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Central Nervous System Control of Circadian Rhythmicity in the Cockroach

III. The Optic Lobes, Locus of the Driving Oscillation?

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Summary. 1. We found no evidence that the thoracic ganglia which effect locomotory movements are themselves autonomously (circadian) rhythmic in their activity.

2. The abdominal ganglia play no role in effecting the rhythmicity of the thoracic ganglia, and hence, of locomotion.

3. We found positive evidence that the suboesophageal ganglion does *not* control the locomotory rhythm by a rhythmic secretion of a hormonal agent. It does, on the other hand, control activity *level* by a neural channel.

4. The evidence is strong that the driving oscillation is in the brain, in fact in the protocerebrum.

5. The *pars intercerebralis* suppresses (by a hormonal channel) the level of activity. It also, and separately, mediates locomotory activity by a hormonal channel.

6. The *pars intercerebralis* can however only cause rhythmicity of locomotion when it has intact neural connections with the optic lobes.

7. The driving oscillation in the nervous system responsible for the circadian rhythm of locomotory activity is thus — probably — localized in the optic lobes.

8. Animals in which the left optic tract and the right optic nerve have been severed display a freerunning rhythm in a 24 hour light/dark cycle: the driving oscillation in the left optic lobe is uncoupled from the *pars intercerebralis* which it therefore cannot drive; the oscillation in the right optic lobe can drive the *pars intercerebralis* but is uncoupled from the right compound eye.

Zusammenfassung. 1. Wir fanden keinen Anhaltspunkt dafür, daß die Thorakalganglien, die die lokomotorische Aktivität beeinflussen, eine eigene autonome (circadiane) Rhythmik ihrer Aktivität besitzen.

2. Die Abdominalganglien spielen keine Rolle bei der Beeinflussung der Rhythmik der Thorakalganglien — und damit der Lokomotion.

3. Wir konnten zeigen, daß das Suboesophagalganglion die Bewegungsrhythmik *nicht* durch rhythmische Sekretion eines hormonalen Stoffes steuert. Es kontrolliert dagegen die *Stärke* der Aktivität auf nervösem Wege.

4. Starke Anhaltspunkte sprechen dafür, daß der steuernde Oszillator im Gehirn liegt, und zwar im Protecerebrum.

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5. Die *Pars intercerebralis* erniedrigt (auf hormonalem Wege) die Aktivität. Sie bewirkt ebenfalls — getrennt davon — lokomotorische Aktivität auf hormonalem Wege.

6. Die *Pars intercerebralis* kann jedoch nur dann eine Bewegungsrhythmik bewirken, wenn die nervösen Verbindungen mit den optischen Lappen intakt sind.

7. Der für die circadiane Rhythmik der Bewegungsaktivität verantwortliche steuernde Oscillator im Nervensystem ist deshalb wahrscheinlich in den optischen Lappen lokalisiert.

8. Tiere, deren linke optische Bahnen und rechte optische Nerven durchtrennt waren, zeigen eine freilaufende Rhythmik im 24 Std-Licht-Dunkel-Zyklus: Der steuernde Oscillator im linken optischen Lappen ist von der *Pars intercerebralis* entkoppelt, und er kann sie deshalb nicht steuern; der Oscillator im rechten optischen Lappen kann zwar die *Pars intercerebralis* steuern, aber er ist vom rechten Komplexauge entkoppelt.

Introduction

The work reported here was prompted by the following questions: 1. What is the fuller meaning of our earlier demonstration (NISHIITSUTSUJI-UWO, PETROPULOS and PITTENDRIGH, 1967) that the *pars intercerebralis* is essential to the expression of circadian rhythmicity in the locomotion of cockroaches? 2. Does the driving oscillation responsible for the observed rhythm originate in that fraction of the protocerebrum? — or is the (certainly necessary) rhythmic output of the *pars intercerebralis* driven by an oscillation originating elsewhere? 3. Are the thoracic ganglia that immediately control locomotion themselves autonomously rhythmic? 4. What is the role of the suboesophageal ganglion?

Our experiments fall into two major groups: Group I is concerned with the ventral cords and their ganglia including the suboesophageal ganglion (SG) which HARKER (1956, 1960a, b) has concluded can autonomously sustain a circadian oscillation which is responsible for the rhythm of locomotion. The facts from our experiments are incompatible with her position. We find no evidence that SG is a rhythmic center and we find, more positively, that the ganglion cannot mediate rhythmicity by endocrine means.

Group II is concerned with the brain. Here we confirm our earlier conclusion that an endocrine output from the *pars intercerebralis* (PIC) mediates locomotory rhythmicity; but that region (the PIC) is evidently not itself autonomously rhythmic: rhythmicity of its output depends on intact neural connections with the optic lobes.

Materials and Methods

We have used newly emerged adult males of *Leucophaea madeirae* for the bulk of our experiments. *Periplaneta americana* (fresh males also) was used only occasionally. Rearing methods and conditions, the nature of our recording techniques, and the principal surgical procedures we use have all been presented in the two previous papers of this series (NISHIITSUTSUJI-UWO and PITTENDRIGH, 1967; and NISHIITSUTSUJI-UWO and PITTENDRIGH, 1968).

The significant detail of surgical procedure is presented for each of the 19 separate experimental series in the next section. The descriptions given there are facilitated by reference to Figs. 1 and 2. Fig. 1 left gives a posterior view of the head and a ventral view of the neck (cervix) of *Leucophaea madeirae*; and Fig. 1 right concerns the central nervous system in the thorax and abdomen of *Periplaneta americana*.

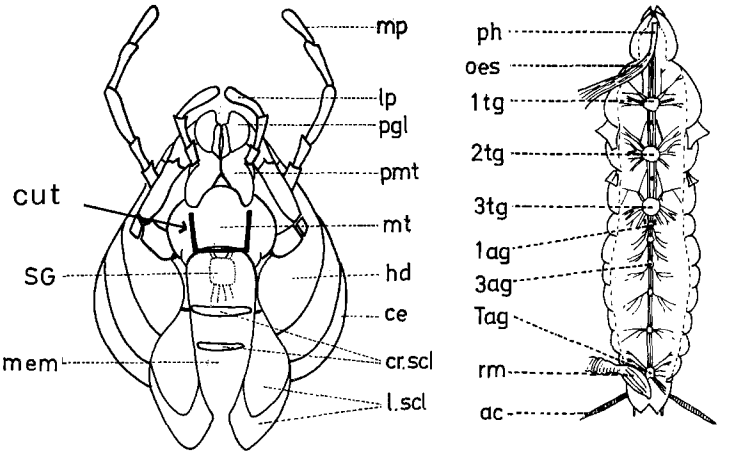

Fig. 1. Left. Posterior view of head and ventral view of neck (Cervix) of *Leucophaea madeirae* showing position of U-shaped cut (see text) and of the suboesophageal ganglion. *mp* maxillary palp; *lp* labial palp; *p.gl* paraglossa; *pmt* prementum; *mt* postmentum; *hd* head; *ce* compound eye; *cr.scl* crescent-shaped sclerites; *l.scl* lateral sclerites; *SG* position of suboesophageal ganglion under membrane; *mem* neck membrane. Right. The nervous system in thorax and abdomen of *Periplaneta americana*; re-drawn from Cameron (1961). *ph* pharynx; *oes* oesophagus; *1*, *2* and *3* tg 1st, 2nd and 3rd thoracic ganglion; *1ag* 1st abdominal ganglion; *3ag* 3rd abdominal ganglion; *Tag*, terminal abdominal ganglion; *rm* rectum; *ac* cercus

Fig. 2 is a diagrammatic representation of a frontal view of the brain (protocerebrum and deuterocerebrum only) of *Leucophaea madeirae*. The numbers identify the site of the cuts involved in the experimental series of Group II. The pattern of operations is schematically summarized below in diagrams which represent the two protocerebral lobes by large ovoids, and the two optic lobes by smaller ovoids.

Experimental Procedures and Results

Group I. Ventral Cords and their Ganglia

I-1. 4 Animals. Complete Decapitation (Table 1, I-1). The decapitating cut was made in the neck and the wound was rapidly sealed with paraffin wax. The ventral cords were cut between the suboesophageal and the first thoracic ganglion. The suboesophageal ganglion was removed with the head.

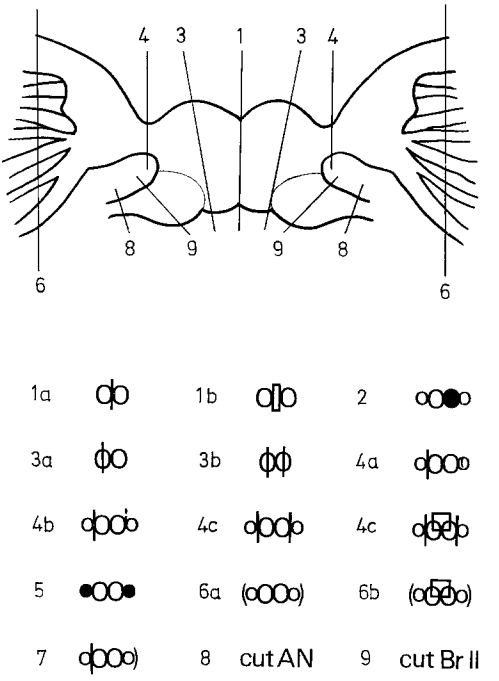

Fig. 2. Above: frontal view (semi-schematic) of the brain to identify the location of the cuts involved in the operations performed in Group II. Below: schematic representations of the operations performed in the nine experimental series in Group II. *1a* Midsagittal bisection of the protocerebrum; cut at location 1. *1b* Same, with addition of a glass-plate separator. *2* Excision of right main protocerebral lobe; cuts at locations 1 and 4, the latter extended across the boundary of the deuterocerebrum (location 9). *3a* Unilateral section through mid-protocerebrum; cut at location 3. *3b* Bilateral section through mid-protocerebrum; cuts at location 3 on each side. *4a* Complete section of optic tract on the left side; cut at location 4 (left). *4b* Same as 4a; with additional incomplete section at location 4 on the right side. *4c* Same as 4b, but section complete at both locations 4. *5* Optic lobes excised from both sides; cuts at locations 4 and 6. *6a* Section of both optic nerves; cuts at location 6 on each side. *6b* Same as 6a; a glass window added above protocerebrum. *7* Section of optic tract (cut location 4) on the left side and section of the optic nerve (cut location 6) on the right side. *8* Bilateral section of the sensory and motor antennal nerves; cuts at location 8 on each side. *9* Bilateral transection of the deuterocerebrum (Br II), by cuts at location 9 on each side

Leucophaea shows even less activity than *Periplaneta* when decapitated. The headless animals can and do move legs, wings and abdomen but they do not complete effective walking movements spontaneously; they must be pushed or otherwise mechanically stressed. Our assay of locomotion requires effective walking to displace the running wheel. The virtually complete absence of any locomotion in all four animals in this

group renders the absence of rhythmicity in the data meaningless (Fig. 3, 1934L).

Evidence presented below (Group I-2) indicates that the loss of locomotion following decapitation is due to severance of the ventral cords between the suboesophageal and thoracic ganglia. Headless animals are thus very different from brainless animals which show abnormally high activity (arrhythmic) due to the loss of a suppressor from the *pars intercerebralis*.

All four of our decapitated animals were dead within 11 days.

