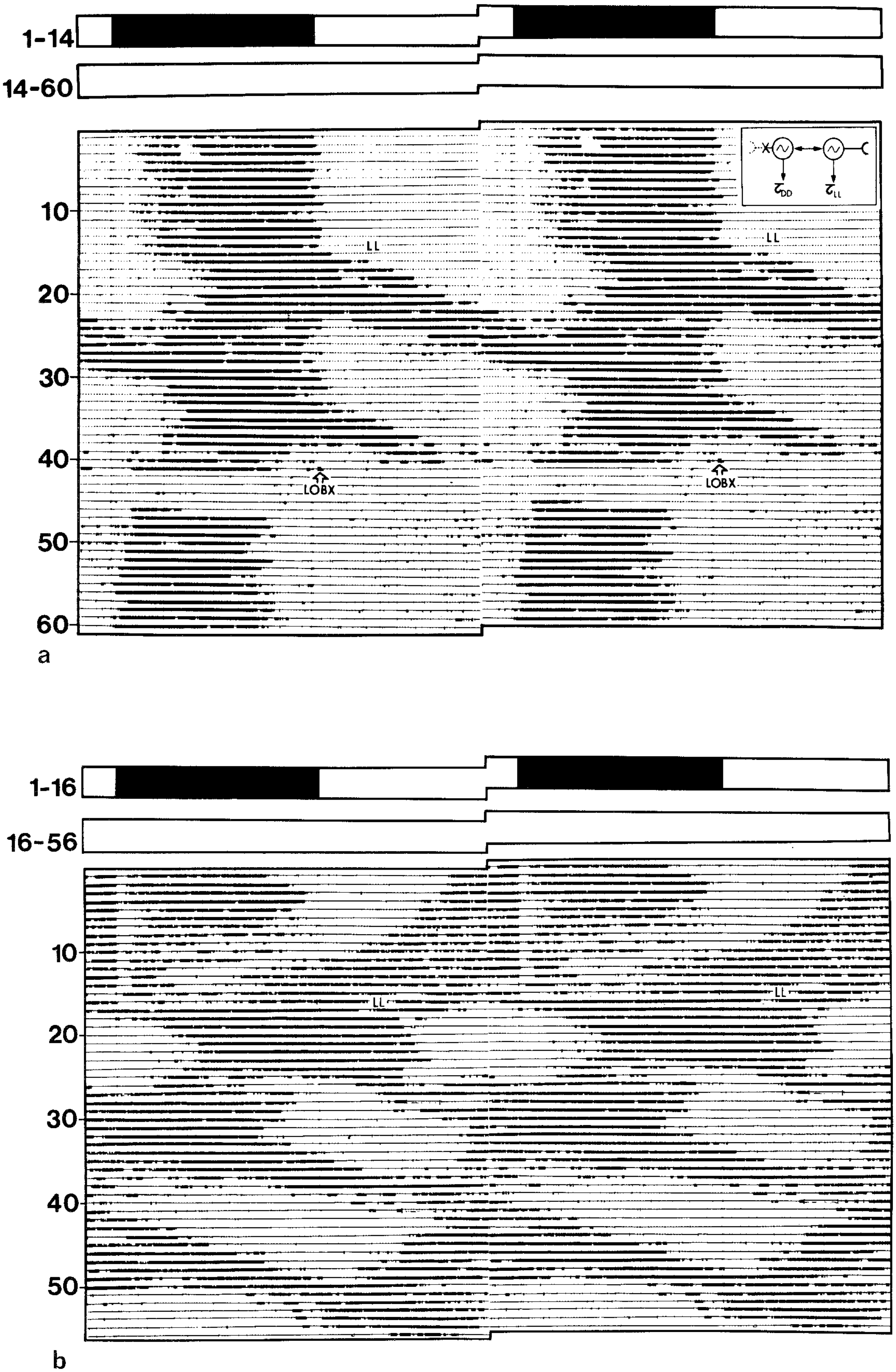
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Fig. 2a, b

