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Regeneration of the optic tracts and circadian pacemaker activity in the cockroach *Leucophaea maderae*

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Summary. Recovery of the circadian rhythm of locomotor activity after bilateral section of the optic tracts (OT) of the cockroach *Leucophaea maderae* was investigated. After OT section rhythmicity consistently reappeared in 3–5 weeks (29 ± 6.2 days, $n=22$) (Fig. 1), while removal of the optic lobes caused permanent (>100 days) arrythmicity ($n=13$) (Fig. 2A). Recovery of rhythmicity after OT section was likely due to regeneration since: (1) Histological examination showed structural regeneration had occurred (Fig. 3A). (2) Insertion of a glass barrier between the OL and midbrain prevented (>75 days, $n=6$) or slowed (46 ± 14.9 days, $n=3$) rhythm recovery (Fig. 2B). (3) Extracellular recording after optic tract section showed recovery of light evoked activity in the cervical connectives (Fig. 4) whose time course paralleled the recovery of behavioral rhythmicity (Fig. 5).

The freerunning period (τ) of the rhythm after regeneration was strongly correlated with τ before surgery ($r=0.87$) but was slightly longer ($\overline{\Delta\tau}=0.2 \pm 0.35$ h) (Fig. 6). Also the phase of the rhythm, projected back to the day of surgery, was correlated with preoperative phase ($r=0.61$) (Fig. 7). Exposure to light cycles the first 10 days after OT section shifted the phase of the subsequent rhythm (Fig. 8). These results suggest that an entrainable circadian oscillation persists in the optic lobes after OT section.

Introduction

Two major problems in understanding the regulation of physiological, metabolic, or behavioral processes by circadian systems in animals are (1) localization of the pacemaking oscillations that time the periodicity and (2) discovery of the mechanism by which the pacemaker is coupled to the process it controls. In the cockroach the finding that bilateral severence of the optic tracts abolished the circadian rhythm of locomotor activity led to the hypothesis that the circadian pacemaker was located in the optic lobes and controlled activity via neural connections between the optic lobes and midbrain structures of the protocerebrum (Nishiitsutsuji-Uwo and Pittendrigh 1968a).

Subsequent work has verified that optic tract section abolishes the activity rhythm in cockroaches for several weeks (e.g. Roberts 1974). However, it has recently been reported for the cockroach *Leucophaea maderae* that if the optic lobes are left in situ rhythmicity consistently returns (Page 1982). This observation had prompted a series of experiments in which optic lobes were transplanted between individuals whose activity rhythms had quite different freerunning periods. Several weeks after transplantation a persistent freerunning rhythm of activity was restored in the host animals and the period of the rhythm was near that exhibited by the donor animal prior to transplantation (Page 1982). This demonstration that the optic lobes determined the period of the activity rhythm, in conjunction with the results of earlier experiments utilizing localized low temperature pulses that showed the optic lobe also controlled the phase of the rhythm (Page 1981a), provided strong support for the notion that the optic lobes were the source of the pacemaking oscillation.

These results raise two other questions which are the focus of this paper. The first concerns the pathway between the optic lobe and midbrain structures that couples the pacemaker to activity. Following optic lobe transplantation the long time course of return of rhythmic locomotor activity and the observation that structural connections

Abbreviations: *CST* central standard time; *OT* optic tracts

were established between the donor optic lobe and the midbrain of the host indicated that the coupling pathway involved neural connections between the optic lobe and midbrain (Page 1982). However, the possibility that the optic lobes can drive the activity rhythm via a humoral pathway has not been ruled out. This alternative should be investigated, particularly in view of the results from parabiosis experiments in cockroaches (Cymborowski and Brady 1972) and brain transplantation experiments in fruit flies (Handler and Konopka 1979) and crickets (Cymborowski 1981) that suggest that at least in some insects the brain can periodically release a diffusable substance capable of driving an activity rhythm.

A second question raised by the discovery that locomotor rhythmicity returns following optic tract section is whether the optic lobe pacemaker continues its motion between the time it is surgically isolated from the midbrain and the time of the return of a rhythm in activity. Positive evidence for the persistence of a freerunning oscillation would strengthen the argument that the circadian pacemaker activity of the optic lobes is independent of midbrain structures.

Evidence presented here suggests that the oscillation does persist in the neurally isolated optic lobe, that it can be entrained by light, and that it regenerates neural connections with the midbrain which are necessary to drive the circadian rhythm of locomotor activity.

Materials and methods

Activity recording. All experiments were performed with adult male *Leucophaea maderae* obtained from laboratory colonies. As noted below some experiments involved animals raised in 22 h or 26 h light cycles (Page and Block 1980). Activity was monitored at constant temperature (25±0.5 °C) in Lucite running wheels whose rotation, caused by the moving insect, was sensed by a magnetic reed switch connected to an event recorder (Esterline-Angus). Experimental animals were housed in light-tight boxes. Light cycles were provided by a water-jacketed 4 W fluorescent bulb. Food and water were available ad libitum.