I-2. 6 Animals. Bilateral Section of the Circumoesophageal Connectives (Table 1, I-1). Under exposure to CO_2 (maintained moist and at 25° C by passage through temperature-controlled wash-bottles), the animal was placed, ventral side up, on a paraffin bed which had been moulded to provide a cavity of appropriate depth, length and width to receive the insect's body. The CO_2 supply vented from a hole in the bottom of this cavity. The animal was taped across the thorax onto the bed and a second tape tied the mouth-parts so as to stretch the neck from the ventral side as much as possible. The suboesophageal ganglion and the circumoesophageal connectives were then readily visible through the unpigmented, intersegmentary integument. A U-shaped cut was made (see Fig. 1 left) on the mentum which then was folded back and taped to get it out of the way. The circumoesophageal connectives were then cut with a fine forceps.

When the circumoesophageal connectives were bilaterally severed near the suboesophageal ganglion, five of the six animals so treated immediately resumed their normal rhythm of activity; two of them at a higher than normal activity level, two of them at a normal level and one at a lower than normal level (Fig. 3, 1957L). In only one out of the six was rhythmicity lost.

I-3. 8 Animals. Bilateral Section of the Ventral Cords between the Suboesophageal and the First Thoracic Ganglia (Table 1, I-3). The animal was placed on the paraffin bed described under I-2. The basisternum of the prothoracic segment was cut and the flap created by the U-shaped

Fig. 3. *1934L* (*Leucophaea*). Loss of activity following decapitation. *1957L* Persistence of the rhythm following bilateral section of the circumoesophageal connectives (cc). This was one of the two animals (out of 6) in which the nightly activity increased following section of both connectives. On day 4, the ventral cords were cut between the suboesophageal and 1st thoracic ganglia (SG+ 1 ThG). Animal lost activity and died 2 weeks after the operation. *1958L.* Loss of rhythm and activity following bilateral section of the ventral cords between the suboesophageal and 1st thoracic ganglia (SG, 1 ThG). This animal died 22 days after the operation. *1965L* Persistence of rhythm following bilateral section of the ventral cords between 2nd and 3rd abdominal ganglia (2, 3 AG) on day 0 and between 1st and 2nd thoracic ganglia (1, 2 ThG) on day 13

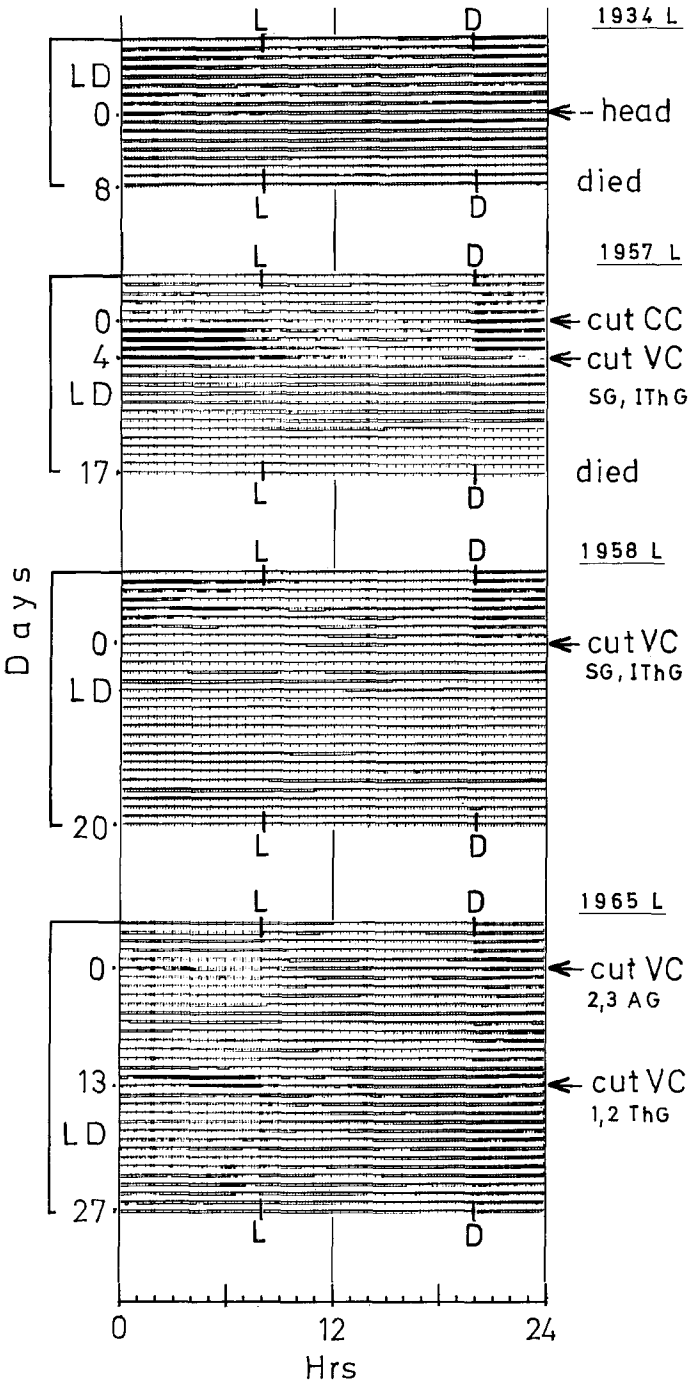

Fig. 3 (for legend see p. 18)

incision was carefully lifted to expose the ventral cords both of which were cut with a fine forceps. The flap of basisternum was then replaced and the cut sealed with paraffin.

This operation — like those for Groups I-4 and I-5 — necessitates some very slight cuttings of the musculature. In this group (I-3) the salivary ducts were commonly cut.

The section of both cords was made just below the suboesophageal ganglion. Most animals (7/8) were almost as motionless as the headless animals (Fig. 3, 1957L, 1958L); one achieved movement but still atypically little. All died within 32 days (7—32 range) after the operation, obviously from hunger. All alimentary canals were completely empty, no droppings were made postoperatively and the fat body was greatly reduced.

We suspect the presence of rhythmicity in what little activity there was but the evidence is not fully convincing.

I-4. 6 Animals. Bilateral Section of the Ventral Cords between the Pro- and Mesothoracic Ganglia (Table 1, I-4). The operation was the same as that described under I-3, but the U-shaped cut in the basisternum was made in the mesothorax.

When the ventral cords were cut, bilaterally, between the first and second thoracic ganglia, three types of result were observed: a) (3 animals) very low activity, two of them with no rhythmicity discernible, one some rhythmicity; animal surely dying of hunger; b) (1 animal) low activity with some rhythmicity becoming clear from 5 to 12 days postoperatively; c) (2 animals) clear rhythmicity and normal activity level. Animals ate normally and continued to live indefinitely (Fig. 3, 1965L).

I-5. 6 Animals. Bilateral Section of the Ventral Cords between the Meso- and Metathoracic Ganglia (Table 1, I-5). The operation followed the procedure given for Group I-3; but the basisternum was cut in the metathorax.

The results of this severance of the ventral cord were qualitatively the same as in the previous group, but a greater proportion (4/6) were of the third type in which activity level and rhythmicity were normal.

I-6. 7 Animals. Bilateral Section of the Ventral Cords between Second and Third Abdominal Ganglia (Table 1, I-6). The animal was placed on the paraffin bed described under Group I-2, ventral side up. The ventral

Fig. 4. *1953L* (*Leucophaea*). Persistence of the rhythm following ablation of the last abdominal ganglion (— T.AG). On day 6, the animal was put in constant darkness and showed a typical freerunning rhythm which continued following the section of the ventral cords between 2nd and 3rd abdominal ganglia (2, 3 AG). *1963L.* After complete ablation of the suboesophageal ganglion, the animal is so inactive that the presence or absence of rhythmicity cannot be resolved. Section of the ventral cord under the suboesophageal ganglion causes the same result

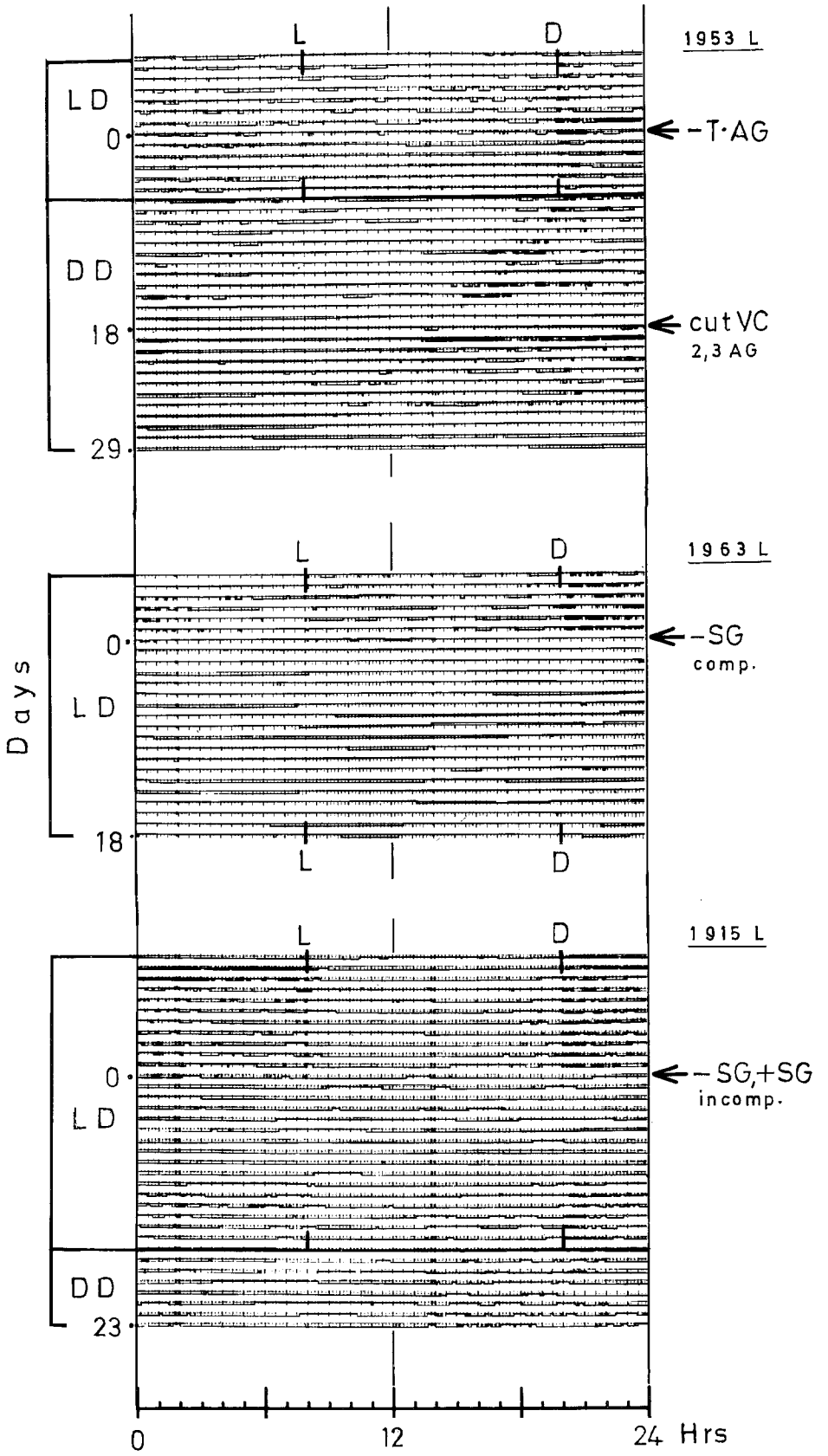

Fig. 4 (for legend see p. 20)