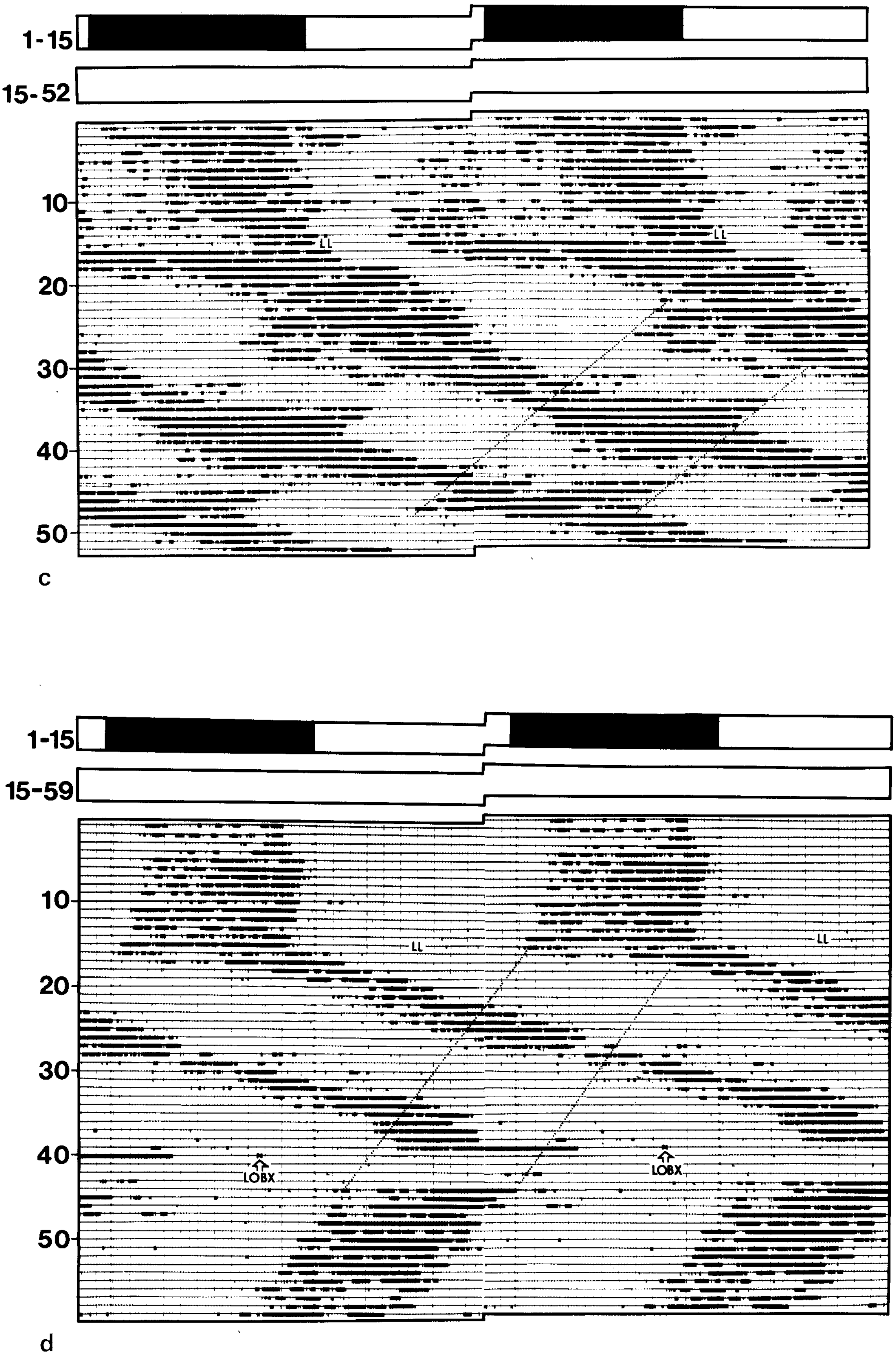
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Fig. 2c, d

both, $\tau_l$- and $\tau_s$-component ranged between continuous and interupted patterns (Fig. 2a–c). In two cases one component only was distinct, the appearance of which altered characteristically: the period of the rhythm changed repeatedly connected with an increase and decrease of the amount of activity for several cycles. In the actogram shown in Fig. 2d the end-slope of activity remained rather constant while the onset-slope varied.

