

CIRCADIAN ACTIVITY RHYTHM OF THE HOUSE FLY CONTINUES AFTER OPTIC TRACT SEVERANCE AND LOBECTOMY

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Abstract—Under constant conditions, locomotor activity in about 50% of 63 adult *Musca domestica* continued to be rhythmic after bilateral severance of optic tracts or bilateral lobectomy. Apparently, the optic lobes of *Musca* do not contain the oscillator for rhythmic control of locomotor activity as has been proposed for other insects. In 20% of the individuals, several circadian components of activity rhythms were found after operation indicating a role of the optic lobes in the coupling of oscillators. The remaining 30% of the flies with severed optic tracts appeared to be arrhythmic. Most of these flies had vacuolized tissue in the central brain. However, disruption of rhythmicity did not correlate with a common pattern of degeneration. Therefore no conclusions can be drawn as to the localization of the circadian control of locomotor activity in the brain. Flies showing an arrhythmic activity pattern could still be synchronized by LD cycles. Activity did not occur solely during the light period as is the case in controls; but was phase delayed by about 6 hr towards the dark period. Since all flies with severed optic tracts could be synchronized by LD cycles, *Musca domestica* must possess extraocular photoreceptors.

Key words—Activity, circadian rhythm, *Musca domestica*, optic lobe, insect brain, photoreceptor.

Introduction

Quite a number of studies of the physiology of insect circadian rhythms have focused on its anatomical localization (1, 2). Nishiitsutsuji-Uwo and Pittendrigh (3) presented evidence for the involvement of optic lobes of cockroaches (*Periplaneta americana* and *Leucophaea maderae*) in regulating the locomotor activity rhythm. Thus, bilateral ablation of the optic lobes or sectioning of the optic tracts caused arrhythmicity. The same operation, performed unilaterally, did not abolish the rhythms. These findings have been confirmed (4-6) and extended to other insect species (*Teleogryllus commodus*: 7, *Gryllus bimaculatus*: 8, *Carabus problematicus*: 9). The region within the optic lobes controlling the activity rhythm seems to be the lobula in the case of cockroaches (1) and the lamina/medulla region in the case of *Gryllus* (8). In the cockroach rhythmic activity depends on the structural connections between optic lobes: 4-8 wk after

implantation new neural connections between optic lobes and midbrain were established and the rhythm restored. The period length was imposed by the donor lobe (6). Regeneration of neural connections restores rhythmicity also in *Gryllus* (8).

Results of parabiosis experiments in cockroaches (*Periplaneta americana*) and *Acheta domestica* (10) indicate, however, that the locomotor activity rhythm can be driven via a hormonal pathway. Apparently the brain can release a diffusible factor capable of driving circadian activity rhythms. At least in *Acheta domestica* (11, 12) and in *Drosophila melanogaster* (13, 14) this factor seems to be produced rhythmically by neurosecretory cells of the brain. In silkmoths both eclosion and flight activity are rhythmic and controlled by a circadian pacemaker in the cerebral lobes (15, 16): ablation of the optic lobes did not disrupt rhythmicity.

Optic lobes seem to be dispensable for the expression of rhythmic locomotor activity in

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Abbreviations: LL, continuous illumination with white light; RR, continuous red light; LD, light dark cycle; α , activity time; ρ , rest time; $\alpha : \rho$, proportion of activity to rest time; τ , period length; min, minute (s); d, day(s); wk, week (s).

Drosophila (17), in the mosquito *Culex pipiens* (18), and, as shown in the following, in house flies.

Materials and Methods

Experiments were performed on the house fly *Musca domestica*. The insects were reared in LD 16:8 at 25°C. The adult flies taken for the experiments were not older than 10 d.

Locomotor activity was monitored separately in each fly under RR at $20 \pm 1^\circ\text{C}$ (19). Recordings were carried out for about 10 d before treatment. All arrhythmic animals were discarded; only those with clear circadian activity rhythms were used as experimental or control animals.

Experimental groups consisted of insects in which either both optic tracts were severed or both optic lobes were removed at different circadian times. During the operation which lasted usually 5–10 min, insects were exposed to cold fiber glass light of 6×10^4 lx. Controls were either sham operated or received illumination alone.

In order to transect the optic tracts the insect was mounted in soft plasticine and illuminated. The head was bent forward to facilitate access to the optic tracts through the rear part of the head capsule. The cuts were performed with a fine scalpel using two cuticular landmarks on the surface of the rear head capsule (Figure 1). Lobectomy was performed after previously severing the optic tracts. Following this, the optic lobes were separated from the eye cup with a needle and then removed using fine forceps. In control flies all steps of operation were performed prior to severing the optic tracts. The wounds were sealed with wax (melting point 30°C).

After surgical treatment the activity of the insect was monitored for about two wk under RR and in some cases for a further wk under LD 12:12 conditions. In order to see whether blind, but unoperated flies can be synchronized by a LD cycle, the complex eyes of some flies were covered with black wax and illuminated with different intensities.