Table 1. *Effects of various nerve sections of the central nervous system on the circadian locomotory rhythm in cockroaches*

Experimental groups	Experimental procedure	No. of Animals	Post-operative observations			
			Days of observation	Activity level	Rhythm	Days until rhythm redevelops
I-1	Headless	4	× 8 (6—11)	Almost none	−4	—
2	Cut CC	6	18 (4—38)	Low 2 Normal 2 High 2	 −1 +5	 — 1 (0—5)
3	cut vent. cord between: SG and 1 ThG	8	× 21 (7—32)	Very low 7 Low 1	−8	—
4	cut vent. cord between: 1 and 2 ThG	6	× and ○ 24 (15—35)	Very low 3 Low 1 Normal 2	−2 ±2 +2	— 5, 12 0
5	cut vent. cord between: 2 and 3 ThG	6	○ 19 (9—28)	Very low 1 Low 1 Normal 4	 ±2 +4	 4, 12 3 (0—9)
6	cut vent. cord between: 2 and 3 AG	7	○ 12 (9—13)	Normal 7	+7	0 (0—1)
7	— T AG	4	○ 24 (17—30)	Normal 4	+4	0 (0—1)

8	— SG comp	15	× 23 (7—44)	Very low 11 Low 4	−15	—	
9	— SG incomp	6	× 21 (17—26)	Very low 4 Low 1 Normal 1	−4 +2	— 9, 8	
10	— SG incomp., then, + SG	4	× 22 (11—35)	Very low 3 Low 1	−3 +1	— 11	

Experimental procedure: CC = circumoesophageal connectives; SG = suboesophageal ganglion; vent. cord = ventral cord; 1, 2, 3 ThG = pro-, meso-, meta-thoracic ganglion; 2, 3 T AG = 2nd, 3rd, terminal abdominal ganglion; − = ablation; + = implantation; incomp = incomplete (see text); comp = complete. *Post-operative observation:* ○ = animals alive at the end of the experiment; × = most animals died at the end of experiment; — = no rhythm; + = some rhythm; ± = doubtful rhythm.

cords were severed with a fine forceps after being exposed by U-shaped cutin the middle of the second abdominal segment.

A fully normal rhythm returned in all seven animals almost immediately (Fig. 4, 1953 L). Post-operative suppression of rhythmicity attributable to the "trauma" of surgery is here, and in the next group, minimal, and very different from that following brain surgery.

I-7. 4 Animals. Ablation of the Terminal Abdominal Ganglion (Table 1, I-7). The operation proceeded as described for the previous group but the U-shaped cut was made in the integument of the sixth abdominal segment. The ganglion was excised with fine forceps.

The removal of this ganglion has no discernible effect whatsoever on roach activity — on its level, its rhythmicity, or the phase of the rhythm relative to the light cycle. (Fig. 4, 1953 L concerns an animal whose terminal abdominal ganglion was removed on day 0; the rhythm in LD is normal for six days with no post-operative effects at all; it enters DD on day 6 when it proceeds to display a free running rhythm which persists after day 18 when the ventral cords were severed between the second and third abdominal ganglia.)

I-8. 15 Animals. Complete Ablation of the Suboesophageal Ganglion (Table 1, I-8). The operation, detailed for Group I-2,

begins with a U-shaped cut in the mentum. Complete excision of the suboesophageal ganglion was effected by two cuts: one of them severs the circumoesophageal connectives and the second severs the ventral cords. Great care was taken not to cut muscle or other nerves, but the recurrent nerve is occasionally severed. The replaced flap of mentum was sealed with paraffin.

When the suboesophageal ganglion was completely excised, the animals lived from 7 to 44 days but showed extremely little locomotory activity, like those in Group I-3. Like those animals, too, their alimentary canals were empty when they died (Fig. 4, 1963 L).

I-9. 6 Animals. Incomplete Ablation of the Suboesophageal Ganglion (Table 1, I-9). The operation proceeded as described for Group 7, up to the U-shaped cut in the mentum. The suboesophageal ganglion shows little external trace of segmentation, but, in sections, the fusion of three ganglia (mandibular, maxillary and labial) is evident. The cell bodies are mainly ventral, as in all the cord ganglia. The ventral bulge of the ganglion containing the cell bodies was removed from animals in this group, using a fine forceps. The intent was to leave the long connectives of the cords themselves intact. Postmortem histological check showed the operation was generally successful in this respect but in some animals a few cell bodies remained.

Four out of the six animals showed very little activity postoperatively and no rhythmicity was discernible in the sparsely scattered movements. Two, however, displayed rhythmicity (8 and 9 days postoperatively) in their normal-to-low activity.

I-10. 4 Animals. Incomplete Ablation of the Suboesophageal Ganglion with Immediate Re-implantation of the Tissue Removed into the Abdominal Haemocoel (Table 1, I-10). The operations on this group followed the procedures outlined above for Group I-9. The excised ventral mass of cell bodies was then *immediately* inserted into a V-shaped opening in the dorsal integument of the fifth abdominal segment. That wound was then sealed with paraffin (Fig. 4).

Re-implantation had no discernible effect. The four animals died from 11 to 25 days after the operation and in the meantime displayed very low to low activity. In only one animal did normal rhythmicity return (11 days postoperatively).

We note again that all animals in Groups I-1, 3, 8, 9, and 10, and some of the animals from Group 4 died obviously from hunger: feeding as well as locomotory activity ceased following severance of the cords between the suboesophageal and first thoracic ganglia. Fat bodies were greatly reduced at postmortem examination, and tumors were occasionally found in the salivary reservoir, fore-gut and/or hind-gut con-

firming our previous observation (NISHIITSUTSUJI-UWO and PITTENDRIGH, 1967 b) that operations in which the probability of severing the recurrent nerve is high tend to induce such tumors.

Group II. Brain

All the brain operations involved in Group II employed the adjustable operating-stage described in the first paper of this series. All of them began, too, with the removal of a square piece of integument above the protocerebrum; when necessary, further access to the brain was facilitated by extending the upper edge of the window bilaterally, to create a liftable flap of integument. At the end of each operation the replacement of the flap and excised square of integument was followed by sealing-off with paraffin (see previous papers).

II-1a. 9 Animals. Simple Bisection of the Protocerebrum (Table 2, II-1 a). Complete separation of the lobes was assured by making, with a fresh fragment of razor-edge, three successive cuts, each judged of itself adequate. Within about a week (2—11 days) six of the nine animals resumed a normal rhythmicity that persisted in light and darkness 12:12 hours, constant light and constant darkness (Fig. 5). Three animals did not recover normal rhythmicity and two of them died within three weeks. With the exception of these latter two, all animals were sacrificed at the end of the observation period for histological examination of the brain.

We note that while the bisection plane appeared exactly midsagittal in all cases under the 50× magnification used for the operation, histological study at 400× magnification always showed the cut had — of course — been made slightly off center.

In one animal which had resumed rhythmicity postoperatively a connective tissue sheath had developed separating the two protocerebral lobes completely into physically isolated entities. However in all six others (five had resumed rhythmicity, one had not) the two lobes of the protocerebrum had fused: the regenerative processes produced a brain that *appeared* normal anatomically and histologically. The only indications of the former mid-sagittal bisection plane were: a) cracks on fissures in the brain sheath toward the central body; and b) the neurosecretory cells and their tracts were usually filled with secretory granules. Some of the tracts from the median groups apparently stopped after they emerged from the perikaryon.

II-1b. 4 Animals. Bisection of the Protocerebrum followed by Insertion of a Glass Separating Plate (Table 2, II-1 b). The glass plate (1 × 1 × 0.14 mm thick) inserted between the separated lobes in these four animals was

Table 2. *Effects of various nerve sections of the brain on the circadian locomotory rhythm in cockroaches*

Experimental group	Operation procedure	No. of animals	Post-operative observations				
			Days of observation	Activity level	Rhythm in LD	Days until reappearance of rhythm	Animals' state at end of experiment
II-1a		9	39 (13—84)	Normal 4 Higher 5	Normal 6 Loss 3	7 (2—11)	+7 −2
1b		4	36	Normal 1 Lower 1 Higher 2	Normal 2 Loss 2	6, 12	+4
2		6	35 (32—36)	Normal 1 Lower 1 Higher 4	Normal 4 Loss 2	8 (1—12)	+6
3a		3	16 (14—43)	Normal 2 Higher 1	Normal 3	0 (0—1)	+3
3b		10[a]	18 (2—36)	Normal 4 Lower 2 Higher 4	Loss 10	—	+1 −9

Experimental group and operation procedure: see Fig. 2. Activity level lower and higher than normal pre-operative activity level. LD = light and darkness 12:12 hours; + = alive; − = dead.

[a] Seven in which the lobes were cut simultaneously, plus the three animals from Group 3a in which the second lobe was cut one to two weeks after the first.

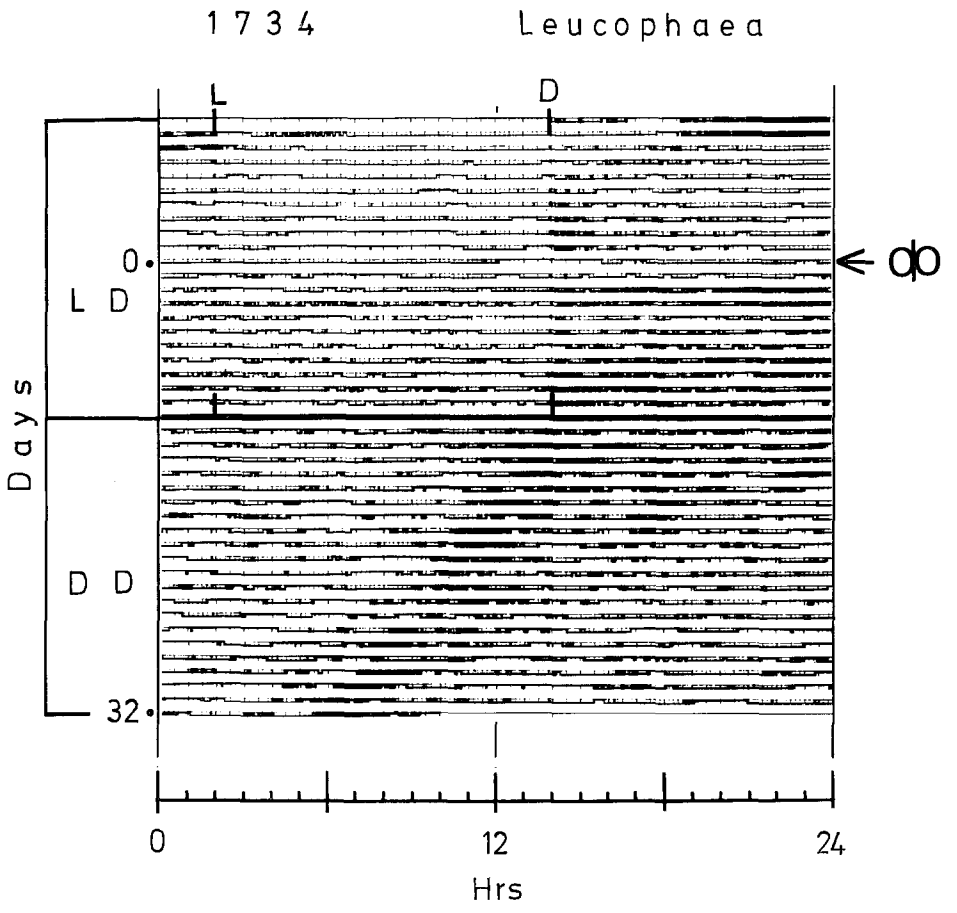

Fig. 5. Persistence of the activity rhythm following the midsagittal bisection of the protocerebrum (on day 0). The rhythm persisted in DD (constant dark). *L* light; *D* darkness

intended to prevent their subsequent fusion and the regeneration of normal pathways. After 36 days of postoperative observation, all animals were sacrificed and it was found in all four animals that the glass plate stood where it had been placed, with the protocerebral lobes tightly pressed to its two surfaces (as if two coffee cups were attached to a single saucer, one on each side). Tissue, especially brain sheath, had grown along each surface but we found no evidence of nerve connections having developed between the two lobes.