At this point it is difficult to explain the spectrum of variability in the appearance of the activity components as illustrated in Fig. 2a–d. The side of blinding did not correlate with the pattern expressed. The distal cup-shaped part of the optic lobe which contains the neuropiles of lamina and medulla, was frequently found to be reduced in size on the disconnected side at the termination of the experiment. The reduction could not, however, be correlated with interrupted components in the activity pattern. Histological examination of the brains revealed, that in these cases the lamina was regularly reduced in size, sometimes the medulla, as well, but to a smaller degree. The lobula, the most proximal neuropile, which in *T. commodus* is located close to the midbrain has always been found of normal size.

Similar to the split rhythms with both periods longer than 24 h (Fig. 1a) surgically induced splitting was modified by unilateral lobectomy (Fig. 2a). Only one component reappeared after the operation. Lobectomy of the intact side always extinguished the $\tau_l$-component (longer than 24 h) while the $\tau_s$-component (shorter than 24 h) remained ($n=8$). The latter disappeared, however, when the optic lobe of the *blinded* side was removed instead ($n=2$). In a case where only a single period, longer than 24 h, was expressed (Fig. 2d), the optic lobe of the intact side was removed. Instead of the original rhythm, which disappeared after the operation, a new rhythm, shorter than 24 h appeared and its extrapolation backwards (dotted line) connected to the entrained activity in LD. Sham-operation did not interfere with the split pattern – *both* activity components reappeared.

Splitting induced by unilateral disconnection of the ommatidial nerves thus allows one to relate predictably either activity component to a particular side of the brain.

## Discussion

*Two Bilaterally Redundant Circadian Pacemakers.* Male *T. commodus* exhibit a circadian rhythm in their calling song activity (Loher 1972). Free-running periods are longer than 24 h in LL (30–50 lx, $\bar{\tau}_{LL}=25.3$ h) and shorter than 24 h in DD ($\bar{\tau}_{DD}=23.6$ h; Loher 1972). Disconnection of the ommatidial nerves between retina and optic lobe on *both* sides results in free-running patterns similar to DD. Rhythms are then independent of external light regimes, proving that the compound eyes are necessary for LD-entrainment (Loher 1972). Based on these results the findings reported here are interpreted as follows (see inset of Fig. 2a):

Split activity patterns induced surgically by unilateral disconnection of the ommatidial nerves, indicate – despite their variability (Fig. 2a–d) – two rather independent circadian pacemakers. One is cut off from its direct optical input, while the other receives information on external light regimes. Thus one finds a DD-rhythm ($\tau_s < 24$ h) in addition to an LL-rhythm ($\tau_l > 24$ h) in the same cricket under continuous light. These $\tau_s$- and $\tau_l$-values lie in the same range as the $\tau_{DD}$ and $\tau_{LL}$ of intact insects.

This interpretation is clearly compatible with actograms of the type shown in Fig. 2a and b. Yet, it can also explain such records as Fig. 2c, where at the first sight only one component is evident in the pattern. Its spontaneous phase-shifts and alterations in the free-running periods of onset and end of activity, however, can be deduced to an interrupted second component criss-crossing the first (dotted lines in Fig. 2c). The second component appeared only when it was closely overlapping the other, and hence masking it. The regularity in the occurrence of spontaneous phase shifts and $\tau$-changes in the continuous component also argues against an otherwise possible interpretation which would attribute such patterns to a single but very sloppy rhythm.