Data analysis. Daily event records of each individual were pasted one below the other in chronological order, photographed, and 'double-plotted' in the conventional manner. An example of the raw data is shown in Fig. 1. Estimates of freerunning period (τ) or phase were made by eye-fitting a line through daily onsets of activity. In the experiments reported below, where estimates of phase and period were made both before and after surgery, pre- and postoperative records of the photographically reduced data were separated, coded, and scored 'blind' by an independent observer to prevent postoperative estimates from being biased by preoperative data.

Of the 44 τ values obtained by this method 41 differed by less than 0.1 h from τ estimates obtained independently by a second observer. Other studies on the accuracy of estimating τ in *Leucophaea* by eye-fitting lines have also shown differences between estimates made by several individuals (Caldarola 1974), or between estimates made by eye and estimates obtained by periodogram analysis (Page, unpublished), are consistently less than 0.1 h.

Surgery. Surgical lesions were performed on animals anesthetized with CO_2. Access to the optic tract was obtained by removal of an approximately rectangular piece of cuticle overlying the optic lobe (Page 1978). Surgery usually involved removal of the ocellus and slight damage to the dorso-medial portion of the compound eye. This damage by itself does not disrupt entrainment of the activity rhythm by light (Page 1978). Exposed optic tracts were cut with fine iridectomy scissors and the completeness of the lesion was visually verified at the time of surgery. Following the lesion the cuticle was replaced and sealed with a small amount of melted wax.

Electrophysiology. Extracellular recordings of electrical activity in the cervical connectives (between the subesophageal and first thoracic ganglia) were made with suction electrodes. Animals were anesthetized with CO_2 and pinned ventral side up in a wax dish housed in a light-tight box. The cervical connectives were exposed through a small incision in the cuticle, taking care to avoid damage to the tracheae, and were cut with iridectomy scissors. The cut end of the nerve was drawn into a glass suction electrode. Signals were amplified (Tektronix 122 preamplifier), displayed on a storage oscilloscope (Tektronix 5111) and led in parallel to an audio monitor. Records were photographed with a Tektronix C5-C oscilloscope camera. Light pulses for stimulating visual interneurons in the connectives were provided by a 6 V tungsten lamp. All animals were dark adapted for at least 30 min prior to testing for light evoked activity. Control preparations exhibited robust responses to light for several hours under these conditions.

Histology. Brains were fixed in a solution of 70% ethanol:formalin:acetic acid (20:1:1). Tissue was dehydrated and embedded in paraffin. 15 µm serial sections were cut and stained with 1% methylene blue.

Results

Recovery of the freerunning rhythm

Both left and right optic tracts (OT) were sectioned in 28 cockroaches that were freerunning in constant darkness (DD) at constant temperature. In every case the activity rhythm was severely disrupted (Fig. 1). Five of the animals died within 2 weeks after surgery and another animal remained aperiodic until his death 56 days after surgery. All of the remaining 22 animals recovered a clear freerunning rhythm of activity between 19 and 42 days after surgery (Fig. 1).[1]

1 Determining the precise day on which the rhythm first reappeared was highly subjective. The values reported represent the shortest and longest times at which a clear rhythm was judged to have been present after OT section. The average number of days to return of rhythmicity was 29±6.2 (mean± standard deviation)

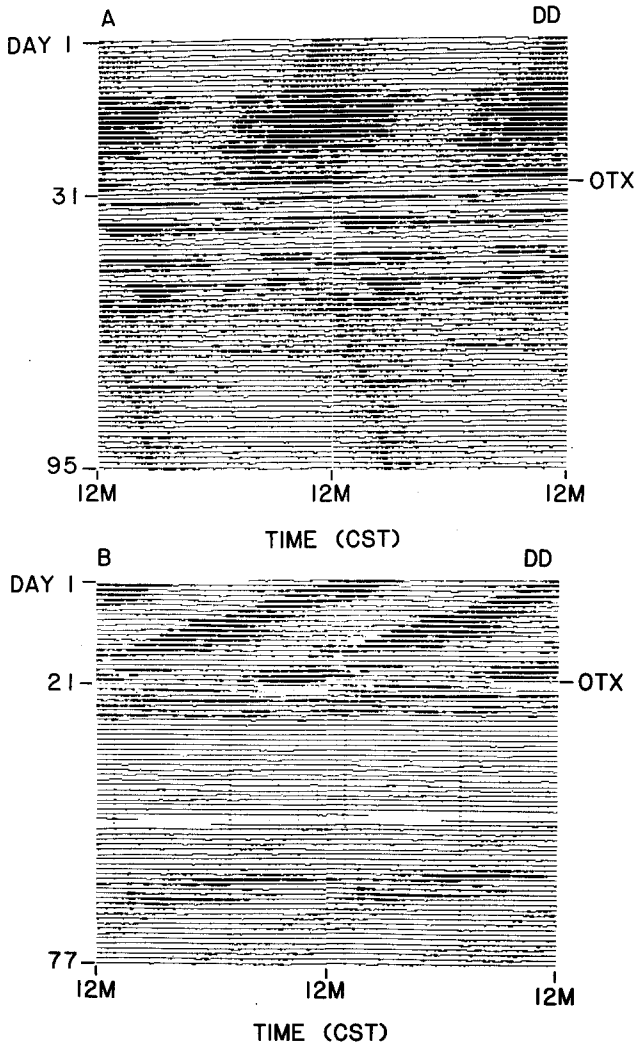

Fig. 1. Activity records of 2 animals showing loss of the activity rhythm and its subsequent reappearance following bilateral severence of the optic tracts. Animals were maintained in constant darkness at constant temperature throughout

The data leave no doubt about the ability of the circadian system to consistently recover functional control of the temporal distribution of activity after the optic tracts have been cut. The results raise several questions that are addressed in the following sections. Specific features of the activity rhythms of these animals are also described below.