Two of the four animals had resumed a fully normal rhythmicity, 6 and 12 days respectively, after the operation (Fig. 6, 1770).

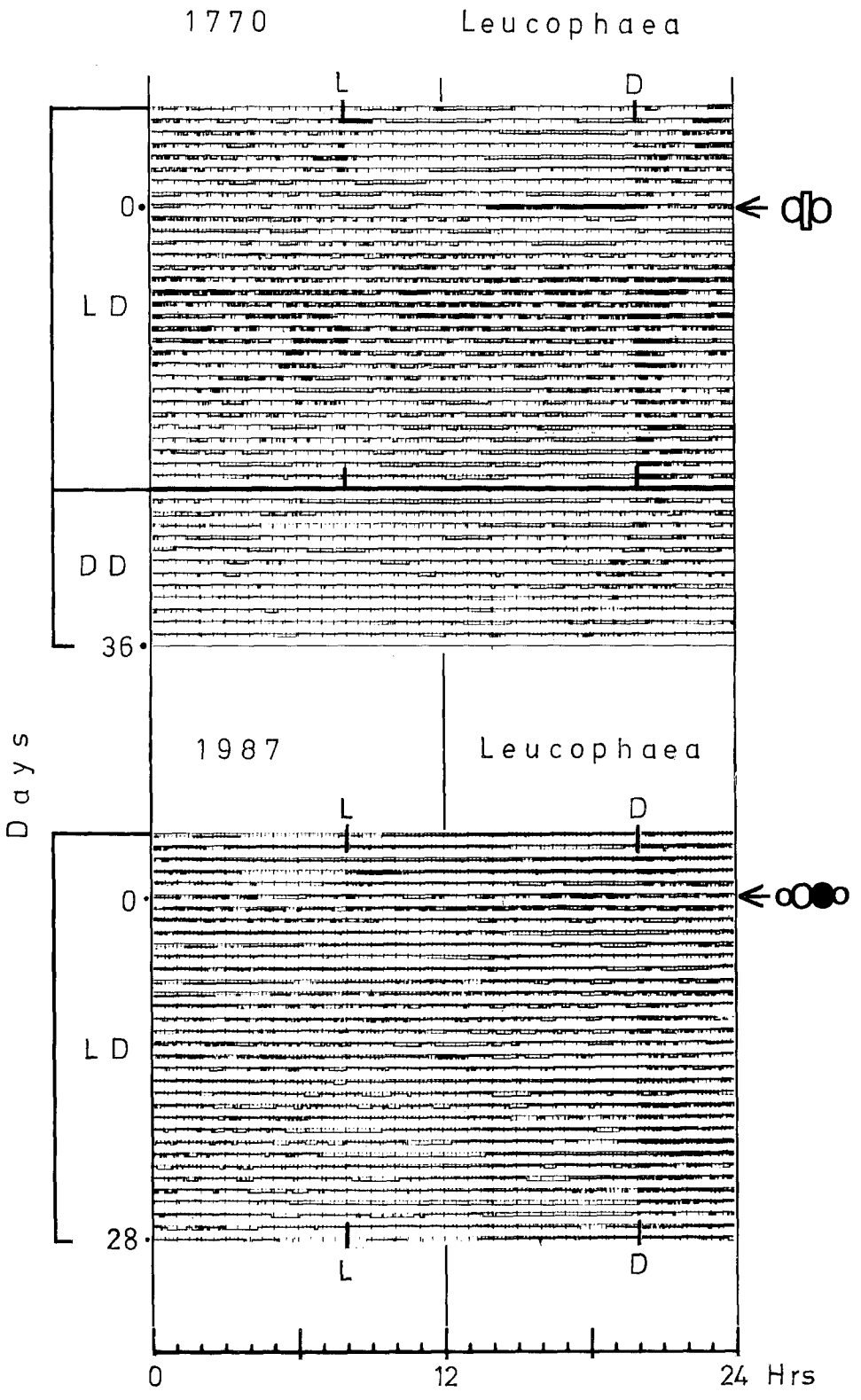

Fig. 6 (for legend see p. 29)

II-2. 6 Animals. Complete Excision of one Lobe of the Protocerebrum (Table 2, II-2). The brain was exposed by removing a square of integument, slightly larger than usual. Mid-sagittal bisection of the protocerebrum was first performed with a razor-fragment scalpel, and the right optic tract was then severed with fine forceps permitting removal of the entire right optic lobe.

The normally entrained rhythm returned (in 1 to 12 days) in four of the six animals (Fig. 6, 1987), generally with an activity level higher than usual.

II-3a. 3 Animals. Lateral Sagittal Section of One Lobe of the Protocerebrum: Subsequent Section of the Other Lobe (Table 2, II-3a). The brain was exposed, as usual, by temporary removal of a square of integument. The section of the protocerebral lobe was made with a fragment of razor-edge. The subsequent operation on the other lobe was performed by temporary removal of the old window of integument.

All three animals resumed rhythmicity at a normal level of activity with, at most, one day of postoperative arrhythmia following severance of the first lobe. When the second lobe was cut all three animals lost their rhythm and died within a week (Fig. 7, 1678).

It is worth noting here that when the square of cuticle was removed for the second operation (1 or 2 weeks after the first) the wound of the first operation appeared to be healed and it was difficult to discern the location of the first cut.

II-3b. 7 Animals. Lateral Sagittal Sections of Both Lobes of the Protocerebrum Simultaneously (Table 2, II-3b). The surgical procedure was that described for Group II-3a.

All seven animals in which both lobes were severed in the same operation failed to recover any rhythmicity before they died. Deaths occurred between one and five weeks postoperatively. Only one animal (Fig. 7, 1833) was sacrificed (33 days postoperatively) before natural death permitting useful histological study. In it, both wounds had "healed" reconstituting a single "brain" but this was completely deformed, and it was impossible to recognize normal tissue relationships.

II-4a. 6 Animals. Unilateral Section of the Optic Tracts (Table 3, II-4a). Access to the brain was again via a square of integument, larger than usual, which was replaced and sealed after sectioning one optic tract with fine forceps.

Fig. 6. *Animal 1770.* A glass plate was inserted into the midsagittal bisection plane (day 0). Activity level increased post-operatively but animal was re-entrained within a week. Rhythm persisted in constant dark (DD) beginning day 23. *Animal 1987.* Right main protocerebral lobe was excised (day 0). The normal rhythm resumed within a week with normal activity level

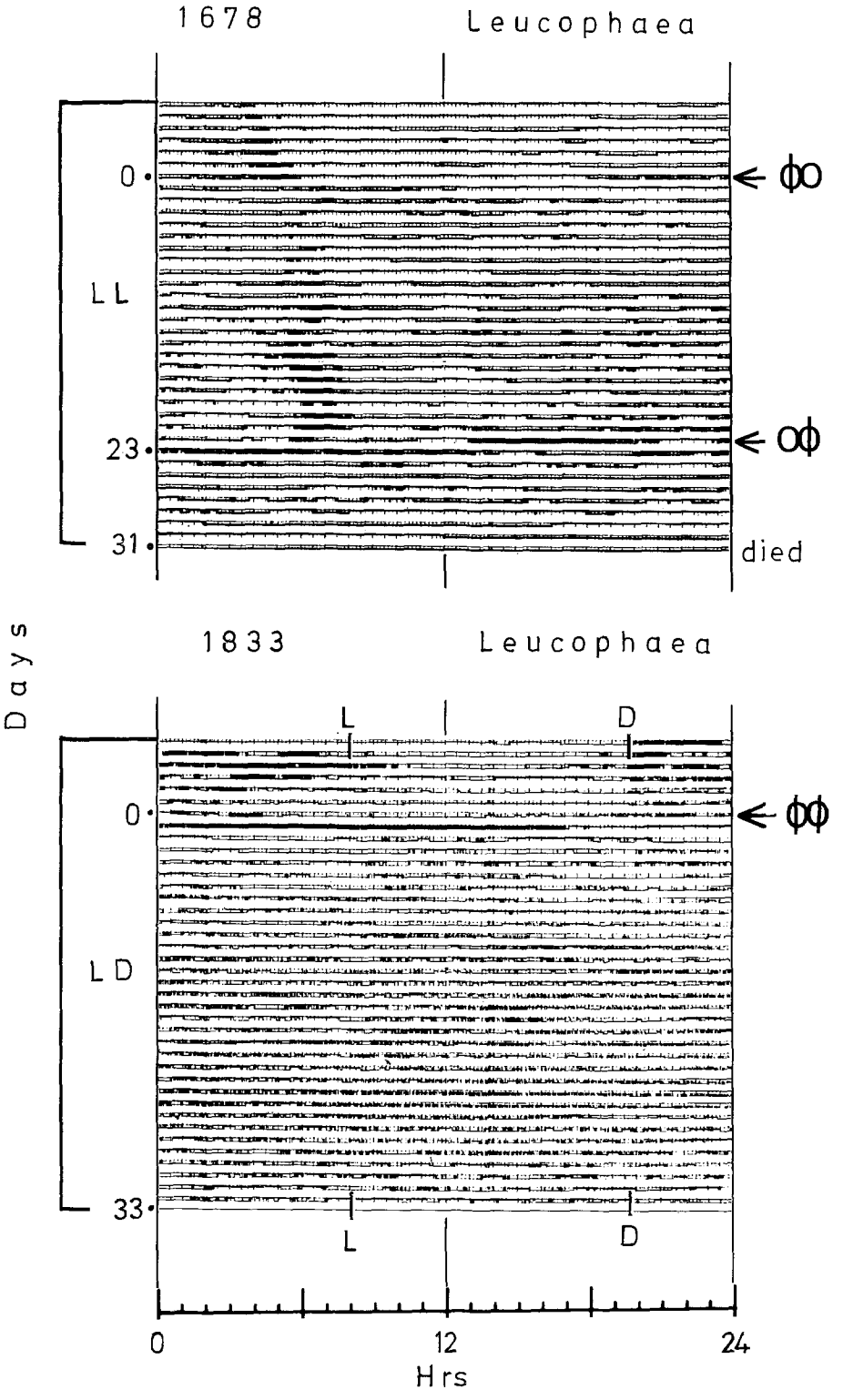

Fig. 7 (for legend see p. 31)

Five of the animals in which the optic tract was severed on one side resumed normal activity levels and normal rhythmicity within three days after the operation (Fig. 8, 1296).