Even the two crickets that showed only one activity component per circadian cycle following unilateral blinding, confirm this interpretation on further examination. When the optic lobe of the intact side was removed (Fig. 2d, day 40) the LL-rhythm vanished and, instead, a DD-component appeared. Its extrapolation (dotted lines) leads back to the LD-entrained rhythm indicating its origin in LL. In addition, whenever the LL-rhythm overlapped with the extrapolation, the activity pattern widened due to changes in the period of the onset. This suggests that the overt rhythm can be influenced by both underlying components even though only one of them (in this case the LL-pacemaker) is the main source. Figure 2c illustrates this feature more clearly since there the DD-component is more pronounced.

Circadian stridulation patterns of *T. commodus* are permanently abolished when both optic lobes are removed whereas unimodal rhythms persist following unilateral lobectomy (Loher 1972). The average free-running periods for intact and unilateral lobectomized crickets are, however, significantly different (Wiedenmann, unpublished), indicating a change in the underlying oscillatory system (compare Page et al. 1977). The results presented here add to the evidence supporting a bilateral organization in the circadian control mechanism, and reveal again an important involvement of the optic lobes. The presence of both is obviously required for the occurrence of splitting. As in unilaterally blinded insects the intact side becomes associated with the LL-oscillation and the disconnected side with the DD-oscillation, it is possible to assign either activity component to the proper side of the brain. This opens up many opportunities for analyzing the circadian system of *T. commodus*.

The split rhythms with two periods both longer than 24 h (Fig. 1a, b) which appeared only in intact crickets, can be explained by extension of the present reasoning. Two pacemakers with different periods, each driving one activity component, drift apart. This is released by low temperature treatment (Fig. 1b) or by other unknown reasons (Fig. 1a). Since the experiments were carried out in LL and both compound eyes were intact, the free-running period of each component is expected to be longer than 24 h. Additional unilateral lobectomy (Fig. 1a, day 21, LOBX) has the same effect as in crickets with surgically induced splitting: only one activity component persisted while the other disappeared. Although one cannot predict which one will be extinguished, this experiment still verifies the bilateral organization in the underlying control mechanism.

*Possible Localization.* It is easy to deduce from the results discussed above that the circadian control mechanism of *T. commodus* is localized in the optic lobes. The fact, that in several crickets the blinded lobe was reduced in size seems to be crucial in this connection. However, this reduction did not correlate with irregularities in the split activity pattern. Moreover, the lobula, which in some other insect species obviously is the most important neuropil of the optic lobe concerning the control of circadian rhythms (Roberts 1974; Sokolove 1975; Fleissner 1982) was located outside the reduced distal part of the lobe. Nevertheless, one has to remark that the experiments reported here do not prove conclusively that the optic lobes are the loci of the circadian pacemakers. As has been stated in connection with other lesioning experiments which eliminated circadian rhythms in orthopteran insects (Nishiitsutsuji-Uwo and Pittendrigh 1968; Loher 1972; Roberts 1974; Sokolove 1975; Sokolove and Loher 1975; Page et al. 1977), one must be cautious. Such experiments do not exclude the possibility that at least part of the oscillatory system is located outside the lesioned part of the brain. Lobectomy may interrupt connections between such subunits in- and outside the lobes, or between pacemaker and executive organs of the overt rhythm, and thus lead to the deletion of the distinct pattern. Evidence for oscillatory circadian structures outside the optic lobes of *T. commodus* was given by Rence and Loher (1975); bilaterally lobectomized (and therefore arrhythmic) crickets were entrained by temperature cycles of 24 h but not of 30 h. Their interpretation, which is also compatible with findings reported here, was that master oscillators in the optic lobes normally drive subordinate centers in the midbrain; in the absence of the lobes these centers can be entrained otherwise.

*Mutually Coupled Pacemakers?* The two bilaterally distributed oscillators driving the circadian stridulation rhythm of *T. commodus* are, however, not independent of each other. Interactions have to be postulated, at least in unimodal rhythms. Otherwise, spontaneous splitting would occur more frequently under constant conditions. Even insects that simultaneously exhibited different free-running periods in the onset and end slope of unimodal rhythms, indicating two different pacemakers (Wiedenmann and Loher, in preparation), did not split into two distinct activity components. Moreover, in seven of those ten cases where splitting was induced by cold treatment the periods of *both* components were different from $\tau$ of the original rhythm. Such a change in oscillatory properties, in addition to the mere split, is a well-known phenomenon in systems of coupled pacemakers. It suggests mutual interactions between the subunits, which are altered during splitting.