Mechanism of recovery

There are several possible mechanisms by which a freerunning rhythm of activity might be reestablished after OT section. (1) A circadian pacemaker outside the optic lobes may assume control of the activity. (2) The pacemaker may reside in the optic lobe and drive the activity rhythm via some humoral pathway. (3) There may be regeneration of functional neural connections that couple the optic lobe pacemaker to the midbrain.

While the first of these explanations seems unlikely in view of the results from optic lobe transplantation experiments (see Introduction), its plausibility is increased by two factors. First, there are very few reported cases of long term (e.g. >60 days) studies showing persistence of arrhythmicity following complete removal of the optic lobes (e.g., Nishiitsutsuji-Uwo and Pittendrigh 1968a; Lukat and Weber 1979). Thus there is little evidence to suggest that the optic lobes are required for recovery of locomotor rhythmicity after OT section. The possibility has been raised, for example, that the optic lobes simply modulate the period of an oscillator located elsewhere and that rhythm recovery could occur even in the absence of the optic lobes (Page 1982). Second, there is good evidence that circadian oscillators do exist outside the optic lobes in the cockroach. Lukat (1978) has shown that the circadian rhythm in cuticle deposition in the cockroach *Blaberus fuscus* is independent of the optic lobes, and there is evidence from *Leucophaea* that a damped oscillator that is capable of controlling activity survives optic lobe ablation and can be driven by temperature cycles (Page 1981b, and unpublished observations).

To examine the possibility that rhythm recovery after OT section might be independent of the optic lobes, these structures were removed from 13 animals and activity was monitored for 100 or more days in DD (Fig. 2A). In no case was there evidence in the activity record for recovery of a persistent rhythm of activity. The results show that the return of rhythmicity that consistently occurs within 42 days after optic tract section depends on the presence of the optic lobes.

The question remains as to whether the effect of the optic lobes is mediated via a humoral pathway or by regeneration of neural connections with the midbrain. The brains of 12 of the 22 OT sectioned animals that recovered rhythmicity were examined histologically. In every case at least one, and usually both, of the optic lobes had reestablished structural connections with the midbrain and appeared relatively normal under light microscope examination (Fig. 3A). Typically there was some distortion in the shape of the optic lobe and midbrain; however, all three regions of the optic lobe neuropil were generally easily identified and there was no indication of significant degeneration.

To determine whether the regeneration of structural connections between the optic lobe and midbrain was necessary for recovery of rhythmicity an effort was made to substantially slow regenera-

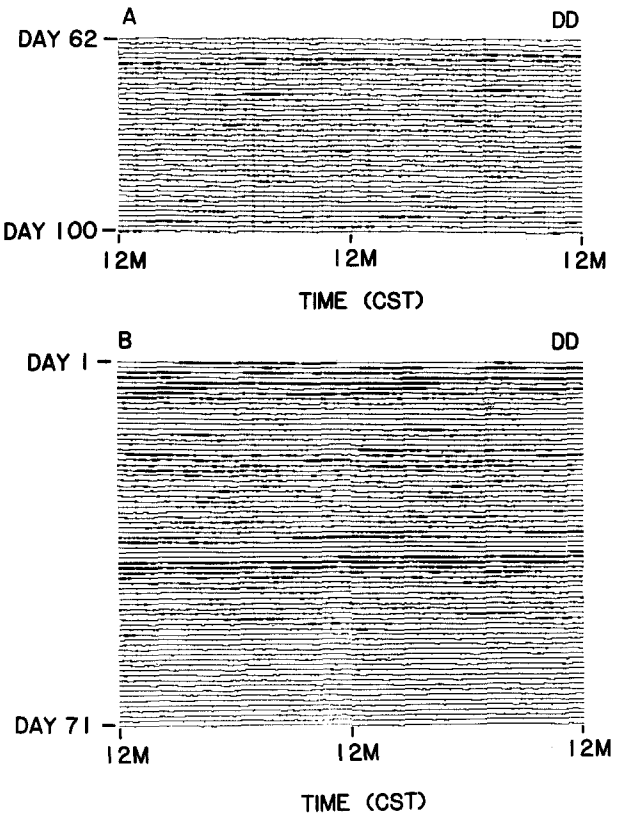

Fig. 2. Activity records showing the persistence of aperiodicity following (**A**) optic lobe removal or (**B**) after optic tract section and insertion of a piece of glass coverslip between the optic lobe and the midbrain. Records begin 62 and 2 days after surgery respectively. Animals were maintained in constant darkness and constant temperature throughout