II-4b. 2 Animals. Complete Section of the Left Optic Tract, Incomplete Section of the Right (Table 3, II-4b). The operation was the same as in Group II-4a except for the addition of incomplete severance of the right optic tract.

Normal rhythmicity was resumed in both animals at normal activity levels, 8 and 13 days respectively after the operation. In both cases the cut in the right optic tract extended about halfway through its diameter (Fig. 8, 1258).

II-4c. 18 Animals. Complete Section of Both Optic Tracts (Table 3, II-4c). The six animals in Group 4a (Fig. 8, 1296) were eventually subjected to a second operation in which the remaining tract was severed. In twelve other animals both optic tracts were severed in one operation (Fig. 9). We have, therefore, data on 18 animals subjected to complete bilateral section of the optic tracts.

Fourteen of these (18) lost rhythmicity permanently. One retained normal rhythmicity and *its* behavior remains unexplained by our subsequent discussion. Three animals retained a weak and irregular rhythmicity which also eludes simple explanation in terms of our general interpretations developed below.

Activity levels were in general normal, in contrast with the hyperactivity developed, typically, by pars intercerebralis ablation.

II-5. 6 Animals. Complete Excision of Both Optic Lobes (Table 3, II-5). The brain was exposed more than usual for this operation, by bilateral extensions of the upper edge of the window cut from the integument over the protocerebrum (see Fig. 1, NISHITSUTSUJI-UWO *et al.*, 1968). Each lobe was removed by first severing the optic nerve entering it, and then by separating the proximal boundary of the optic lobe from the adjacent remainder of the protocerebrum. Both cuts were made with fine forceps.

In contrast to the previous group in which only the optic tracts were severed none of the six animals from which the lobes were entirely removed ever recovered rhythmicity. And this result is the more impressive because of their longevity. One animal died in 16 days but the

Fig. 7. *Animal 1678.* Persistence of the activity rhythm following lateral sagittal section through mid-protocerebrum in the left lobe on day 0. The rhythm persisted in LL (constant light) to day 22. The other lobe of the protocerebrum was cut on day 23. The animal lost the rhythm immediately after the second cut and died on day 31. *Animal 1833.* Lateral sagittal section of both protocerebral lobes on day 0 results in permanent arrhythmia (even in LD *12*:12)

Table 3. *Effects of various nerve sections of the brain on the circadian locomotory rhythm in cockroaches (continued)*

Experimental group	Operation procedure	No. of animals	Post-operative observations				
			Days of observation	Activity level	Rhythm in LD	Days until reappearance of rhythm	Animals' state at end of experiment
II-4a		6	12 (8—15)	Normal 5	Normal 5	1 (0—3)	+6
4b		2	20	Normal 2	Normal 2	8, 13	+2
4c		18	51	Normal 10	Normal 1	6	
			37 (4—119)	Lower 3	Loss 14	—	+11
				Higher 3	± 3	35 (26—40)	−7
				Special 2			
5		6	55 (16—156)	Normal 3	Loss 6	—	+5
				Special 3			−1
6		17	94 (18—312)	Normal 15	Normal 3	13 (6—19)	+13
					Free		
				Higher 2	Run 14	6 (0—15)	−4
7		8	46 (36—45)	Normal 4	Loss 2	—	+7
				Lower 1	Free		
				Higher 3	Run 6	13 (3—22)	−1
8	cut A. N.	3	23 (22—24)	Normal 3	Normal 3	6 (6—7)	+3
9	cut Br. II	3	45 (25—47)	Normal	Normal 2	22, 33	+1
				Higher	Loss 1	—	−2

For diagrams, see Fig. 2. Rhythm ±.

others lived many months without any return to normal rhythmicity (Fig. 10).

II-6. 17 Animals. Section of the Optic Nerves (Table 3, II-6). This group has already been reported and discussed in the previous paper of this series which gave a description of the operation procedure. Its behavior is recalled here because of its bearing on that of the following group. Severance of the optic nerves between the ommatidia and the distal sections of the optic lobes uncouples the oscillating system in the brain from the entraining influence of the light cycle, which is exerted via the compound eyes.

II-7. 8 Animals. Section of the Left Optic Tract and the Right Optic Nerve (Table 3, II-7). Both cuts followed the procedures described for earlier experimental groups.

Six out of the eight animals showed a clear circadian rhythmicity which freeran in spite of (was unentrained by) the continuing light/dark cycle (Fig. 11). Two of the animals failed to recover rhythmicity.

II-8. 3 Animals. Complete Section of Both Antennal Nerves (Table 3, II-8). The window cut out of the integument to expose the brain for this operation, was extended frontally more than usual to facilitate severance of both the sensory and motor antennal nerves with fine forceps.

All three animals regained normal rhythmicity within a week after the operation (Fig. 12, 1875).

II-9. 3 Animals. Bilateral Section of the Deuterocerebrum (Table 3, II-9). A razor-fragment scalpel, introduced through the usual window in the integument over the protocerebrum, was used to cut the deutero-cerebrum proximal to the glomerulus of antennal nerve endings, and close, therefore, to the boundary between proto- and deuterocerebrum.

Two of the animals regained normal rhythmicity but only after three (Fig. 12, 1868) and four weeks respectively, of postoperative arrhythmia. The third animal which died on the 25th day after the operation never regained rhythmicity.

Discussion

The Thoracic Ganglia and Ventral Cords

The autonomy of circadian rhythmicity in the thoracic ganglia could, in principle, be established by determining: 1. the presence of rhythmicity, at normal activity levels, in animals whose thoracic ganglia had been isolated by cuts immediately anterior to the first, and posterior to the third thoracic ganglia; and 2. by then showing that the phase of such rhythmicity remained unchanged following a shift in an LD cycle (Group I-3, 4, 5).

That line of analysis was closed to us when we found that severance of the ventral cords between the first thoracic and the suboesophageal (SG) ganglion caused nearly total inactivity (Group I-3).

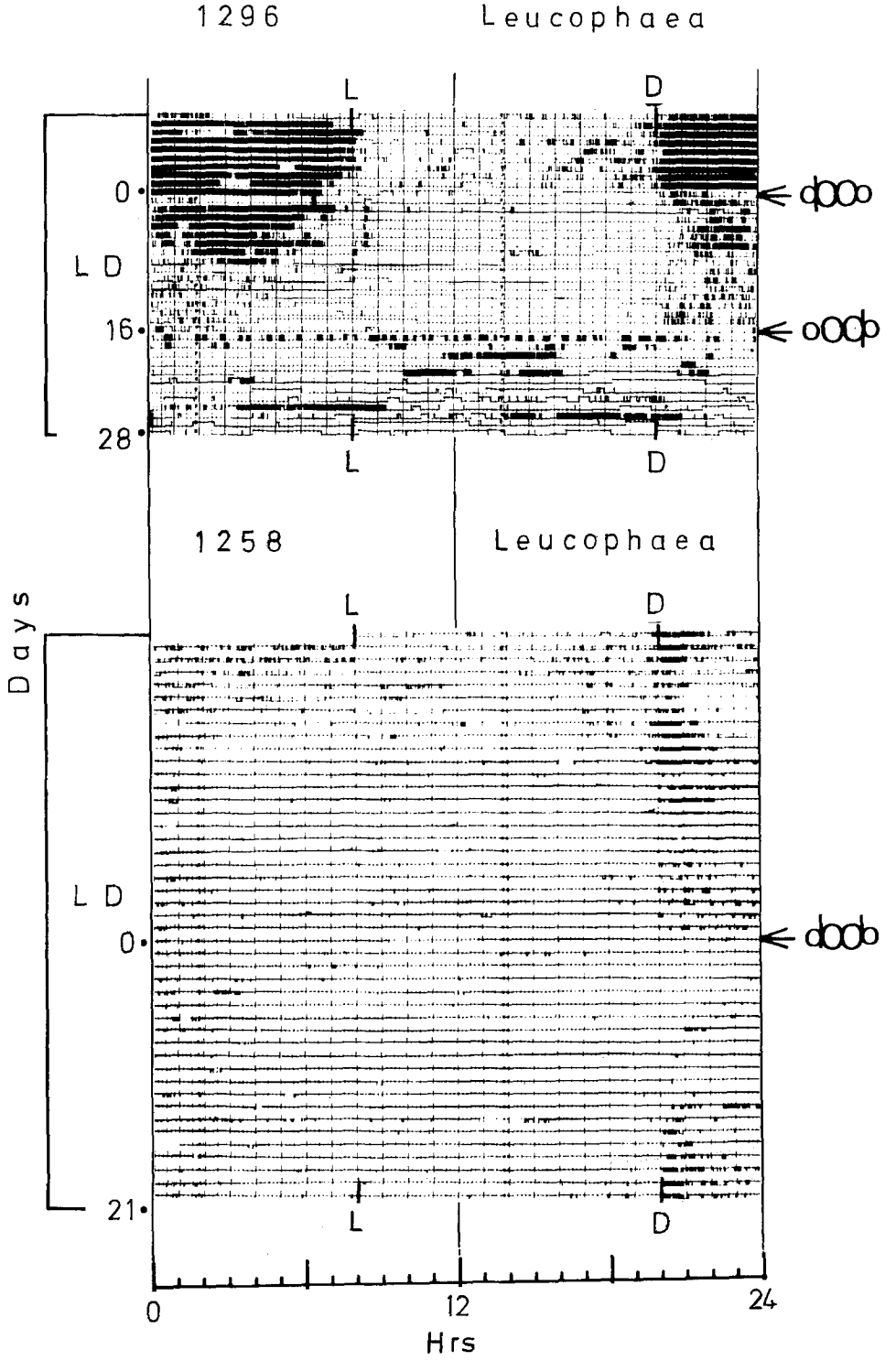

Fig. 8 (for legend see p. 35)

It is, however, fully clear that the ventral cord in the abdomen plays no role: neither severance of the cord between the second and third abdominal ganglia (Group I-6) nor surgical ablation of the terminal ganglion (Group I-7) has any detectable effect on the rhythm whatsoever. Nor did we find in an earlier study (NISHITSUTSUJI-UWO *et al.*, 1967a) any effect on the rhythm attributable to chemical ablation of the third abdominal ganglion by gels of actinomycin-D, which did, however, destroy rhythmicity when inserted into the *pars intercerebralis*.

Severance of the cords between the thoracic ganglia themselves only depresses the activity of the animals. Part of this depression may be due to a resultant reduction of coordination in leg movement, but it is also very probably due to the isolation of one or more ganglia from the suboesophageal ganglion (SG) which appears to be the origin of an activity stimulating influence.

The Suboesophageal Ganglion (SG)

The origin in the SG of an activity stimulator affecting the thoracic ganglia is suggested by two of our observations.

First, complete section of the ventral cord between the SG and the first thoracic ganglion leads to nearly total inactivity. Decapitation which involves the same break in the ventral cords has the same effect; and so has complete ablation of the SG. Section of the cord here thus deprives the ganglia of a stimulus to activity. When the cord is severed it promptly contracts and we cannot exclude the possibility that reduced activity is in part due to a resultant atypical mechanical stress (or its absence) on the complex of thoracic ganglia. But this cannot explain all the facts: activity level increases steadily from Group 3 to 4 to 5; in other words, the more thoracic ganglia that retain neural connections with the SG the higher the level of activity (Table 1). It is difficult to account for this clear trend on the basis of the abnormal mechanical state of the severed cords.