Activity records were analysed using different methods of time series analysis such as

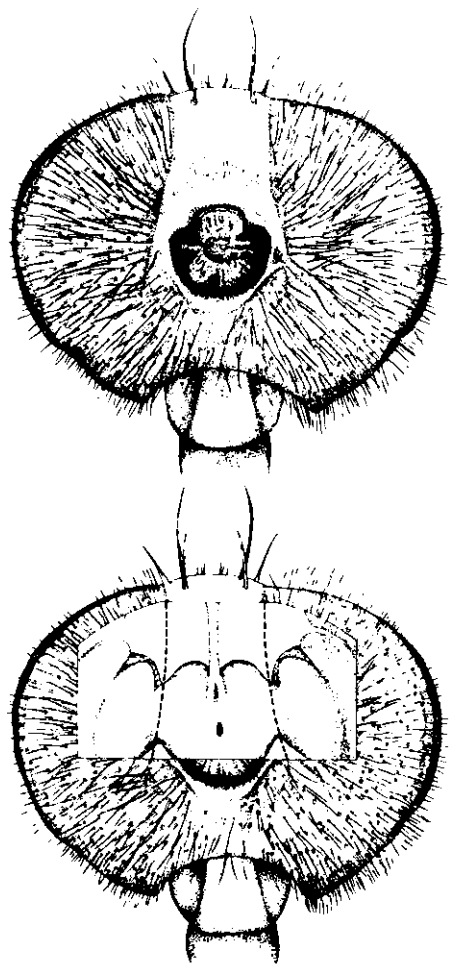


Figure 1. Caudal view of head of adult *Musca domestica*. (a) To sever the optic tracts, a landmark between the central and peripheral area of the dorsal part was used. (b) The position of the brain and the path of the cut is shown through a window in the lower drawing. Modified after Straussfeld (36).

periodogram, signal average, and complex demodulation (20). Activity levels were determined from the Esterline Angus recordings by assigning an activity score to each 20 min interval in units ranging from 0 (no activity) to 4 (hyperactive). To test the significance of the results, nonparametric tests such as χ^2 test, Mann-Whitney-U-test, Wilcoxon test and Spearman rank correlation test were used (21).

The position of optic tract severance was determined by histological examination. After

postoperative locomotor activity the head was cut off and mounted upside down on a paraffin block. The caudal wall of the head capsule was removed taking care not to destroy any major nerve connections. The exposed brain was stained *in situ* for 10 min with Chicago blue dissolved in insect saline (22). The stained brain was rinsed with fresh saline and then fixed for 3 hr in Carnoy fluid. After dehydration the head was embedded in paraffin, sectioned (10 μ m), cleared with xylene and embedded in Entellan (Merck) prior to microscopical examination.

Results

(1) Characteristics of locomotor activity rhythm

In order to characterize the locomotor activity rhythm of *Musca domestica*, flies were recorded under LD 12 : 12, under RR and LL (1.8 lx). Under all recording conditions no difference was found in the circadian behaviour of males and females. Under LD conditions activity was restricted to the light span, starting immediately after lights on and stopping immediately after lights off. The highest amount of activity occurred at the end of the light span. The majority of flies (85%) showed unimodal activity patterns under LD and shortened their activity

time when transferred to free running conditions. Some flies (15%) showed a bimodal distribution with some activity after lights on and a more pronounced peak before lights went off. After transfer to RR, only the second component persisted.

During the first few d of RR 70% of the flies had a period length of about 23 hr, which then increased to about 25 hr without further change. About 75% of all flies monitored showed clear free running rhythms. The remaining 25% of the flies were either arrhythmic or had a complex rhythm with more than one component as revealed by periodogram analysis. Under LL conditions (1.8 lx) 77% of the flies were hyperactive and appeared to be arrhythmic.

(2) Effect of optic tract severance on activity rhythm

Most of the flies with transected optic tracts had less defined activity rhythms after operation compared with the preoperative behaviour. This was due to increased mean activity levels (in 75% of all operated flies) and lack of clear rest time (ρ) (Table 1). Changes were also observed in the activity patterns of operated flies. Three major types could be distinguished (Table 2): about half of the operated flies showed a clear circadian

Table 1. Activity levels, changes in activity level, ratio of activity time (α) to rest time (ρ) of *Musca domestica* before and after treatment. Mean values and standard errors. Number of cases in parentheses

	Activity level	Mean increase of activity level	$\alpha : \rho$
Preoperative	1.64 \pm 0.08 (53)		0.774 \pm 0.094 (20)
Postoperative	2.31 \pm 0.10 (53)	0.70 \pm 0.12* (53)	2.428 \pm 0.407 (20)
Before sham	1.53 \pm 0.10 (20)		0.708 \pm 0.096 (20)
After sham	1.62 \pm 0.17 (20)	0.09 \pm 0.16 (20)	0.721 \pm 0.060 (20)
Before light pulse	1.61 \pm 0.16 (15)		0.727 \pm 0.045 (15)
After light pulse	1.75 \pm 0.17 (15)	0.27 \pm 0.13 (15)	0.940 \pm 0.148 (15)

*Significant at 0.005 (Wilcoxon test).

Table 2. Activity patterns of *Musca domestica* after bilateral severance of optic tracts, lobectomy, sham operation and light pulse treatment only. Except for the first column all figures are percentages of each group (rows)

	Number of cases	Rhythmic pattern	Complex rhythmicity	Arrhythmic pattern
Optic tracts severed	53	52.8	18.9	28.3
Lobectomy	10	50.0	30.0	20.0
Sham	20	100.0	0.0	0.0
Light pulse	15	100.0	0.0	0.0

rhythmicity after bilateral optic tract transection (example: Figure 2) or after lobectomy (see Figure 7a). About 30% of the insects with both optic tracts severed and 20% of the lobectomized flies were arrhythmic (example see Figure 7c). Complex rhythmicity was found in 20% of the flies with transected optic tracts (example see Figure 7b) and in 30% of lobectomized animals. All sham operated and light pulsed insects were rhythmic.