tion by inserting a small piece of glass coverslip between the optic lobe and midbrain after OT section. Nine of ten animals survived the surgery. This procedure prevented the recovery of rhythmicity in 6 of the 9 animals (activity was monitored for 75–90 days) (Fig. 2B) and delayed it in 3 animals (average number of days to rhythmicity was 46 ± 14.9). Insertion of the coverslip elsewhere in the head capsule of OT sectioned animals had no effect on the recovery of rhythmicity (days to recovery $= 31 \pm 12.9$, $n = 6$). For six of the animals in which the coverslip had been placed between the optic lobe and midbrain activity recording was terminated prior to the animal's death and the brains and optic lobes were examined histologically. In all cases, including both 2 of the rhythmic and 4 arrhythmic animals, some tissue connections between the optic lobes and midbrain had developed around the coverslip. These connections were weak and were invariably broken upon removal of the brain tissue from the head capsule. In all cases the optic lobes appeared normal in histological section (Fig. 3B), and there was no indication the presence of the coverslip had damaged the optic lobe or midbrain.

These data suggest that blocking normal regeneration of connections between the optic lobe and the midbrain can prevent or slow the recovery of the activity rhythm after the optic tracts are severed. The results prompted an effort to determine whether or not the structural regeneration that was observed histologically following optic tract section reflected functional regeneration of neural connections between the optic lobe and the midbrain.

As a simple assay the recovery of visually stimulated electrical activity recorded from the cut ends of the cervical connectives with suction electrodes was used as an indication of regeneration of functional connections. In intact animals several cells that respond to changes in light intensity are readily observed in these multi-unit recordings (Fig. 4A). This activity is abolished by section of the optic tracts (Fig. 4B).

In these experiments the optic tracts were cut and the ocelli completely removed in a group of 30 animals. At various times after surgery animals were assayed for the presence of light evoked activity in the cervical connectives (Fig. 4B, C). The results are summarized in Fig. 5. There was no evidence of regeneration for 2 weeks after optic tract section; between 2 and 4 weeks a significant proportion of the animals tested exhibited recovery of light evoked activity in the connectives; and by 7 weeks all animals showed a positive response. In 3 animals from the 36–42 day group the optic tracts were cut a second time and the light evoked activity was abolished. Typical latency from light onset to the appearance of increased impulse activity was 200–300 ms which is similar to that observed in intact animals. These results demonstrate that functional neural connections between the optic lobes and midbrain are reestablished (for at least one class of cells) within 42 days following severence of the optic tracts. For comparison the time course of recovery of the locomotor activity rhythm is also shown in Fig. 5. The return of behavioral rhythmicity shows a time course that is remarkably similar to the recovery of light evoked activity in the cervical connectives.

In summary, the results show (1) the optic lobes are required for rhythm recovery after optic tract section, (2) structural and functional connections are regenerated between the optic lobes and the midbrain, (3) mechanically blocking regeneration of these connections can prevent the recovery of a circadian rhythm of activity, and (4) the time