Second, bilateral section of the circumoesophageal connectives isolates the SG from the brain but fails to reduce either the level or the rhythmicity of locomotion: the activity stimulus blocked by severing the cord below the SG originates in that ganglion.

The recorded arrhythmia (Table 1) of animals in which one or more of the thoracic ganglia are isolated from the SG have little bearing on the possible role of that ganglion as the site of the rhythm's driving

Fig. 8. *Animal 1296.* Persistence of the activity rhythm following the first section of the left optic tract (on day 0). On day 16, the right optic tract was cut and animal lost its rhythmicity. *Animal 1258.* Re-development of rhythm following complete section of the left optic tract and incomplete section of the right tract

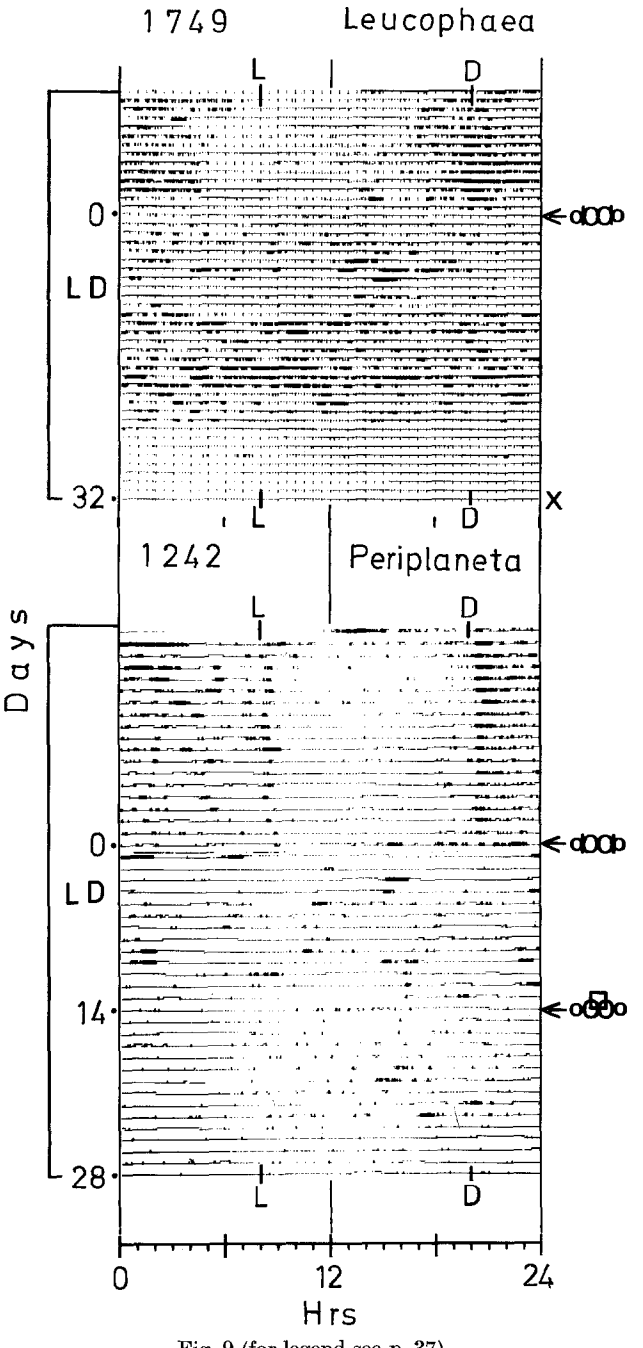

Fig. 9 (for legend see p. 37)

oscillation: their "arrhythmia" means only that there was so little activity at any time of day that the influence of any rhythmic modulator — were it present — would never have been detectable. However, the loss of discernible rhythmicity in Group 3 and 4 does have considerable bearing on HARKER'S well-known view that not only is the SG the site of the responsible oscillation but that the ganglion exerts its influence (on the thoracic ganglia) by rhythmic secretion of a humoral agent. The parabiosis and ganglion transplant techniques that led to this conclusion would certainly demand a humoral agent for any role the SG might have in this regard; but the arrhythmia of 10 out of 14 animals in Group 3 and 4 is quite incompatible with her position. In all those animals an intact ganglion was not only present but had not even been subject to the trauma of excision and transplant; and to that extent there is no reason to attribute the observed arrhythmia to interference with the capacity of the SG to secrete its humoral agents which HARKER has concluded mediate rhythmicity. And, finally, the "loss of rhythmicity" in our animals was, in fact, "loss of activity" and that, as its graded decrease in the series Group 3, Group 4 and Group 5 clearly indicates, derives from severance of a neural not humoral channel.

The behavior of our animals subjected to complete and incomplete ablation of the SG (with and without reimplantation of the excised perikaryon mass) adds little to the evidence from other groups on the role of the SG. Reimplantation had no effect; the incompletely ablated animals were generally inactive and a weakly expressed rhythmicity was discernible in those sufficiently active; complete ablation had the same effect as severing the cords below the ganglion. There is no support, from any of our data, for HARKER'S view of the role of the SG. ROBERTS' (1965) rather full critique of her work on this issue accompanied a report of evidence that was negative in the sense he could not confirm her observations from experiments that followed her original design. We add the positive evidence against her position that an intact, untouched suboesophageal ganglion fails to mediate rhythmicity.

The Brain

The autonomy of the thoracic ganglia themselves was, as we saw, something we could not settle by surgical isolation which inactivated the

Fig. 9. *Animal 1749.* Loss of the activity following bilateral section of the optic tract (on day 0). Animal 1749 showed no rhythmicity with higher activity level for 3 weeks and then low activity for 10 days and he died on day 32. *Animal 1242.* 14 days after bilateral section of both optic tract, a glass window was put above the protocerebrum without effect. This animal's activity level was normal (a typical example of this kind of operation)

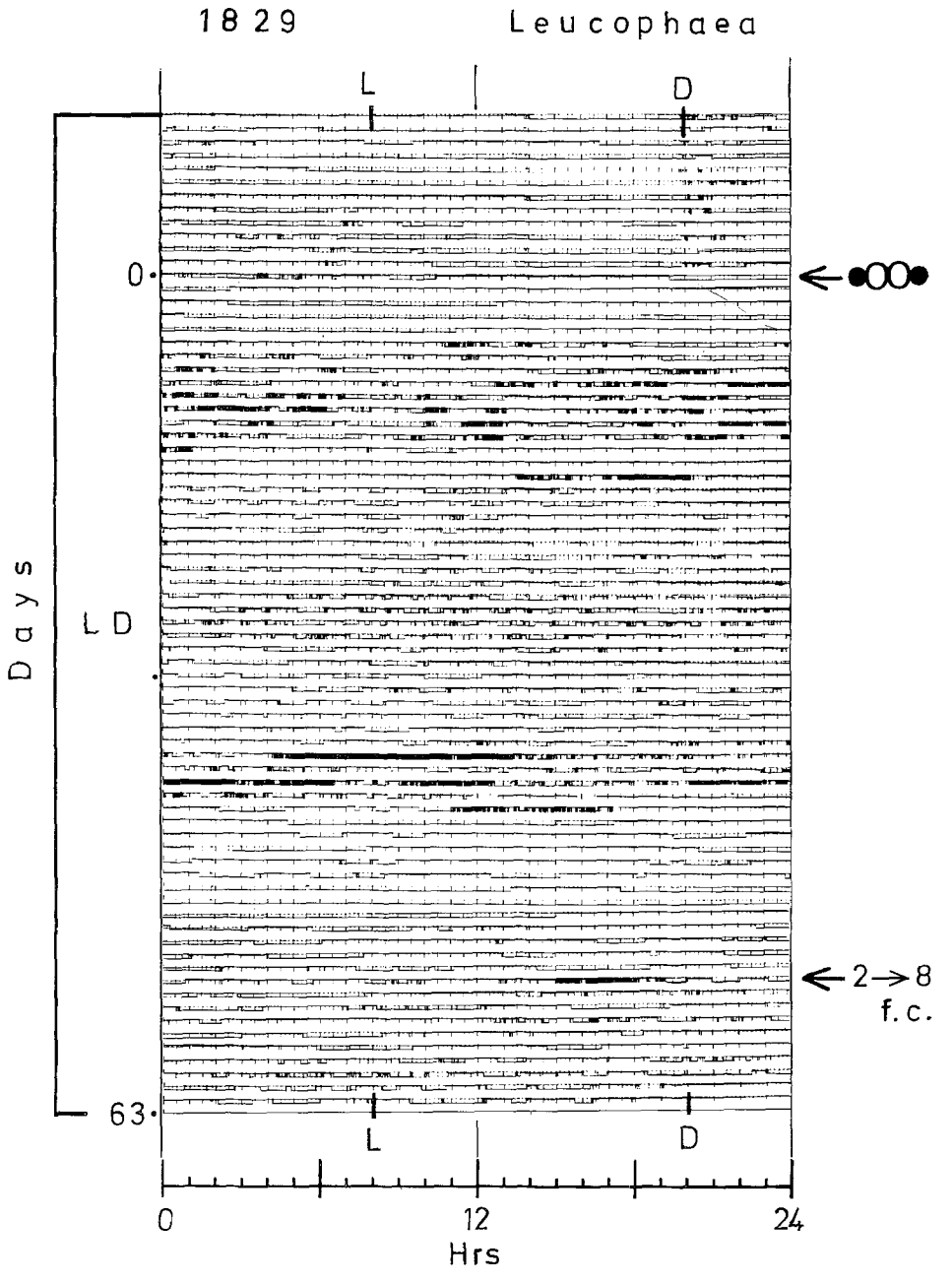

Fig. 10. Loss of the activity rhythm following bilateral ablation of the optic lobe (on day 0). This animal showed occasionally higher activity level or normal level, without showing any overt rhythm. On day 53 the light intensity was increased from 2 to 8 foot candles without effect