(3) Histological examinations.

Of the 53 flies with severed optic tracts, 28 had complete cuts on both sides. In nine individuals transection of the optic tract was complete and at the proper position on one side, but on the contralateral side the cut was more distal than anticipated. The remaining 16 flies revealed cuts through optic tracts or lobula complexes on one

side and incomplete severance of optic tracts contralaterally (compare Figure 5a).

Neural connections between brain and optic lobes were not established in any of the flies examined histologically. In the majority of the operated flies the optic lobes had degenerated as shown by a large number of small vacuoles (Figures 3 and 4). These degenerated regions usually stained much deeper than the rest of the brain tissue (Figures 3a, 3b, 4a). Areas of degeneration were found to varying extents even in distal parts of the central brain (Figures 3c, 4b, 4d) but never in mushroom bodies, central body and in antennal lobes. Degeneration in the brain was also observed in some flies with cuts performed through the lobula complexes (Figure 3b) and in individuals with incomplete severance of optic tracts (Figure 3c).

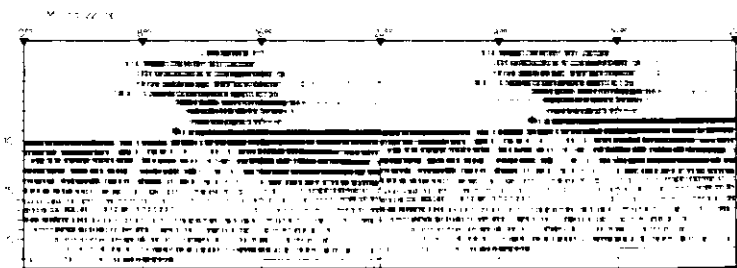


Figure 2. Actogram of an adult *Musca domestica* under conditions of continuous weak red light. On day 9 both optic lobes were transected (*) as confirmed histologically. The activity rhythm continues with a longer period, increased activity level, and an increased ratio of activity to rest time.



Figure 3. Examples for vacuolized areas (considered to represent degenerated tissue) in the optic lobes (a, b) and the distal part of the central brain (c). (a) Cut between brain and lobula complex with deeply stained optic lobe. Animal remained rhythmic. (b) Cut through lobula complex. Deeply stained optic lobe degenerated on both sides of transection. Animal remained rhythmic. (c) Incomplete cut with degenerations in parts of the optic lobe and distal part of the brain. Animal showed arrhythmic behaviour after operation.