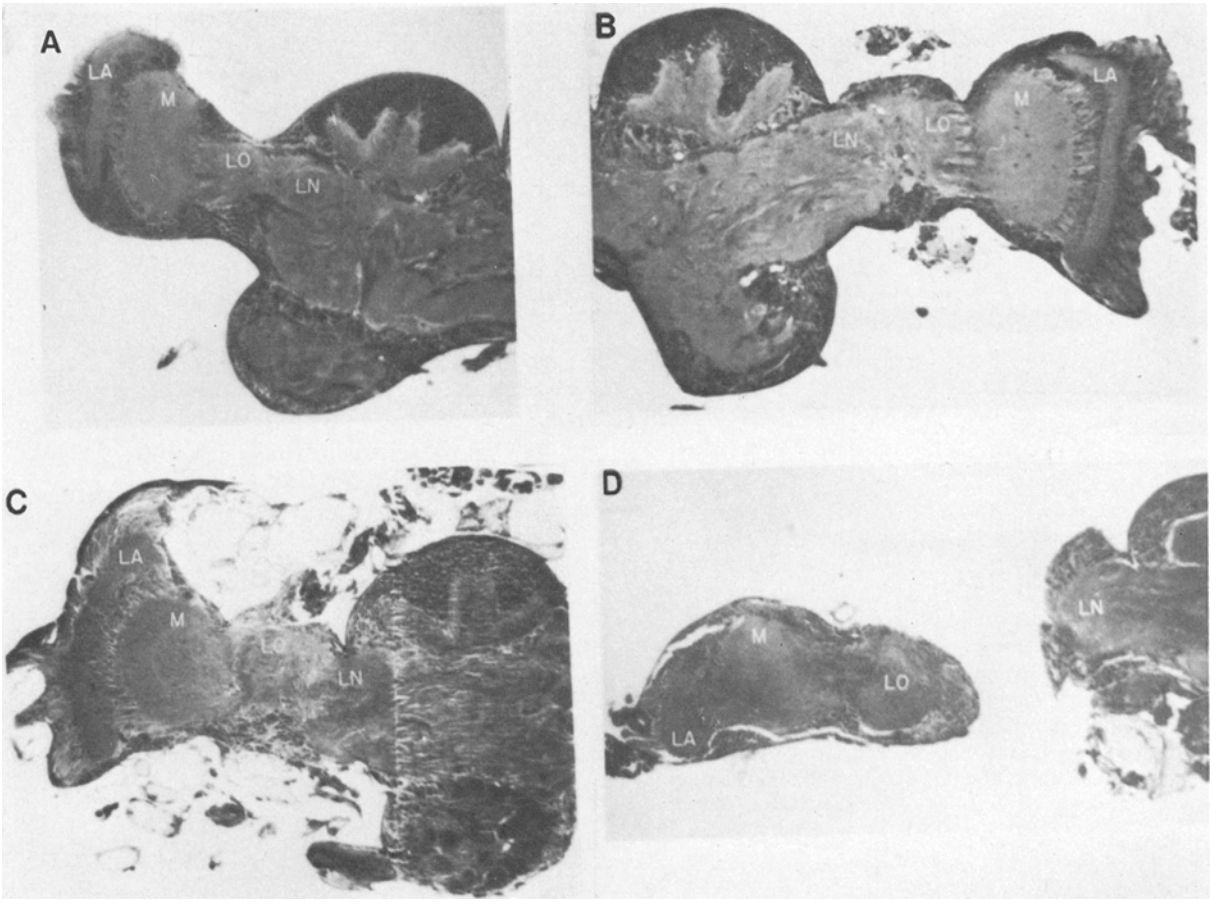

Fig. 3A–D. Histological sections of the optic lobes and the midbrain. **A** Frontal section through an intact control. **B, C** Structural regeneration between the optic lobe and the midbrain several weeks after bilateral section of the optic tracts in 2 animals. **D** Regeneration prevented by insertion of a glass barrier between the optic lobe (horizontal section) and the midbrain. Following optic tract section the lateral neuropil (*LN*) of the midbrain bulges outward causing some distortion of the shape of the brain. If regeneration is allowed the optic lobe invariably becomes attached to the lateral neuropil (*LN*). *LA* lamina; *M* medulla; *LO* lobula

course of recovery of circadian rhythmicity and the time course of regeneration of functional neural connections are similar. These results constitute strong, if circumstantial, evidence that the return of a circadian rhythm in locomotor activity after bilateral section of the optic tracts depends on the regeneration of neural connections between the optic lobe and midbrain, and that humoral pathways alone are not sufficient.

Conservation of period and phase during regeneration. The return of behavioral rhythmicity following regeneration of the optic tracts raises 2 additional questions. (1) To what extent, if any, is the pacemaking oscillation altered by optic tract section and regeneration? (2) Does the optic lobe oscillator continue its periodic motion during the period of regeneration?

The periods of the freerunning rhythms of activity before and after optic tract section were measured in the 22 animals in which a rhythm regenerated in DD. This group included 5 animals that had been raised in a 22 h light cycle (*LD 11*:11) and 4 animals that had been raised in a 26 h light cycle (*LD 13*:13). Cockroaches raised in these conditions have freerunning rhythms whose periods are shorter and longer, respectively than those of animals raised in *LD 12*:12 (Page and Block 1980; Page 1982). Figure 6A plots τ before surgery vs τ after regeneration for these animals. The two values for τ were strongly correlated ($r=0.87$), but OT section and regeneration led to an increase in τ in 15 of the 22 animals. The increase, which averaged 0.2 ± 0.30 h (standard deviation), was significant ($P<0.05$, signs test). Similar results were obtained in experiments in which optic lobes were transplanted from one animal to another. In these experiments optic lobes were exchanged between animals raised on different or the same LD cycle (*LD 11*:11 or *LD 13*:13) and τ of the host animal

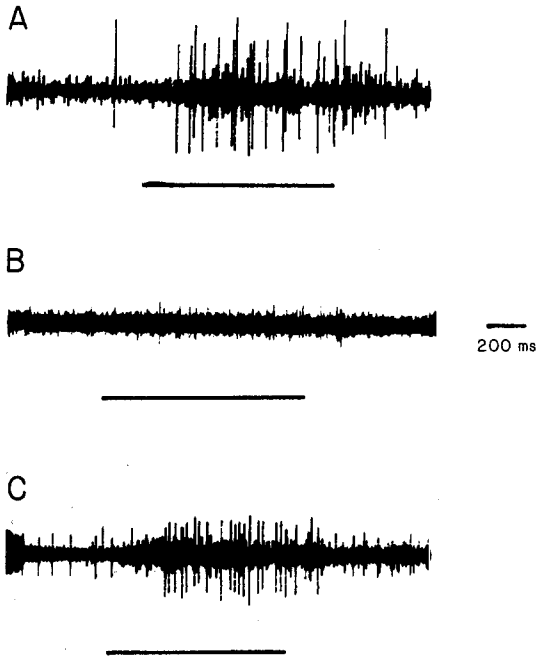

Fig. 4A–C. Multi-unit activity in the cervical connectives evoked by a light pulse. *Bar* below each oscilloscope trace designates the light pulse. **A** Intact control. **B** 1 week after bilateral section of the optic tracts and ablation of the ocelli light evoked activity is absent. **C** 8 weeks after OT section and ocelli ablation light evoked activity of several units has reappeared. Calibration: 200 ms

