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Temporal Changes in the Ommatidial Structure of the Cockroach, Leucophaea Maderae

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TEMPORAL CHANGES IN THE OMMATIDIAL STRUCTURE
OF THE COCKROACH, *LEUCOPHAEA MADERAE*

A Thesis

Presented to

the Faculty of the Department of Biology

Western Kentucky University

Bowling Green, Kentucky

In Partial Fulfillment of

the Requirements for the Degree

Master of Science

by

Becky Green Reitcheck

June, 1990

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TEMPORAL CHANGES IN THE OMMATIDIAL STRUCTURE
OF THE COCKROACH, *LEUCOPHAEA MADERAE*

Recommended _June 21, 1990_

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Approved _July 20, 1990_

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ACKNOWLEDGMENTS

I would like to express sincere gratitude to my advisor, Dr. Blaine Ferrell, for all his guidance. Thanks also to Mr. J. Rodney McCurry for all his technical knowledge and advice. I would also like to thank the members of my committee, Dr. Rudolph Prins and Dr. Kenneth J. Balak.

This thesis is dedicated to my husband, Allen Reitcheck, and my parents, Thurston and Betty Green, whose love, encouragement and support made this effort possible.

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OF THE COCKROACH, <u>LEUCOPHAEA</u> <u>MADERAE</u>

Becky G. Reitcheck   July, 1990   33 pages

Directed by: Drs. Blaine R. Ferrell, Rudolph Prins and Kenneth Balak

Department of Biology   Western Kentucky University

A circadian rhythm in eye sensitivity to light has been previously reported for *Leucophaea maderae*. Temporal changes in eye cell morphology that could be correlated with those changes in eye sensitivity to light were examined. Rhabdom area, screening pigment organization and palisade layer area about the rhabdom were the parameters measured to detect structural change through time. Measurements of those parameters from tissue samples obtained from the anterior one-third of compound eyes surgically removed at midday, light offset, midnight and light onset from roaches entrained to a 12-h light / 12-h dark photoperiodic cycle were used to assess the daily pattern of morphological changes. Eyes were removed at subjective midday and subjective midnight from roaches free-running under constant conditions of temperature and darkness to detect circadian changes. All roaches received food and water *ad libitum*. Tissue samples were fixed, embedded, sectioned and the sections were examined and photographed using a Zeiss transmission electron microscope to test for time-related morphological differences. The extent of pigment organization was determined by counting the number of pigment granules found within a 10μm diameter circle centered about the rhabdom. The rhabdom area and the palisade layer area were determined by the Jandel PC3-D computer program. The rhabdom area did not vary throughout the day. The organization of screening pigment granules and the

palisade layer area did vary on a daily basis. In animals maintained under constant environmental conditions the rhythm of the organization of the pigment granules did not persist. In contrast, temporal changes in the palisade layer area did persist for three cycles with a pattern similar to that in roaches held under LD12:12 and thus could be considered a circadian rhythm controlled by a pacemaker.

INTRODUCTION

Circadian rhythms in behavior (e.g. locomotor activity) and physiology
(e.g. metabolism) are present in unicellular to complex multicellular
organisms. To be considered circadian a rhythm must have a period length of
approximately 24 h and be repeated at least two times in the absence of
external timing cues, such as photoperiod and temperature cycles. The
persistence of such a rhythm in the absence of environmental entrainment is
indicative of internal regulation by an endogenous timer, the pacemaker.
Locomotor activity is an easily monitored event that recurs on a circadian
basis and as such has been used to assess the operation of such a pacemaker.
Evidence from studies of circadian locomotor activity in the cockroach,
Periplaneta americana, has indicated that an efferent output from the brain
imposes a circadian rhythmicity on the activity of the thoracic ganglia which
control walking (Roberts 1974). When the optic lobes of the brain are
removed, the animals express total arrhythmicity of locomotor activity,
indicating that the pacemaker that drives this rhythm is located in the optic
lobes and is connected via a neural pathway (Nishiitsutsuji-Uwo and
Pittendrigh 1968). Transplantation experiments involving the removal of the
optic lobes from a donor organism and placement of these optic lobes into a
host organism of the cockroach species *Leucophaea maderae* have confirmed
that the pacemaker is located in the optic lobes. Although the host animal
will express arrhythmicity for a few weeks until the neural connections are
regenerated, the host animal expresses the periodicity of the rhythm of the
donor animal (Page 1982, 1983).