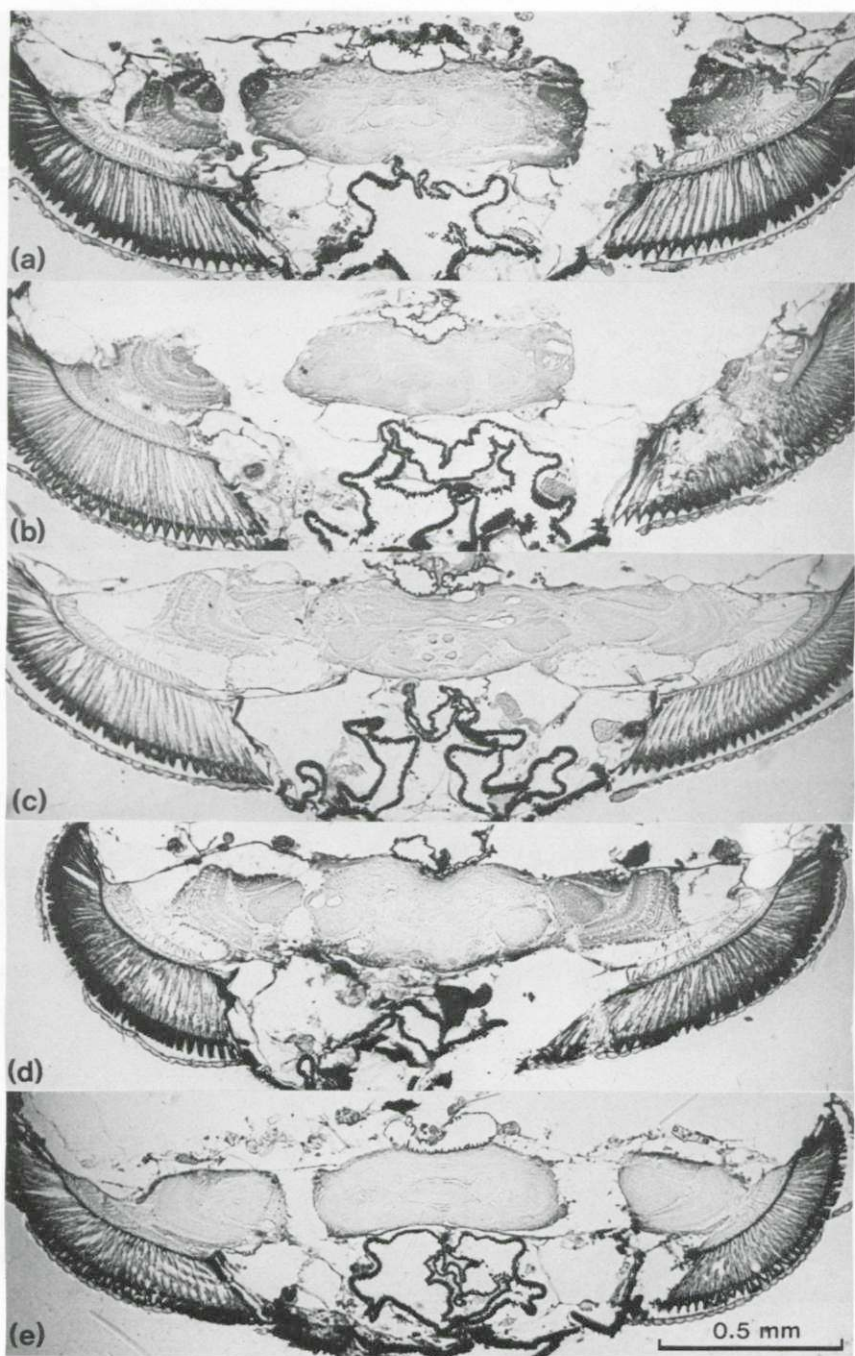


Figure 4. Examples of histological examinations of optic lobes and central brain in *Musca domestica* illustrating the relationship between location of degenerated tissue and rhythmic behaviour.

- (a) Complete cut, optic lobes degenerated, central brain normal appearance. Animal remained rhythmic.
 (b) Lobectomy. Parts of the optic lobe still present, but degenerated. No vacuolized areas in brain. Animal remained rhythmic.
 (c) A few large vacuoles close to the great commissure. Animal remained rhythmic.
 (d) Complete cuts on both sides, optic lobes degenerated, extensive degenerated areas in ventrolateral and posterior protocerebrum. Postoperative behaviour arrhythmic.
 (e) Control fly. Staining and fixation immediately after the cut. No vacuolized areas neither in the brain nor in the optic lobes.

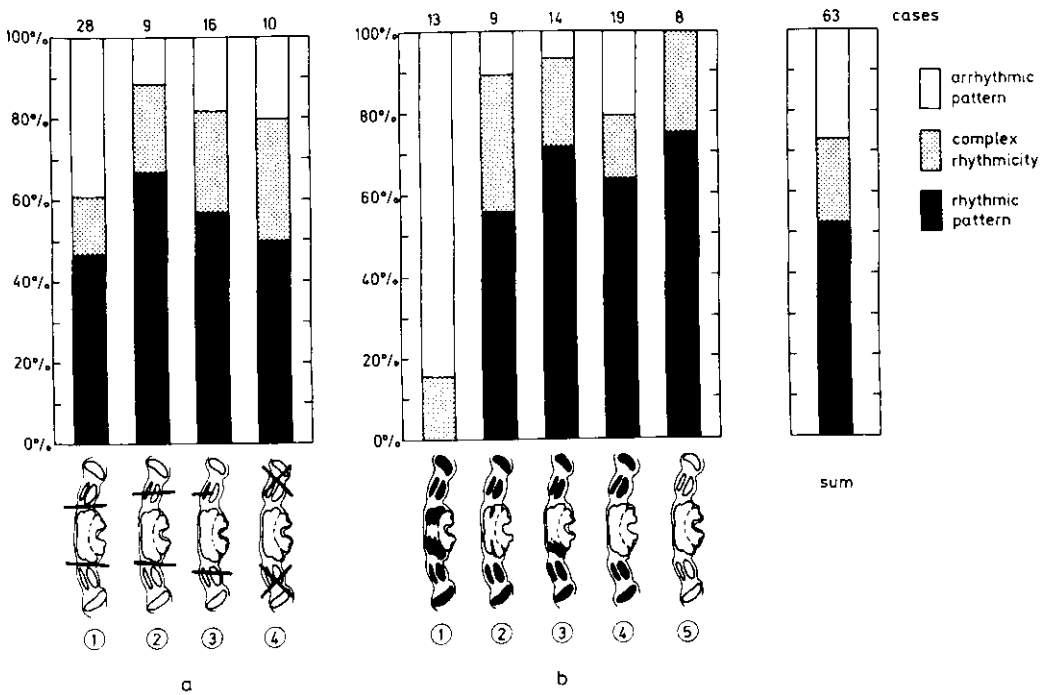


Figure 5. (a) Quantitative relationship between location of optic tract severance (shown below the graph, group 1 to 3 cuts, 4 lobectomy) and kind of locomotor pattern (as explained on the right margin). Number of cases at the top of graph, results in percentage of cases. (b) Quantitative relationship between degree of degeneration (shown below the graph, black areas representing tissue with vacuoles) and kind of locomotor pattern (as explained on the right margin). Last histogram shows sum of (b).

Among lobectomized animals, histological examination revealed that in no case ablation was complete. Small portions of the lamina and sometimes fragments of the lobula complex remained. However, these fragments had no connections with the brain and were completely disorganized (Figure 4b).

(4) Correlation between postoperative rhythms and histological findings.

After recording the locomotor activity of operated flies their brains were carefully examined histologically in order to establish the position of the cut and the degree of degeneration in the brain. No correlation was found between the position of optic tract severance and the percentage of flies exhibiting rhythmicity, complex rhythmicity or arrhythmicity (Figure 5a). However, these types of postoperative behaviour were correlated with the presence of vacuolized regions in the central

brain (Figure 5b): The percentage of arrhythmic flies was significantly higher in group 1 (Figure 5b) with vacuolized regions in both lateral parts of the brain as compared to the other groups. All flies lacking vacuolized regions (group 5, Figure 5b) were rhythmic. Of all arrhythmic flies 65% showed such degenerations within the brain. 30% of the arrhythmic flies had no such degenerated areas within the brain. However, these insects were extremely hyperactive (Figure 7d) which makes the detection of rhythmicity in the actograms difficult.