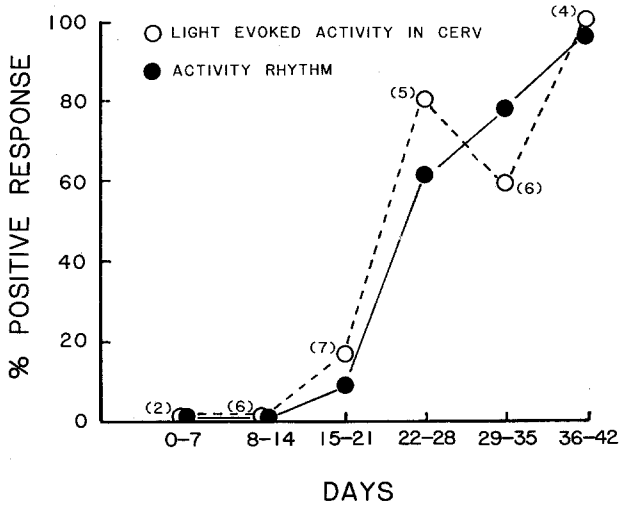

Fig. 5. Time course of recovery of light evoked activity in the cervical connectives (*CERV*) (*open circles*) and of the locomotor activity rhythm (*filled circles*) following bilateral severence of the optic tracts. Activity of 23 animals was followed continuously (Fig. 1) to determine the time of recovery of the activity rhythm. Numbers in parentheses refer to the number of animals examined for recovery of light evoked activity at each time point. In both cases there was little functional recovery prior to the beginning of the 4th week and recovery was essentially complete by the end of the 7th week

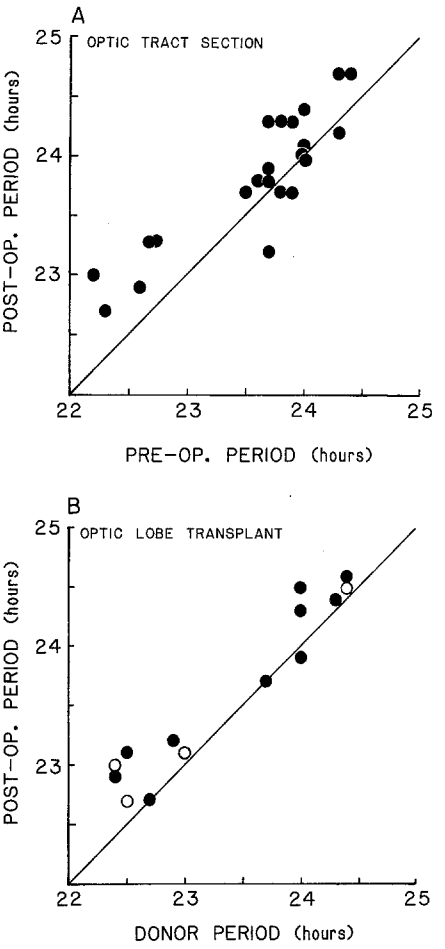

Fig. 6. Effects of optic tract section (**A**) or optic lobe transplantation (**B**) on the freerunning period of the locomotor activity rhythm. In **A** the period after regeneration of the optic tracts is correlated with the period prior to surgery, and in **B** the period of the host animal after regeneration of the transplanted optic lobe is correlated with the period exhibited by the donor prior to surgery. However, in both cases τ after regeneration is typically slightly longer. *Diagonal lines* show values expected if the pre- and postoperative periods were identical. In **B** animals were raised on *LD 11*:11 or *LD 13*:13. *Filled circles:* data from transplants between these two groups (Page 1982); *open circles:* from transplants within groups

after recovery of rhythmicity was compared to τ of the donor prior to surgery (Fig. 6B). The freerunning periods before and after surgery were strongly correlated ($r=0.96$), but on the average τ of the host after regeneration of the transplanted optic lobes was slightly longer than τ of the donor ($\Delta\tau=0.2\pm0.21$ h, $n=14$, $P<0.05$, signs test). τ increased in 11 of the 14 cases.

The inrease in τ is comparable to the increase seen following lesions that destroy pacemaker function in one optic lobe leaving a rhythm driven by the single remaining lobe (Page et al. 1977; Page 1978). Interpretation of this latter result has been

based on the hypothesis that the 2 pacemakers in the optic lobes are mutually coupled and that the freerunning period of each pacemaker is slightly longer than the period of the coupled pair (Page et al. 1977; Page 1981a, b). One possible explanation then of the increase in τ following optic tract section and regeneration is that it is due to a loss of coupling between the two optic lobes. Evidence has been obtained that the coupling pathway between the optic lobe pacemakers does not regenerate following optic tract section or optic lobe transplantation (Page, in preparation).

Another question of interest is whether or not a circadian oscillation in the optic lobe persists between the time of optic tract section and recovery of rhythmicity. Indication that the oscillator does continue in motion is provided by comparison of the phase of the freerunning rhythm after regeneration with the phase of the rhythm at the time of surgery.