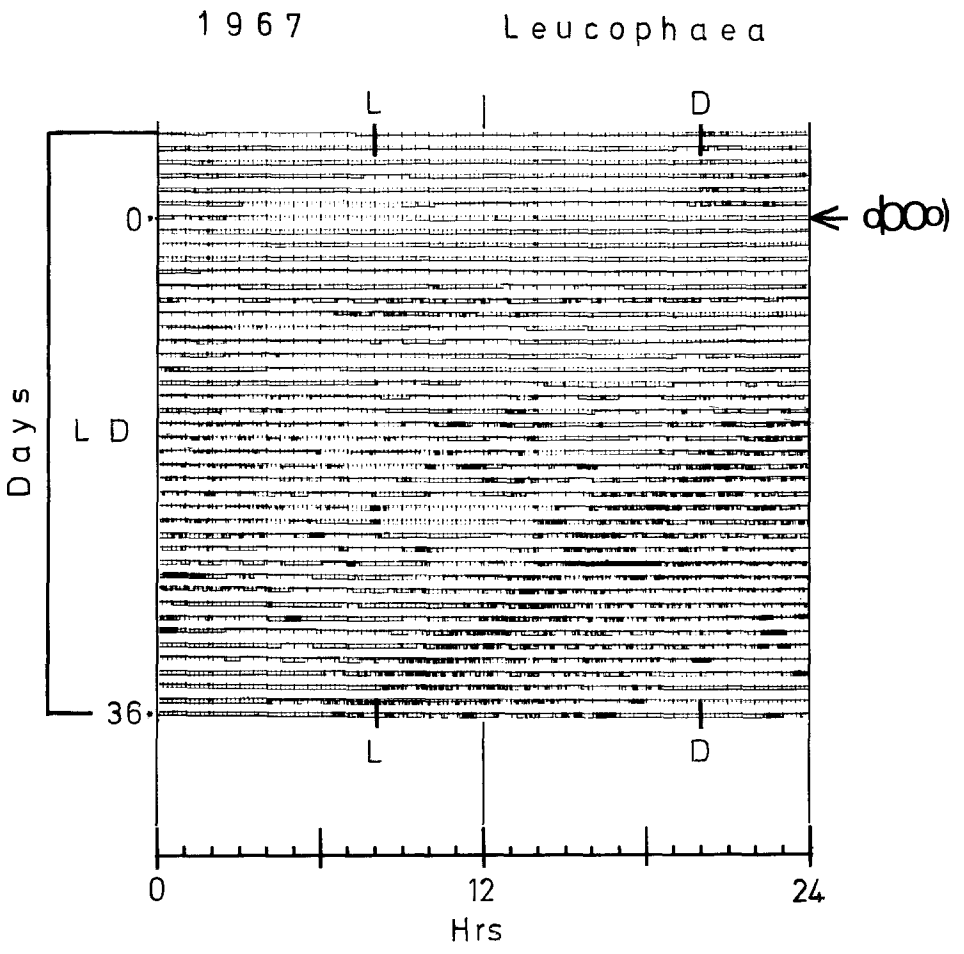

Fig. 11. Freerunning rhythm in LD (light and darkness 12:12 hours) following sections of optic tract in the left lobe and of optic nerve in the right lobe

animals. The conclusion that they are *not* autonomous in their rhythmicity is however strongly indicated by aspects of our earlier data (Table 1, NISHIITSUTSUJI-UWO, PETROPULOS and PITTENDRIGH, 1967) on the effects of *pars intercerebralis* ablation. Thirteen of the 19 animals made arrhythmic by *pars intercerebralis* ablation were also made so hyperactive (Fig. 13) that, on the basis of their behavior alone, one could not exclude the possibility that a rhythmic modulation continued but was simply masked by the uniformly high activity. However, in six of the 19 animals the arrhythmia developed without hyperactivity and these roaches seem crucial in excluding the hypothesis of autonomous rhythmicity in the

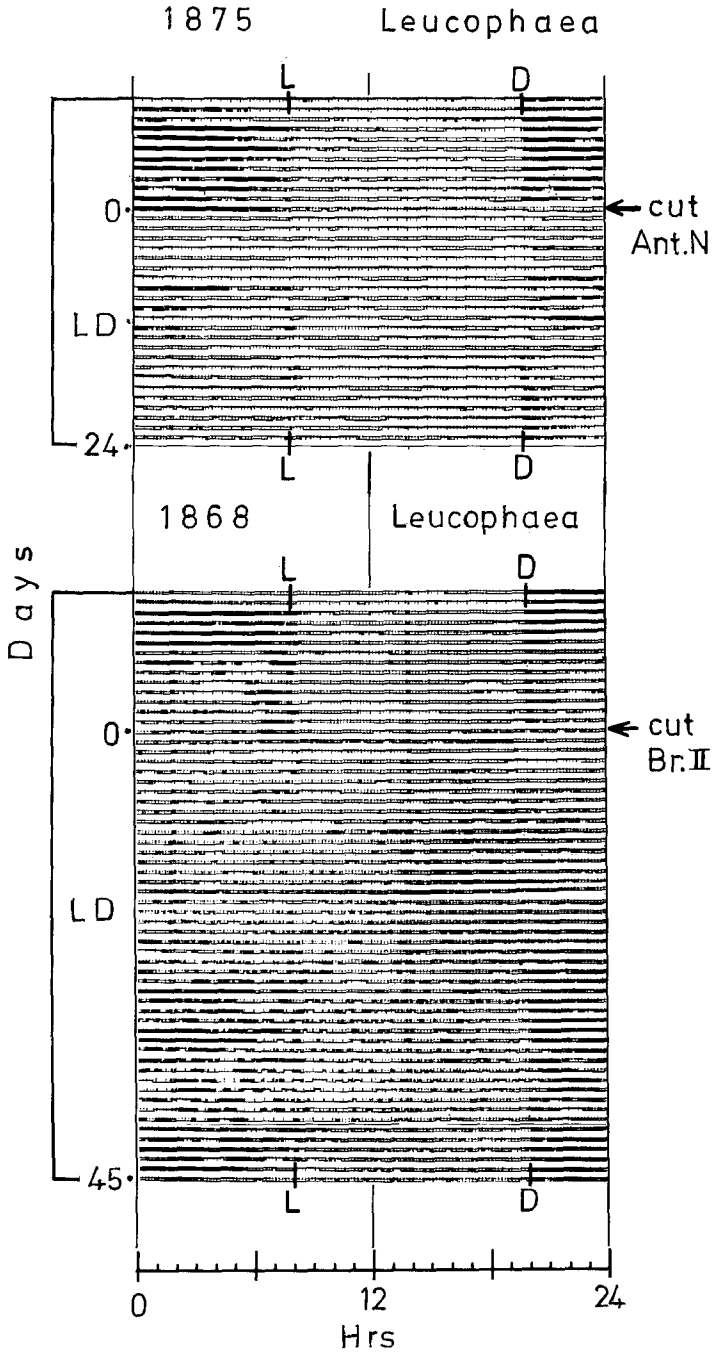

Fig. 12 (for legend see p. 41)

thoracic ganglia, and for that matter in the suboesophageal ganglion also: the presence of a wholly intact complex of suboesophageal and thoracic ganglia is inadequate to assure rhythmicity at normal activity levels in the absence of the *pars intercerebralis*. The brain therefore is evidently the site of the driving oscillation that causes rhythmicity of locomotion.

The experiments performed on Groups II-1 through II-9, in this paper, were intended to clarify the role of the *pars intercerebralis* initially established by surgical and chemical ablations. The fact that neurosecretory cells were found in 58% of the animals which continued rhythmicity following attempted *pars intercerebralis* ablation led us to conclude that they were involved in the mediation of rhythmicity (see Table 1 of NISHITSUTSUJI-UWO *et al.*, 1967a).

Bisection of the protocerebrum (Group II-1) failed to stop rhythmicity permanently in 67% of the animals so treated. We conclude that the lateral neurosecretory cells of the protocerebrum can drive the rhythm: truly midsagittal bisection should, and evidently does, leave their nerve tracts intact. It is the axons of the medial neurosecretory cells (SCHARRER, 1952) which cross and must be severed by bisection. We attribute loss of rhythmicity in 33% (3/9) of our animals in Group II-1 to inadvertent damage to *both* the lateral and medial groups of cells. ROBERTS (1966) earlier reported arrhythmia following bisection of the protocerebrum and concluded from that observation that neurosecretory cells (without specifying lateral or medial) were involved; but that conclusion is justified from bisection experiments only if the surgery is sufficiently blunt to destroy not only the chiasma of axons from the medial cells but those of the uncrossed laterals too, or if (as our data show is not the case) only the medial cells are involved. It is very likely, we believe, that the higher incidence we report (6/9) of the rhythm persisting after bisection, is attributable to the new razor edge we used for *each* of the cuts we made.

The conclusion that it is the neurosecretory cells of the *pars intercerebralis* that mediate rhythmicity is greatly strengthened by the observations reported here on Group I-2. The rhythm persists as though nothing had been done to animals in which both circumoesophageal connectives are cut: in these animals the ventral cord ganglia, including

Fig. 12. *Animal 1875*. Re-development of the activity rhythm following bilateral section of the antennal nerves (on day 0). Within a week normal activity rhythm resumed. *Animal 1868*. Re-development of the rhythm following bilateral transection of the deuterocerebrum (Br. II). Rhythm did not resume until 22 days postoperatively (see text)

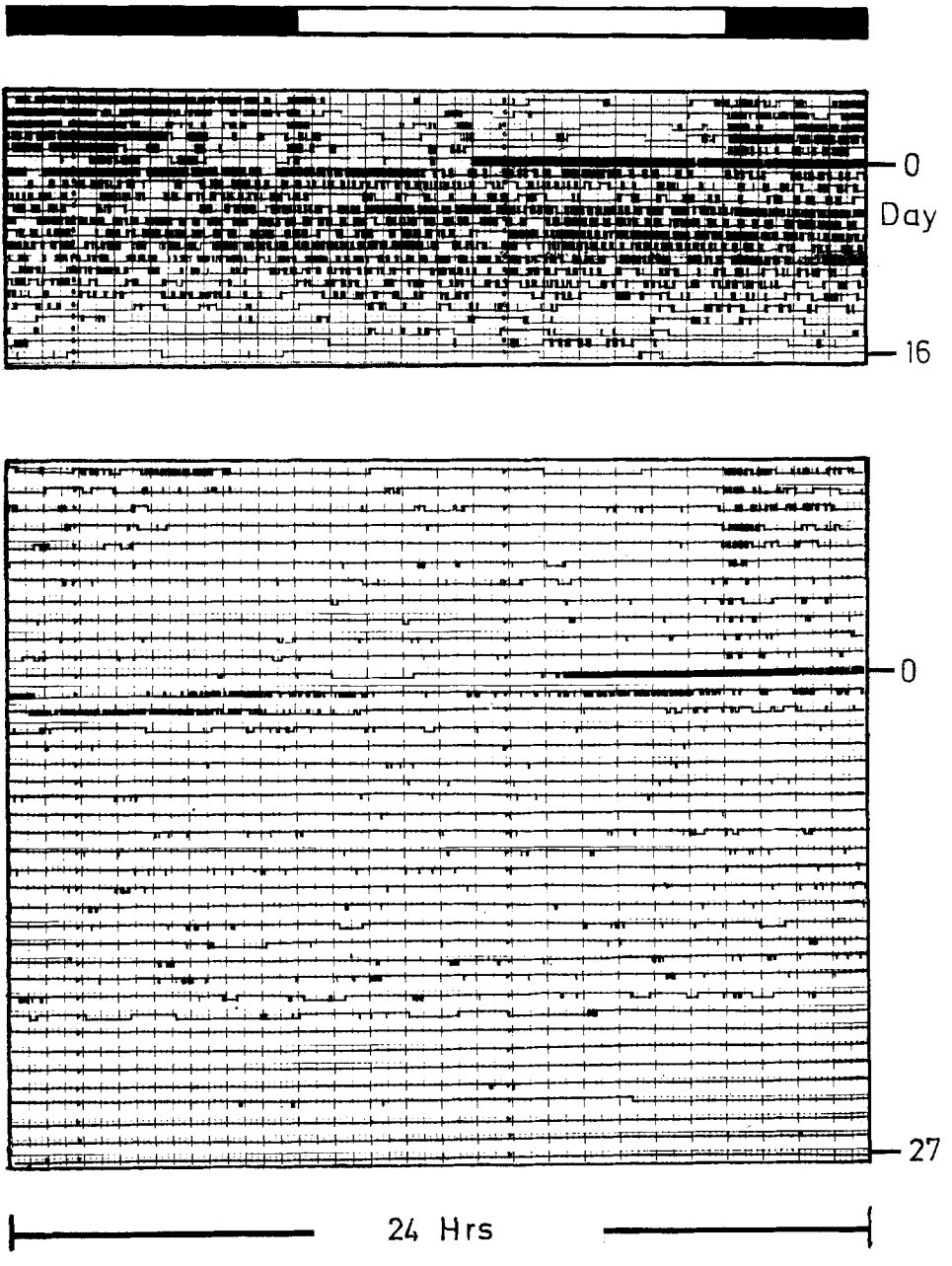

Fig. 13. Loss of the activity rhythm in *Leucophaea madeirae* following complete ablation of the *pars intercerebralis* (see NISHITSUTSUJI-UWO *et al.*, 1967a). The operation was performed on day 0. Loss of rhythmicity is accompanied by hyperactivity in the upper record; but it develops without increased activity in the lower record

the thoracic have *no* neural connectives with the brain whatsoever, and its continuing mediation of rhythmicity must therefore be effected by endocrine not neural channels.