Some flies with rhythmic postoperative behaviour had also vacuolized areas within the brain. However, these were minor as compared to those of arrhythmic flies and never occurred simultaneously on both sides of the brain within ventrolateral and posterior parts of the protocerebrum. Vacuolized areas were mainly located between these two parts of the brain in the rhythmic flies (Figure 4c). In the case of flies

exhibiting complex postoperative rhythmicity no correlation was found between degenerations and its localization within the brain.

(5) *Characteristic of postoperative rhythms*

After severing the optic tracts 25 of the rhythmic flies were recorded long enough to

allow the precise analysis of their postoperative rhythms. This included determination and comparison of preoperative and postoperative period length (Table 3) and phase of the rhythm. Almost all flies lengthened period after the operation. This was, however, also found in flies which were sham operated or illuminated only.

Table 3. Changes in period length of locomotor activity rhythm in *Musca domestica* after bilateral severance of optic tracts, bilobectomy, sham operation and light pulse treatment only. Period length determined with periodogram analysis, peaks above 95% confidence limit. Mean and standard errors given

	Number of cases	Before treatment (hr)	After treatment (hr)	Difference $\Delta \tau$ (hr)
Optic tracts severed	20	24.9 \pm 0.16	25.6 \pm 0.14	0.66 \pm 0.11
Lobectomy	3	24.8 \pm 0.48	26.0 \pm 0.45	1.23 \pm 0.07
Sham	20	25.3 \pm 0.18	25.6 \pm 0.10	0.31 \pm 0.13
Light pulse	15	25.0 \pm 0.23	25.3 \pm 0.23	0.32 \pm 0.05

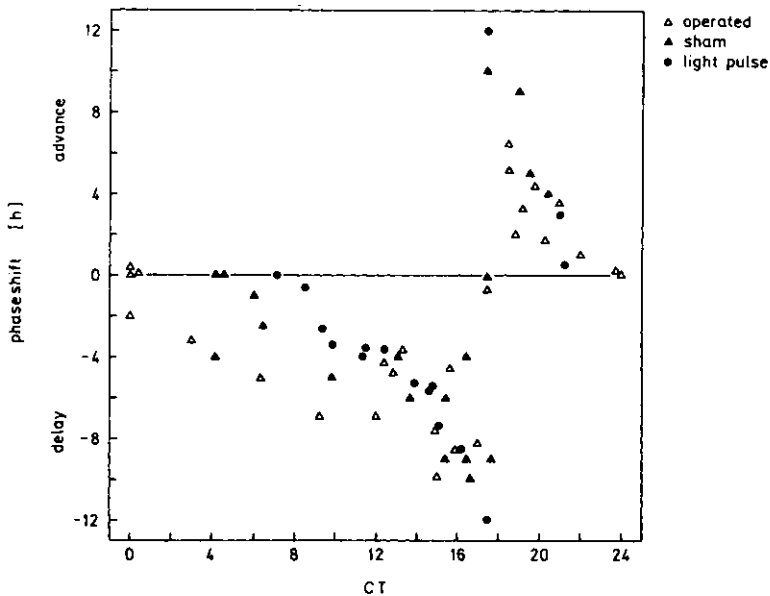


Figure 6. Phase response after 5 min white light (6×10^4 lx) illumination at different circadian times (CT 0 is onset of activity). Operated (optic tract severed or lobectomy), sham operated and unoperated cases in different symbols.