On the assumption that τ of the oscillation during regeneration was the same as τ after recovery of rhythmicity, activity onsets were projected back to the day of surgery in the OT sectioned animals. Figure 7 shows the relation between phase before and after regeneration for 19 of the 22 animals (because this estimate of phase is very sensitive to error in the estimate of τ three animals for which 2 independent estimates of postoperative freerunning period differed by more than 0.1 h were excluded). Although there is substantial scatter in the data, rhythm phase before and after surgery were correlated ($r=0.61$, $P<0.01$), and for the majority of animals (13/19) projected phase was within 4 h of the pre-operative rhythm phase on the day of surgery (Fig. 7, inset). Only 4 of the 19 animals showed phase differences as great as 6–12 h. The average of the absolute values of the differences in phase was 3.8 ± 1.52 h (95% conf. limits). There was no correlation between regenerated rhythm phase and the time of surgery ($r=-0.14$). These results are consistent with the idea that (a) an oscillation with a freerunning period equal to the period of the rhythm after regeneration is present between the time of OT section and the return of the rhythm, and (b) the oscillation conserves information on the phase of the rhythm at the time of surgery.

If an oscillation does persist in the neurally isolated optic lobe it should be possible to entrain it with light since after OT section the optic lobe remains attached to the compound eye which contains the sole photoreceptors for entrainment of the activity rhythm (Roberts 1965; Nishiitsutsuji-Uwo and Pittendrigh 1968b). To examine this possibility 20 intact animals were entrained to *LD 12*:12 (lights-off at 19:00 CST) and following a period of stable entrainment both optic tracts were cut. The day after OT section the LD cycle was phase advanced by 6 h for 10 animals and phase delayed 6 h for 10 animals. After 10 days on the new light regime animals were placed in constant darkness. If the optic lobe contained an entrainable oscillator that continued in motion after OT section then the phase of the rhythm after regeneration should reflect the phase of the last LD cycle – the rhythms in the two groups should reappear on average about 20 days after the entry into DD and should be approximately 12 h out of phase. The results, shown in Fig. 8, match prediction quite well. Eighteen of the 20 animals survived surgery and recovered rhythmicity (average number of days to rhythmicity $=30.2 \pm 10.90$). The average difference of the rhythm phases (determined for post-operative day 30) for the two groups was 12.5 h. For the phase advanced group average phase of activity onset (day 30) with respect to real time was 12.6 ± 3.08 h (CST) (mean $\pm 95\%$ conf. limits). For the phase delayed group mean phase of activity onset was 0.1 ± 2.81 h (CST).

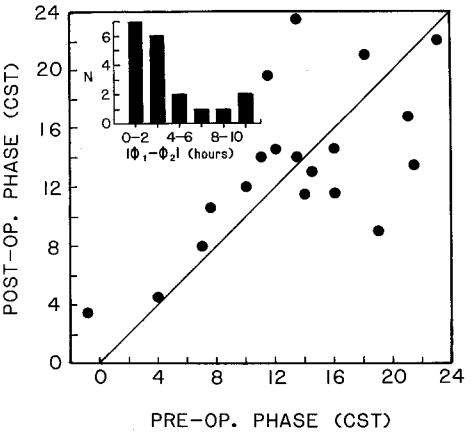

Fig. 7. Plots phase of the activity onset after optic tract section and regeneration (projected back to the day of surgery) as a function of the preoperative rhythm phase on the day of surgery. *Diagonal line* shows the predicted values if the pre- and postoperative phases were identical. *Inset (upper left):* frequency distribution of absolute values of pre- and postoperative phase differences in 2 h bins

The results suggest that even in the absence of behavioral rhythmicity an oscillation persists in the neurally isolated optic lobe, that it can be entrained by light within 10 days from the time the optic tracts are cut, and that the new phase information is retained after entry into constant darkness and ultimately expressed in the freerunning activity rhythm that appears after regeneration.

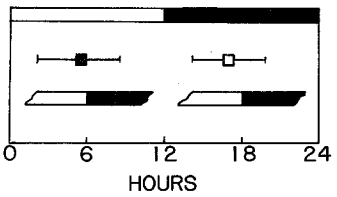

Fig. 8. Shows control of the phase of the activity rhythm by a light cycle presented for 10 days after bilateral severence of the optic tracts. Prior to surgery all animals were exposed to the light cycle illustrated by the bar at the top of the figure (*shaded portion* represents the dark phase of the LD cycle). The day after surgery animals were placed in an LD cycle that was either phase advanced by 6 h ($n=9$) or phase delayed by 6 h ($n=9$). *Lower bars:* time of the L/D transition for the two shifted light cycles. After 10 days animals were placed in constant darkness. *Squares:* mean phases (95% confidence limits) of rhythms on day 30 after surgery of the two groups (*filled square:* advanced; *open square:* delayed)

Discussion

The possibility that the optic lobes were the locus of the driving oscillation that controlled the circadian rhythm of locomotor activity in the cockroach was first suggested by Nishiitsutsuji-Uwo and Pittendrigh (1968a). The suggestion was based on the observation that bilateral severence of the optic tracts or removal of the optic lobe disrupted the activity rhythm. Subsequent work led to a further localization of the cells within the optic lobe that were crucial to sustaining rhythmicity (Roberts 1974; Sokolove 1975; Page 1978) and prompted the hypothesis that the pacemaker was comprised of two, bilaterally redundant oscillators – one located in each optic lobe – that were mutually coupled via a neural pathway in the brain (Page et al. 1977; Page 1978). This hypothesis received substantial support from the demonstration that localized cooling of a single optic lobe caused a phase shift in the activity rhythm and that the phase information was transmitted to the contralateral optic lobe (Page 1981a). More recently it was shown that transplantation of the optic lobe from one animal to another both restored rhythmicity in the host and imposed the freerunning period of the donor animal's rhythm on the rhythm of activity of the host (Page 1982). Thus the optic lobes have been shown to be necessary for the expression of a rhythm in locomotor activity and to be involved in the control of both its phase and freerunning period. These results provide strong support for the view that the temporal information that determines the distribution of activity is derived from cells in the optic lobes.