Another rhythm expressed in *L. maderae*, as in other arthropod species studied (Barlow et al. 1980), that recurs on a circadian basis is a rhythm of eye sensitivity to light (Wills et al. 1985). Eye sensitivity to light was measured by determining the change in an electrical potential in response to a flash of light, an electroretinogram (ERG). The amplitude of the potential changes in response to a brief pulse of light in *L. maderae*, tethered under constant dark conditions, changed on a circadian basis. Furthermore, the pacemaker that controls the expression of this rhythm in *L. maderae* has been located in the same general area as the one that regulates the expression of the locomotor activity rhythm. There appears to be a neural connection between the pacemaker in the brain and the eye that regulates the expression of this light sensitivity rhythm similar to the condition described for other arthropods (Barlow et al. 1980). A series of lesion studies were done in the optic lobes of *L. maderae* to determine what part of the brain was generating the rhythm leading to eye sensitivity (Wills et al. 1985). These studies determined that an efferent pathway from the brain to the eye is involved in generating the rhythm of eye sensitivity and that the pacemaker for this and the rhythm of locomotor activity lie in close proximity to one another. Circadian rhythms of locomotor activity and photoreceptivity have been studied in organisms ranging from *Paramecium* (Nakajima and Nakoaka 1989) to *Limulus* (Chamberlain and Barlow 1979), *Bulla* (Block and Davenport 1982) and *Leucophaea* (Nishiitsutsuji-Uwo and Pittendrigh 1968).

Anatomical changes in ommatidia may be responsible for changes in eye sensitivity to light. Temporal morphological changes have been described for ommatidia in another cockroach species, *P. americana* (Butler 1973), which include the movement of screening pigment granules within retinula cells about the rhabdom and the presence of a palisade layer about the rhabdom.

Three hypotheses have been advanced to explain how an anatomical change could have an influence on eye sensitivity to light. The first proposes that there may be changes in the number of photoreceptor molecules in the rhabdom available to catch photons (Colwell and Page 1989). The second proposes that changes in the organization of screening pigment granules about the rhabdom in the retinula cells could lead to a change in the probability that a photon would reach the photoreceptor (i.e. rhabdom) (Wills et al. 1985). A third hypothesis is that the strength of the signal in response to each photon is altered through a process of amplification. The possibility exists that the palisade layer, which is more prevalent at night, contains a regulatory ion that amplifies the photoreceptor response to each photon (Baumann and Walz 1989). This study was carried out to determine anatomical changes consistent with these hypotheses that account for changes observed in eye sensitivity to light.

In terms of its anatomical and physiological properties, the cockroach is one of the best understood in terms of circadian organization, which makes it a good choice as a model organism in which to study circadian systems. The general location of a pair of mutually-coupled pacemakers within the brain of *L. maderae* has been determined through a series of studies (Page et al. 1977 and Page 1978). Additional studies have employed brain lesions (Wills et al. 1985) or measurements of the rate of metabolic activity (Lavialle et al. 1989) to isolate regions of the optic lobes involved. For example, lesions made proximal to the distal edge of the lobula neuropil of the optic lobes did not affect the rhythm of ERG amplitude, whereas, bisection of the optic lobes distal to the lobula abolished this rhythm (Wills et al. 1985). Evidence from studies carried out in *L. maderae* in which circadian rhythms of cytochrome oxidase activity in various optic lobe structures indicates the site of the clock

is in the lobula plate of the lobula neuropil in the optic lobes of the brain (Lavialle et al. 1989). A pacemaker or pacemakers within this same area of the brain controls the circadian rhythms of locomotor activity, eye sensitivity to light and metabolism described earlier. However, whether one clock controls all three rhythms or that each rhythm is controlled by a separate clock can not be established until the cells of the clock(s) have been located. Determining which of these conditions is in operation is important to understanding circadian organization in this model system. If some anatomical change within a single cell could be determined to occur on a circadian basis, then it should be possible to establish the exact location of the cells comprising the pacemaker by tracing the efferent pathway from its connection with this anatomical unit back to its origin in the pacemaker. Anatomical changes in ommatidia structure do occur on a daily basis in another cockroach species *P. americana* that may have an effect on eye sensitivity (Butler 1973). These changes include the movement of screening pigment granules and palisade layer formation within anatomical units, retinula cells. Because temporal changes in eye sensitivity to light recur on a circadian basis, morphological changes correlated with changes in light sensitivity should also occur on a circadian basis. Therefore, another aim of this study was to determine if these temporal changes in the anatomy of retinula cells within the cockroach, *L. maderae*, compound eye persist on a circadian basis.