We began operations on the optic tracts prompted in part by knowledge that the optic nerves were the principal, indeed exclusive (NISHITSUTSUJI-UWO and PITTENDRIGH, 1968) pathway to the driving oscillation for the effects of light; when they are severed the system freeruns in light/dark cycle. Would severance of the optic tracts, isolating the *pars intercerebralis* from the light cycle, similarly lead to a freerunning rhythm ?

To our surprise removal of the lobes caused total arrhythmicity, and (typically) without effecting either a decrease or increase in activity level. Indeed severance of the lobes from the *pars intercerebralis* (II-4c) is far more effective (74% vs. 40%) in inducing arrhythmicity than the attempt to excise the *pars intercerebralis* surgically.

The *pars intercerebralis* (almost surely its neurosecretory cells) though indispensable for the expression of normal locomotory rhythmicity is clearly not, of itself, an autonomous oscillator: it only *mediates* rhythmicity when it has intact neural connectives with the optic lobes. To be sure, the input from the lobes could be — in principle — arrhythmic and still be essential to the expression of rhythmicity by the *pars intercerebralis*. No technically feasible experiment has occurred to us that could exclude that possibility. Thus it could well be that the *autonomous* system is the entire protocerebrum; but it is also possible, and we feel likely, that the optic lobes are themselves oscillatory in their output to the *pars intercerebralis* and hence the locus of the driving oscillation ultimately responsible for the whole system underlying the rhythmicity of locomotion. We note that EIDMANN's (1956) conclusion that the optic lobes are responsible for the locomotory rhythm in *Carausius morosus* is based on only 24 hours of postoperative observation which, as we have emphasized (on the basis of our experience with roaches) is inadequate for useful evaluation of the consequence in the rhythm system of *any* brain operation.

The *pars intercerebralis*, in this view, is a simple follower; when uncoupled from the lobes its endocrine output reverts to an arrhythmicity. More strictly we should infer that *one* of its endocrine outputs does so, because the facts we have suggest there are two and only one of them concerns the rhythm of locomotion.

We recall here that attempts to excise the entire *pars intercerebralis* generally elevated the activity level as well as causing a loss of rhythm. But the two effects are separable. Table 1 in our 1967 paper showed that six of the 19 animals made arrhythmic by loss of the *pars intercerebralis* continued activity at a normal, unelevated level. Fig. 13 in this paper exemplifies loss of rhythm with and without hyperactivity.

The six animals (out of 19) that became arrhythmic, without hyperactivity (Fig. 13, bottom panel), are important in two respects: 1. they obviously establish the separability of *pars intercerebralis* effects on level and rhythmicity; and 2. in doing so also dispose of a troublesome possibility which the other 13 (out of the 19) *pars intercerebralis*-less animals leave open, viz. that their arrhythmicity is as uninteresting as that of SG-less animals in the sense that the activity *level* obscures the presence of a rhythmic modulator. It is clear from the six arrhythmic animals whose activity level is normal that in losing the *pars intercerebralis* they lost a rhythmic modulator of their activity. That is precisely what we could *not* conclude from the arrhythmicity that follows SG loss.

Both *pars intercerebralis* effects — on level and on rhythmicity — are evidently mediated by endocrine channels as the fully normal behavior (as to level *and* rhythmicity) of Group I-2 indicates: complete surgical isolation of the ventral cord complex by severance of the circumoesophageal connectives leaves *pars intercerebralis* action unimpaired.

The only element in our data which appears in conflict with our conclusion that the driving oscillation is certainly autonomous to the protocerebrum and very probably to its optic lobes, concerns the effects of severing the deuterocerebrum. Arrhythmicity followed that operation for 22 and 33 days in two of our three tested animals before a normal, and entrained, rhythmicity returned. [The one animal which Table 3, (II-9) records as failing to recover rhythmicity died on the 25th day after the operation, well within the range the other two show is necessary for recovery.] One could take these results to imply that the boundary of the autonomously oscillatory system should be extended to include, as necessary, the deutero- as well as the protocerebrum, but we are inclined not to do so for two reasons. First, the operation is very difficult to perform with any real assurance that no damage was imposed on the protocerebrum, close to the optic lobes: we sought to make the cut proximal to the glomeruli of antennal nerve endings and in doing so were forced close to the illdefined boundary of proto- and deuterocerebrum. Second, both surviving animals *did* recover rhythmicity, in sharp contrast with the low incidence of eventual rhythm recovery among animals subjected to bilateral optic tract severance or optic lobe ablation.

The long recovery time required by the two survivors in II-9 is certainly difficult to dismiss as "simple" postoperative "trauma", and we assume that the damage clearly done to the rhythm's driving oscillation was in fact damage at the boundary of the protocerebrum. In this context we reemphasize that almost any operation on the brain (as contrasted with the ventral cords and their ganglia) causes some postoperative loss of rhythm whose variable duration can be so prolonged as

to suggest some general "healing" processes must occur. Whether or not regeneration of broken pathways is involved in the longest recovery times is not known.

The Internal "Clock" and its Coupling to the Environment

It is, thus, our view that the circadian rhythmicity of roach locomotory activity is ultimately caused by an autonomous self-sustaining oscillation in the output of the optic lobes which causes (a non-autonomous) circadian periodicity of secretion by the *pars intercerebralis* which in turn imposes a circadian periodicity on the activity of the thoracic ganglia which control walking.

The driving oscillation in the optic lobes is the internal daily or circadian clock of the cockroach. When the animal is maintained in constant darkness and constant temperature the "natural" frequency (or period) of the optic lobe oscillation dictates the period of the locomotory rhythm: it is close to, but not exactly 24 hours; it is *circadian* (Fig. 11). When the animal experiences — or "sees" — a daily light/dark cycle in the environment its activity rapidly becomes limited to the nightly period of darkness, *not* because light inhibits cockroach activity (it usually stimulates it) but because the oscillation in the optic lobes locks-onto (is entrained by) the 24 hour cycle of light and dark, and in so doing not only "follows" the exact period (24 hours) of the entraining light cycle, but assumes a unique phase relationship to it; that phase is such that locomotory activity (promoted during only a fraction of the physiological cycle initiated by the optic lobes) occurs in the darkness.

The coupling of the internal clock, or oscillation, to the environment is effected by the compound eyes and the optic nerves which couple them directly to the optic lobes. The complex of relationships involved is made very clear by the behavior in a 24 hour light-dark cycle of these animals (Group II-7) in which the optic tract was severed on the left side and the optic nerve on the right. The left optic lobe though still coupled to the light cycle by an intact optic nerve is unable to drive the *pars intercerebralis*. The right optic lobe still coupled to the *pars intercerebralis* can and does impose rhythmicity upon it; but severance of the optic nerve on the right side prevents the environmental light cycle from entraining this one effective clock, or oscillation in the animals, which displays a clear *freerunning* circadian rhythmicity (Fig. 11) in spite of being in LD *12*:12.

Postscript

Following completion of this manuscript two papers by BRADY (1967a, b) have been published, reporting investigations very similar to our own. Several specific issues are explicitly treated by both BRADY and ourselves, and on all of them our conclusions are fully compatible and in some cases identical. Thus,

1. We both find the medial neurosecretory cells of the *pars intercerebralis* are dispensable in the control of circadian rhythmicity in cockroaches. (However our own study demonstrates the lateral neurosecretory cells are involved.)

2. We both find that the abdominal ganglia play no role in the control of rhythmicity.

3. We both find positive evidence against the position taken by HARKER that neurosecretion from the suboesophageal ganglion mediates rhythmicity.

4. BRADY'S position is that the brain is the locus of the pace making oscillation; in this we also concur but go further in a) localizing it to the protocerebrum and indeed to the optic lobes, and b) in implicating neurosecretion from the *pars intercerebralis* as an essential, intermediary, link in the chain of control.

References

BRADY, J.: Control of the circadian rhythm of activity in the cockroach. I. The role of the corpora cardiaca, brain and stress. J. exp. Biol. **47**, 153—163 (1967a).

— Control of the circadian rhythm of activity in the cockroach. II. The role of the suboesophageal ganglion and ventral nerve cord. J. exp. Biol. **47**, 165—178 (1967b).

CAMERON, E.: The cockroach. London: William Heinemann, Medical Books Ltd. 1961.

EIDMAN, H.: Über rhythmische Erscheinungen bei der Stabheuschrecke *Carausius morosus*. Z. vergl. Physiol. **38**, 370—390 (1956).

HARKER, J. E.: Factors controlling the diurnal rhythm of activity in *Periplaneta americana*. J. exp. Biol. **33**, 224—234 (1965).

— The effect of perturbations in the environmental cycle of the diurnal rhythm of activity of *Periplaneta americana* L. J. exp. Biol. **37**, 154—163 (1960a).

— Internal factors controlling the suboesophageal ganglion neurosecretory cycle in *Periplaneta americana* L. J. exp. Biol. **37**, 164—170 (1960b).

NISHIITSUTSUJI-UWO, J., S. F. PETROPULOS, and C. S. PITTENDRIGH: Central nervous system control of circadian rhythmicity in the cockroach. I. Role of the pars intercerebralis. Biol. Bull. Wood's Hole (in press) (1967a).

—, and C. S. PITTENDRIGH: Neuroendocrine basis of midgut tumor induction in cockroaches. J. Insect Physiol. **13**, 851—859 (1967b).

— — Central nervous system control of circadian rhythmicity in the cockroach. II. The pathway of light signals that entrain the rhythm. Submitted to Z. vergl. Physiol. (1968).

ROBERTS, S. K.: Significance of endocrines and central nervous system in circadian rhythms. In: Circadian Clocks (ed. J. ASCHOFF), p. 198—213. Amsterdam: North Holland Publishing Co. 1965.

SCHARRER, B.: Neurosecretion. XI. The effects of nerve section on the intercerebralis-cardiacum-allatum system of the insect *Leucophaea madeirae*. Biol. Bull. Wood's Hole **102**, 261—272 (1952).

STRUMWASSER, F.: The demonstration and manipulation of a circadian rhythm in a single neuron. In: Circadian Clocks (ed. J. ASCHOFF), p. 442—462. Amsterdam: North-Holland Publishing Co. 1965.

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