A major remaining question of interest is whether or not the cockroach optic lobe can sustain a circadian oscillation independent of any humoral or neural connections with other cells in the organism. Two observations reported here bear on this issue. First, it was found that the phase of the freerunning activity rhythm after optic tract section and regeneration, when projected back to the day of surgery, was correlated with the phase of the preoperative activity rhythm. The simplest interpretation of this observation is that the oscillation that controls the activity continued in motion during regeneration with a freerunning period similar to the period of the rhythm after recovery. Thus the phase of the preoperative rhythm was conserved, but drifted with respect to real time with a circadian period. Second, the phase of the freerunning activity rhythm observed after optic tract regeneration was shown to be subject to control by light cycles presented after optic tract section but prior to both recovery of the activity rhythm or the detection of light evoked activity, driven via the compound eyes, in the central nervous system. Photoreceptors sufficient for entrainment of the activity rhythm are contained solely within the compound eyes (Roberts 1965; Nishiitsutsuji-Uwo and Pittendrigh 1968b; Driskill 1974). Since, after optic tract section the eyes remained connected to the optic lobes but were neurally isolated from the rest of the nervous system, it seems likely that the phase information provided by the LD cycle was encoded and, during the subsequent period of constant darkness, conserved in the optic lobe. These results suggest that an entrainable circadian oscillator is contained in the optic lobe and that it continues its periodic motion in the absence of neural input from other CNS structures. The data do not, however, rule out the possibility that humoral factors originating outside the optic lobes are necessary to sustain the oscillation.

Another remaining problem is the delineation of the pathway and mechanism by which circadian information is transmitted from the optic lobes to other central nervous system structures that directly control activity. The discoveries that optic tract section (Nishiitsutsuji-Uwo and Pittendrigh 1968b), or section of the circumesophageal connectives (Roberts et al. 1971; Brady 1967) consistently disrupted rhythmicity have provided the foundation for the generally accepted view that the coupling between the pacemaker and the structures responsible for executing locomotor movements is neural and does not normally involve a humoral step (Brady 1974; Page 1981c). However, the

mechanism by which the temporal information is transmitted is by no means certain (Page 1983). Results of transplantation experiments in *Drosophila melanogaster* (Handler and Konopka 1979) and the house cricket *Acheta domesticus* (Cymborowski 1981) indicate that the brains of these insects produce a diffusable factor that can drive several cycles of rhythmic activity. Similarly, in the cockroach *Periplaneta americana* parabiosis experiments have provided evidence that a blood borne factor can drive rhythmicity for a few cycles (Cymborowski and Brady 1972). These results suggest caution is warranted in ruling out the possibility that a humoral link in the circadian output pathway may play a significant role in the regulation of the activity rhythm of *Leucophaea*.

The results presented here indicate the recovery of rhythmicity following optic tract section is dependent on regeneration of neural connections between the optic lobe and the midbrain. Thus the data suggest that one early step in the output pathway, from the optic lobes to the midbrain, is via axonal tracts, and that release of a humoral factor directly from the optic lobes is not sufficient. The precise mechanism by which the optic tracts communicate temporal information to the midbrain is uncertain. The pathway may involve classical synaptic transmission or the transport and release of a neurohormone (or neuromodulator) in the midbrain. The possibility of the involvement of a hormone in subsequent steps is, of course, also not excluded, and an important direction for future research will be a detailed analysis of the output pathway by which the circadian information derived from the optic lobes regulates activity.

The consistency with which regeneration in the protocerebrum leads to recovery of a circadian rhythm of activity after section of the optic tracts is notable. In the present experiments 96% of the animals that survived more than 2 weeks after surgery recovered a rhythm of activity. An interesting question is to what extent is the functional and structural regeneration complete? In preliminary anatomical studies it appears that after regeneration the vast majority of optic tract cells terminate in the lateral neuropil of the brain near its connection with the optic lobe (Logan and Page, unpublished). Other tracts, which normally terminate in the contralateral optic lobe or midbrain or which end nearer the midline on the ipsilateral side, appear to be absent. Furthermore, there is extensive evidence that suggests the coupling pathway between the optic lobes that mediates mutual entrainment between optic lobe pacemakers and provides for entrainment of a pacemaker by light cycles perceived by the contralateral eye (Page et al. 1977; Page 1978, 1981a) does not regenerate, even after over 150 days following optic tract section (Page, in preparation). Thus it appears on the basis of both structural and functional criteria that although recovery of some connections occurs consistently, regeneration of the optic tracts is, typically, only partially complete.

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