MATERIALS AND METHODS

Cockroaches, *L. maderae*, were reared in a colony maintained under a light/dark cycle of 12 hours of light and 12 hours of dark (LD12:12) and provided with food (i.e. Puppy Chow) and water *ad libitum*. Only male cockroaches were used for the experiments in order to ensure uniformity. Prior to each experiment, the animals were placed in environmental chambers set at the same photoperiodic schedule as the parent colony and a constant temperature of 25 ± 2 °C. Six animals in each environmental chamber were each placed in one of six running wheels equipped with magnetic reed switches wired to an Esterline Angus event recorder. Magnets mounted on the wheel closed the switch with each revolution of the wheel and a dash mark was made on a paper strip chart moving at a constant rate. Using this set-up, the locomotor activity was continuously recorded and the activity patterns of representative cockroaches were determined. Food was placed on a hook attached to a rubber stopper in the center of the stationary face plate of each running wheel. Water was provided via a stoppered tube fitted with a cotton wick that ran through the stationary face plate of the running wheel. Additional animals were kept in small plastic cages.

The daily rhythm of representative morphological changes of ommatidia from *L. maderae* was determined first. Only after the animals in running wheels had become entrained to LD12:12, as determined by analysis of activity recordings, were the eyes surgically removed for analysis. Eyes were

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removed from different cockroaches at four times of day: midday, lights offset, midnight and light onset. At midday the eyes were removed under white light but at midnight the eyes were removed under red light with a wavelength >630 nm. In order to surgically remove eyes, each animal was transferred from a running wheel or plastic cage to a large plastic Petri dish with two small holes cut in the lid. A tube leading from a water trap hooked to a CO_2 cylinder provided the CO_2 anesthesia via one of these holes throughout the surgical procedure. After the animal was anesthesized, the head of the animal was placed through the other triangular shaped hole. A piece of tape was placed under the head to keep it from falling through the hole, and another piece of tape was placed over the body to secure the animal. The anterior one-third of the compound eye was removed using a fractured razor blade scalpel, scissors and a pair of forceps. Small strips of eye tissue were removed from the eye and immediately placed in 4% glutaraldehyde in a microvial for 2.5 h. The tissue samples were then rinsed twice for 15 min. each time in Sorensen's phosphate buffer, pH 7.2. The solution was changed to a 1% osmium tetroxide solution for 1.5 h. After rinsing twice with buffer for 15 min. each time, the tissue samples were subjected to a series of alcohol dehydrations: 20%, 35%, 50%, and 70%. The samples remained in 70% alcohol until all samples were ready for embedding. The eye tissue removed at midnight remained in the dark until the first phosphate buffer rinse. After all samples were ready for embedding, the series of alcohol dehydrations was continued: 85%, 95%, and 100% ethyl alcohol twice. Each alcohol dehydration step was for 10 min. The tissue samples were then subjected to 3 parts 100% alcohol to 1 part Spurr's resin. Spurr's resin was used for embedding the samples for transmission electron microscope evaluation. The eyes were subjected to equal parts of Spurr's resin and alcohol, followed by 3

parts Spurr's resin to 1 part alcohol. The final step was 100% Spurr's resin. Each of the last steps was for 0.5 h. The samples were placed in the bottom of Beem capsules with the convex side facing down. Spurr's resin was placed into the Beem capsule along with the appropriate labels. Care was taken to ensure that no air bubbles were present in the tip of the Beem capsule. The capsules were placed in capsule holders which in turn were placed in an oven set at 60 °C for approximately 11.5 h. not to exceed 12 h. The cured resin was allowed to cool for about 24 h. The Beem capsule was removed from the hardened resin by using a razor blade.