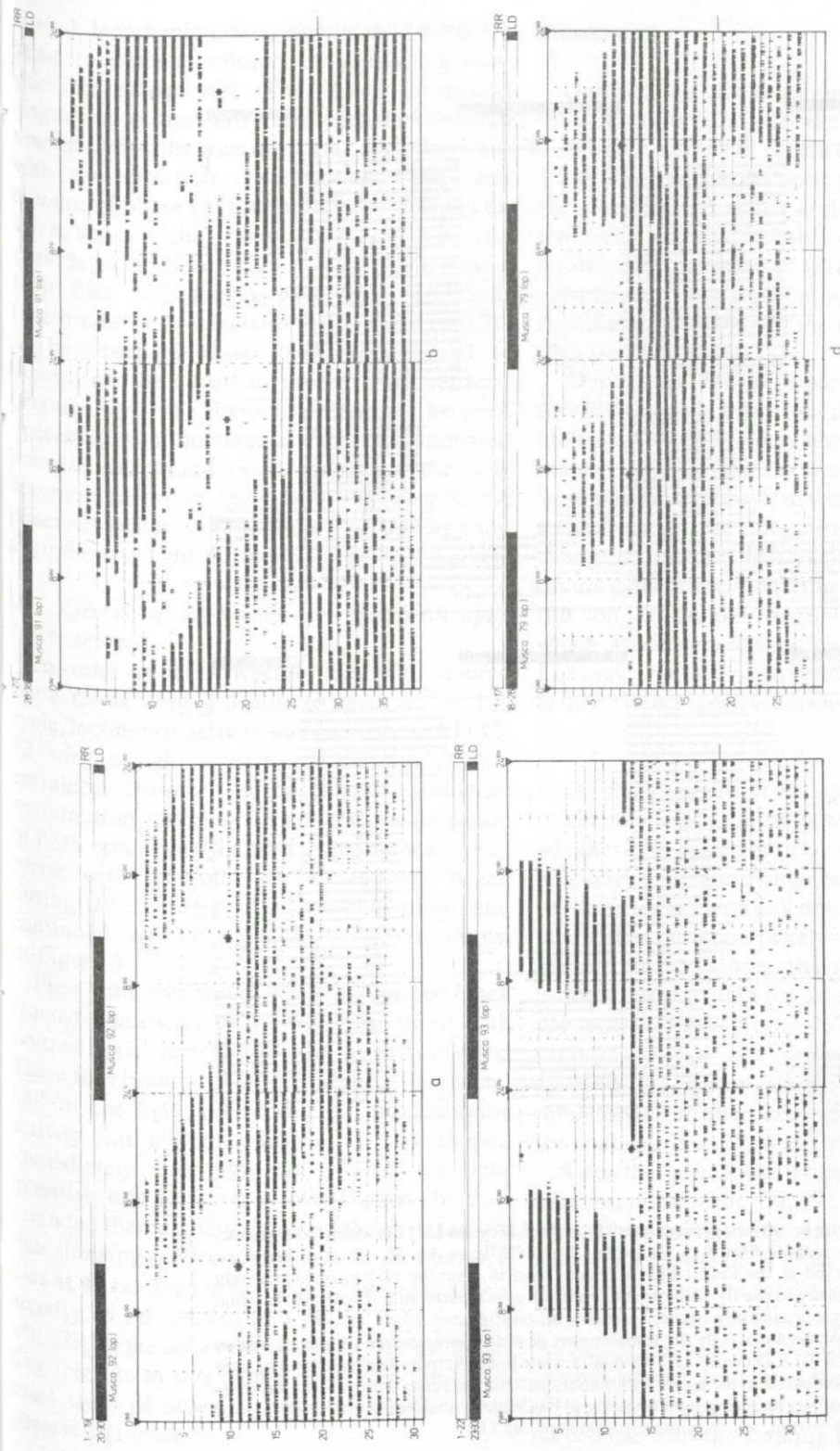


Figure 7. Pre- and postoperative activity pattern of individual flies under constant conditions of continuous weak red light (days shown in upper bar at top of actograms) and (postoperatively) under Ld 12:12 light-dark cycles (position and days shown in lower bar at top of actograms). Time and type of operation indicated by * (optic tract severed) and ⊕ (bilectomy). (a) Complex rhythmic pattern after lobectomy (⊕) on day 11. Synchronization by a LD cycle from day 20 onward with activity onset at middle of light period and end of activity after midnight. (b) Complex rhythmic pattern after severance of optic tracts (⊕) on day 18. Synchronization by a LD cycle from day 28 onward with phase lag of about 4 hr and extension of activity far into the dark period. (c) Arrhythmic activity pattern after optic tract severance (*) on day 14. Synchronization by a LD cycle from day 23 onward with phase delayed activity onset. (d) Hyperactivity after bilectomy (⊕) on day 9. Synchronization by a LD cycle from day 18 onward with phase delayed activity time.

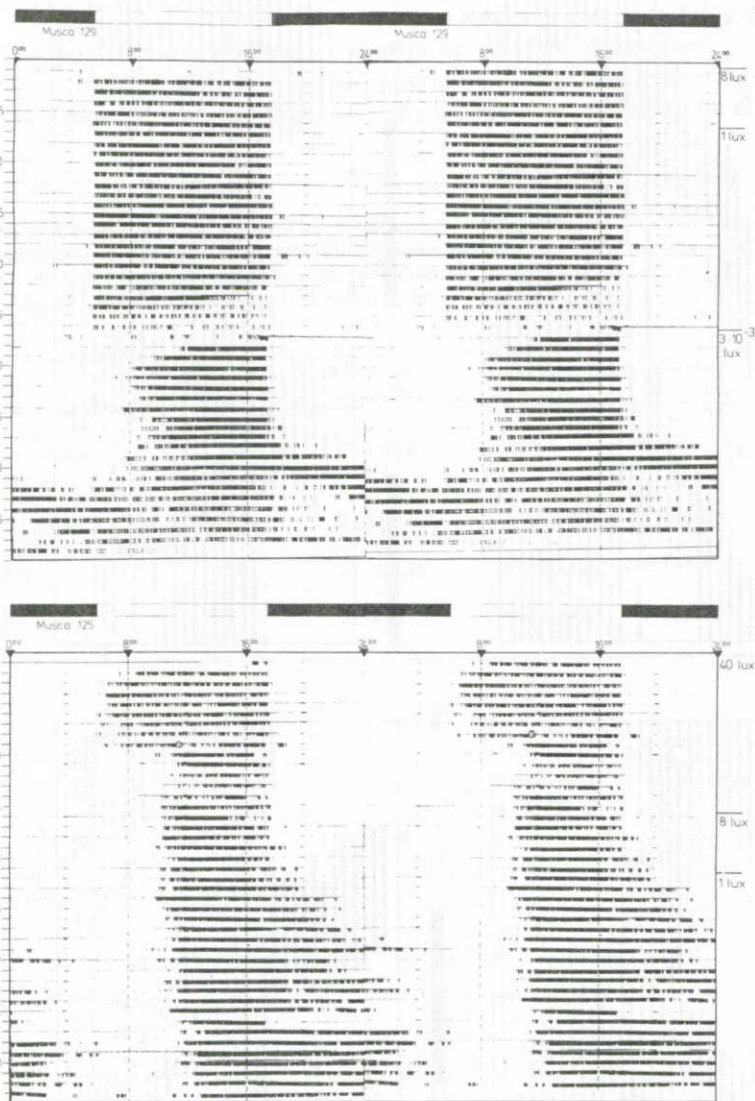


Figure 8. (a) Actogram of intact fly under LD cycles 12:12 in which light intensity was reduced from 8 to 1 lx on day 26. Onset of activity is delayed at the lowest light intensity used in a similar way as at higher light intensity in the fly with covered complex eyes illustrated in Figure 8b. Internal desynchronization occurs from day 38 onward. (b) Actogram of a fly the complex eyes of which were covered with black wax on day 9 (0). LD cycle 12:12 with 40 lx white light. Activity begins several hr later as compared to the intact fly's activity pattern, and ends with onset of darkness as before. Reducing light intensity to 8 lx leads to a widening of activity into the dark period, which is more pronounced at 1 lx.