In about 70% of all cases of surgically induced splitting both components started together after the LD/LL transition, indicating their continuing synchrony with LD (Fig. 2a, c, d). This must be due to a contralateral information transfer since one of the pacemakers was disconnected from its direct optical input. The synchrony can be explained by postulating that the underlying control units are weakly coupled oscillators. Self-sustained pacemakers are entrained by a driving oscillation only in a certain 'range of entrainment', outside of which they show relative coordination or their own frequency (Klotter 1960). Thus the DD-component is usually entrained to 24 h due to weak coupling with the LD-entrained contralateral pacemaker, and a relatively small difference in their periods. In LL, however, when the free-running period of the intact side deviates from 24 h, the difference between $\tau_{LL}$ and $\tau_{DD}$ exceeds the range of mutual entrainment and each rhythm exhibits its own period. In those eight actograms, examplified in Fig. 2b, the entrainment between the contralateral pacemakers failed already in LD, and a free-running DD-rhythm is expressed despite the zeitgeber. In subsequent LL a pattern typical for unilaterally blinded crickets appeared: in addition to a DD-rhythm, which connects to the previous one, a free-running component started with $\tau_{LL}$, clearly indicating its entrainment in LD.

The existence of two pacemakers driving split stridulation rhythms of *T. commodus* leads to explanations of unimodal patterns and their underlying control mechanism: (1) Interactions between the oscillators in constant conditions are usually sufficient to establish mutual entrainment. Small phase angle differences between the components are not evident in the activity pattern, due to their temporal overlap. Separate components appear only if coupling is reduced and the phase angle difference between them is great enough, i.e. the first finishes before the second begins. (2) Although either pacemaker may not always appear explicitely in the activity pattern, the regularity of the overt circadian rhythm can nonetheless be affected by both – acting jointly or in alteration. The behaviour expressed is therefore not necessarily a clear indicator of the state of the underlying compound pacemaker system. The daily onset and end of activity may refer to two components (i.e. pacemakers) which overlap scarcely or completely.

*Comparison with Other Insects.* In the cockroach *Leucophaea maderae* there is considerable evidence for symmetric circadian pacemakers (Page et al. 1977; Page 1978, 1981), which – according to most recent work – in all probability are located in the optic lobes (Page 1982). Aside from various arguments for a bilaterally redundant organization in the control mechanism, further similarities exist in circadian patterns of locomotion in *L. maderae* and of stridulation in *T. commodus*. Following exposure to low temperature pulses, rhythms of both species change repeatedly into patterns with two activity components per circadian cycle. In contrast to crickets, however, such splits appear in cockroaches only when cold pulses were given during certain phases of the cycle (Wiedenmann 1977); moreover, in *Leucophaea* both activity peaks show the same period for most of the time, and both react alike to phase shifting pulses (Wiedenmann 1980). These results suggest a single undivided control mechanism in cockroaches, but they can also derive from strongly coupled pacemakers which appear as one unit. If one assumes bilaterally distributed pacemakers in both, cockroaches and crickets, the two oscillatory units must be more strongly coupled in *Leucophaea* than in *Teleogryllus* (Wiedenmann and Allen, in preparation).

A bilateral organization of circadian control mechanisms has also been postulated for several insect species other than orthopterans (e.g. Köhler and Fleissner 1978; Mack 1981; Balkenohl and Weber 1981). The existence of circadian pacemakers in the optic lobe is proven for the beetle *Anthia* (Fleissner 1982). The occurrence of bilaterally redundant circadian pacemakers which are entrained via the compound eyes, and the involvement of the optic lobes in these pacemakers is therefore not an isolated case in *T. commodus*. Merely the interaction between the subunits and their expression in the overt rhythm may differ.

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