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Period lengthening was significant ($\alpha = 0.01$). Most treatments induced in addition a considerable phase shift. The change in period length was neither correlated with the circadian time at which the treatment was performed nor with the direction (delay or advance) and amount of phase shift induced by the treatment. There was a slight tendency in flies with shorter periods to increase period postoperatively more than flies with longer postoperative periods (Spearman rank correlation coefficient $r_s = 0.5$).

The observed phase shifts were found to depend on the circadian time of the treatment (Figure 6). No obvious difference between operated, sham operated and solely illuminated animals was found. Since light was the only common factor in these three treatments, the observed phase shifts are light responses (and Figure 6 is a light pulse phase response curve).

(6) *Effects of LD conditions flies with optic tracts severed.*

In order to check whether flies with severed optic tracts were still able to react to the LD cycle, locomotor activity was recorded in LD 12:12 with a light intensity of 40 lx. All flies entrained. However, in flies whose histological examination revealed a complete disconnection of both optic lobes the activity period was out of phase with the light period. Activity began during the second part of the light phase and continued until midnight. Examples are shown in Figure 7.

Flies with eyes which had been painted black reacted somewhat differently than those with severed optic tracts at the light intensity of 40 lx. These flies became also active during the second half of the light period but did not continue activity until midnight. Instead activity stopped immediately after lights off. When the light intensity was lowered to 1 lx (Figure 8b) they extended their activity time into the dark period, thus showing a behaviour similar to the operated flies at 40 lx. Even intact flies often shifted their activity to the second half of the light period, when light intensity was reduced to 10^{-3} lx. Some flies free ran at this light intensity and in a few cases signs of internal desynchronization were observed (Figure 8a).

Discussion

Our findings can be discussed with respect to three questions: (1) Is the clock driving the locomotor activity of *Musca* (and probably other *Diptera*) located in the optic lobes as seems to be the case in cockroaches and crickets? Since this question has most probably to be answered with no, the next question is: (2) What is the role of optic lobes in the circadian system of house flies? And finally: (3) How is the circadian system of flies synchronized?

Throughout the discussion it is necessary to consider the possibility that the circadian system of *Musca* is composed of several oscillators. The main arguments for this were the occurrence of 'complex rhythmicity', in which several rhythms were found in an actogram, the spontaneous change in period length of rhythmic flies, which occurs often some days after transfer to the free run conditions, and internal desynchronization which was observed in a few flies under a weak LD cycle. Similar observations have been made in other insect species (23) including flies (17, 24).

Is the clock located in the optic lobe?

The main goal of this work was to clarify whether the optic lobes of the house fly were necessary for maintaining the locomotor activity rhythm. This is certainly not the case. Since 50% of the flies with completely severed optic tracts showed clear postoperative rhythmic activity, it is rather unlikely that the pacemaker is located in the optic lobes. At least, the expression of rhythmicity can not depend entirely on the existence of neural connections between the optic lobes and the midbrain as was postulated for cockroaches (25) and *Gryllus* (8).

Since the optic lobes of the house fly contain neurosecretory cells (26, 27), a hormonal factor controlling rhythmicity can not be ruled out by disconnecting the optic lobes from the brain. We performed therefore lobectomy in rhythmic flies and still found rhythmicity in 50% of them. Although fragments of optic lobe tissue were still present after lobectomy, they were completely disorganized and no neurosecretory cells were detectable with the staining methods used here.

The same was found in most of the flies with optic tracts severed.

It seems therefore that the pacemaker is located in the midbrain of the fly. In order to localize it more precisely we tried to correlate the postoperative behaviour of the flies with the histological appearance of their brains. We found that most arrhythmic flies had vacuolized areas in the brain after severance of both optic tracts. However, the exact location for the pacemaker could not be identified, because degenerations were located in different parts of the brain and varied from fly to fly. The observed degenerations within the brain might be due rather to destruction of the tracheal system supplying oxygen than to the severance of the optic tracts. This could explain the fact that in some flies with incomplete optic tract severance and sometimes in sham operated flies vacuolized areas were found in the optic lobes and in the midbrain. Damage of tracheal system might also be the main factor preventing regeneration of the severed optic tracts of flies. Regeneration of the connection between the optic lobes and the brain has been observed in the case of cockroaches (25) and *Gryllus* (8).

To ascertain that the fixing and staining procedures were not the cause of the observed vacuolisation, the same procedures were applied to brains immediately after optic tract severance. Neither in the brain nor in the optic lobes could any degeneration areas be detected in these cases (fig. 4e). Our results thus suggest that the optic lobes of the house fly are not necessary for maintaining the circadian rhythm of locomotor activity. This is in accordance with findings in the fruit fly in which mutations reducing the optic lobes in size to about 5% did not abolish the locomotor activity rhythm (28) and with recent findings of Kasai and Chiba (18) in *Culex pipiens*.

Role of the optic lobes in the circadian system of house flies

Although the optic lobes of house flies are not the site of the clock, it seems that they play a role in the oscillatory system of this insect. This conclusion is based on the postoperative behaviour of lobectomized flies and flies with optic tracts severed showing three major

changes: (1) appearance of complex rhythmicity, (2) lengthening of the period of the rhythm and (3) increase in activity level.

Appearance of complex rhythmicity: As mentioned already, a certain percentage of the operated flies exhibit a complex activity pattern composed of different rhythms with differing period lengths (periodogram analysis). In *Drosophila* mutants with reduced optic lobes the occurrence of such complex activity patterns is more frequent than in the wild strain (17, 18). However, in *Drosophila* complex rhythmicity usually consisted of two components with period lengths of about 22 and 25 hr. There was not much interindividual variation in these periods. House flies with two or four components however, lacked the regularity of period lengths of the *Drosophila* mutants. Nevertheless the occurrence of complex rhythmicities does support the view that more than one oscillator is involved in the expression of the overt rhythm of locomotor activity and that the optic lobes play a role in the mutual coupling (17).

Lengthening of the period of the rhythm: period lengthening was observed in all groups postoperatively. The period lengthening in lobectomized flies was almost twice as large (+1.23 hr) as in animals with severed optic tracts (+0.66 hr), and this was twice as large as after sham operation (+0.31 hr). There seems therefore to be a slight correlation between the degree of damage to the optic lobes and period lengthening. However, these differences were not statistically significant among each other. Even the light pulsed control flies lengthened their period (+0.32 hr).

Aschoff (23) reported period changes in vertebrates after light pulses. Period was lengthened by light pulses causing phase delays and shortened by light pulses causing phase advances. In the case of the house fly, period was lengthened by both phase advancing and delaying light pulses. Page *et al.* (30) found period lengthening for the locomotor activity rhythm in cockroaches after removal of one optic lobe. They explained this finding by assuming that circadian pacemakers were located in both lobulae and mutually coupled. In the coupled state the period length of the system

was thought to be shorter as compared to each single oscillator. The optic lobes might play also a role with respect to the coupling of oscillators located in the central brain as has been discussed in *Drosophila* mutants with strongly reduced optic lobes in regard to the lengthened period of locomotor activity rhythms. Whether and in which way optic lobes influence the coupling of oscillators in *Musca* need further studies.

Increase in activity level: an increase in activity level has been observed in several insect species after different surgical treatment (3, 31, 32). In the case of crickets (32, 33) an increased level of locomotor activity seems to be due to a disturbance in the functioning of neurosecretory cells. Neurosecretion liberated rhythmically from the brain has an inhibitory effect on the locomotor activity of the insect (33, 12). It is possible, that in house flies severance of the optic tracts causes a disturbance in the normal function of the neurosecretory cells. This possibility is now under investigation.

Photoreception and entrainment

Contrary to the situation in *Drosophila pseudoobscura* (34) the circadian rhythm of the locomotor activity of house flies seems to be quite sensitive to light. Substantial phase shifts of up to 12 hr were found after a single exposure to 5 min of white light. A tentative phase response curve is shown in Figure 6, but needs further work. Both, phase advances and delays were found.

Under LD conditions flies are entrained and active during the light period. Some flies show a bimodal activity as already observed by Parker (35) and, for *Drosophila pseudoobscura*, by Mack (34). Painting the complex eyes black suppresses activity in the first part of the light period (Figure 8b) and in cases of bimodality the first activity peak is missing. In any case flies with covered compound eyes as well as operated flies were still synchronized by LD cycles suggesting that the compound eyes are not the decisive photoreceptors for the oscillatory system controlling activity. However, the compound eyes seem to be responsible for the occurrence of the activity peak immediately after lights on.

Operated flies began activity at about midday

as did flies with eyes painted black, but extended activity into the dark period. Their activity time was therefore much longer than that of flies with black painted complex eyes. This increase in activity time might have been caused by a weakened coupling of normally more tightly coupled oscillators. A tight coupling results in a distinct and narrow band of activity. If the coupling were loosened the activity pattern would lose its distinct onset and end and widen. If coupling were further decreased, several rhythms might occur. Finally, with even less coupling, arrhythmicity would occur. A widening of the activity pattern was observed in some of the flies with covered eyes if light intensity was reduced (Figure 8b). Under these circumstances, the Zeitgeber is apparently not strong enough to synchronize all components of the oscillatory system. Some flies with covered complex eyes and even some normal flies under very low light intensities showed internal desynchronization (Figure 8a).

Leaving speculation aside, our most important result is the fact that even arrhythmic and hyperactive flies can be synchronized to LD cycles. This is probably not a direct response to light, but indicates the participation of an endogenous clock. Otherwise we would expect activity to begin immediately after light on. If this reasoning is correct, even overt arrhythmic flies would possess a clock. However, its amplitude was too weak to maintain a clear rhythm under continuous RR. Or, alternatively, the internal coupling between the oscillators was not strong enough to bring about a distinct activity pattern in RR or DD. An external Zeitgeber, however, was able to synchronize the oscillators.

The photoreceptor for synchronization of blind flies is not yet known. We are planning to determine the action spectrum of phase shifting light pulses in blind house flies and blind mutants of *Drosophila*.

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