A Reichert OM U2 ultramicrotome was used to cut tissue sample sections using a glass knife. The sections were collected on copper grids that were 200 or 300 mesh. The samples on the grids were then examined using a Zeiss 9S2 transmission electron microscope. All examinations were at a magnification of 1950 times. Photomicrographs were made of ommatidial cross sections taken immediately proximal to the crystalline cone. The electron micrographs were printed at an enlargement of 3.4 (i.e. total magnification of 6630) to ensure that all data were uniform.

The same procedure was used to detect the presence of a circadian rhythm, except that the lights in the environmental chambers were turned off following at least one week of acclimation to chamber conditions of LD12:12 and 25 ± 2 °C. The activity recordings were necessary to determine the roach's subjective midday and subjective midnight, times when the eyes were removed to make possible comparisons with the conditions observed at these times of day in entrained animals. All dissections were made under red light. For statistical purposes all samples included nine ommatidia, three ommatidia from each of three separate animals.

Micrographs of cross sections immediately proximal to the crystalline cone were analyzed for three morphological features: 1) organization of pigment granules, 2) rhabdom area and 3) palisade layer area. The organization of pigment granules was determined by centering a 10 μm circle around the rhabdom and counting the number of pigment granules present within the circle. The areas of the rhabdom and palisade layer were determined using a Jandel 3-D computer program. The micrographs were traced into the computer and the computer determined the area of these structures automatically. One-way analysis of variance and Tukey's range test were used to determine significant differences at the 95% confidence level.

RESULTS

Ommatidia of light-adapted eyes (Figure 1) were characterized by rhabdoms with an average area of 23.6 ± 8.8 μm^2 (mean $\pm$ standard deviation), an average of 99 ± 21 screening pigment granules clustered about the rhabdom and an average palisade layer area of 2.1 ± 2.0 μm^2. Ommatidia of dark-adapted eyes (Figure 2) were characterized by a rhabdom area of 32.4 ± 20.3 μm^2 (Figure 3), significantly fewer (mean of 29 ± 16, P<.05) screening pigment granules about the rhabdom (Figure 4) and a significantly greater mean palisade layer area of 26.8 ± 14.3 μm^2 (Figure 5, Table 1) than in light-adapted ommatidia. Rhabdom areas between the two times of day were not significantly different. Measurements of these anatomical parameters at transition times of light offset (Figure 6) and light onset (Figure 7) were not significantly different with the average rhabdom area of 31.3 ± 12.9 μm^2 for light offset and 31.0 ± 6.4 μm^2 for light onset. The organization of screening pigment granules was also not significantly different at transition times with an average of 91 ± 21 for light offset and 73 ± 26 for light onset. The mean palisade layer areas at these two times were significantly different with 5.5 ± 3.5 μm^2 in ommatidia removed at light offset and 22.4 ± 8.3 μm^2 in ommatidia at light onset. Recordings of locomotor activity of representative animals indicated that the animals were entrained to the photoperiodic conditions of LD12:12 (Figure 8).

The same morphological parameters were measured in ommatidia removed at subjective midday (Figure 9) and subjective midnight (Figure 10) over a three day time period in roaches freerunning under constant

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conditions of darkness and temperature in order to detect the expression of these changes on a circadian basis. Consistent with data from entrained roaches, rhabdom area did not vary temporally (Figure 11). The average area varied between 32 ± 11 μm^2 and 45 ± 15 μm^2 (Table 2). Unlike animals held under LD12:12, the daily rhythm in pigment granule organization did not continue to vary temporally within the defined area about the rhabdom (Figure 12). The palisade layer area of ommatidia removed on the third subjective midnight was significantly greater than the area of ommatidia removed on all three consecutive subjective middays and the first subjective midnight. The palisade layer area in ommatidia removed at the first subjective midday was significantly lower than that of the second and third subjective midnights (Figure 13). Thus, it appeared that the palisade layer area varied on a circadian basis. However, palisade layer areas determined at subjective middays were greater than during midday in animals entrained to LD12:12. Locomotor activity recordings of representative animals in constant darkness indicated that they were free of entrainment, (i.e. freerunning) (Figure 14).

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Figure 1. Electron micrograph of an ommatidial cross-section obtained at midday from a cockroach maintained under LD12:12 and 25 ± 2 °C (R=rhabdom, Pi=pigment granules).
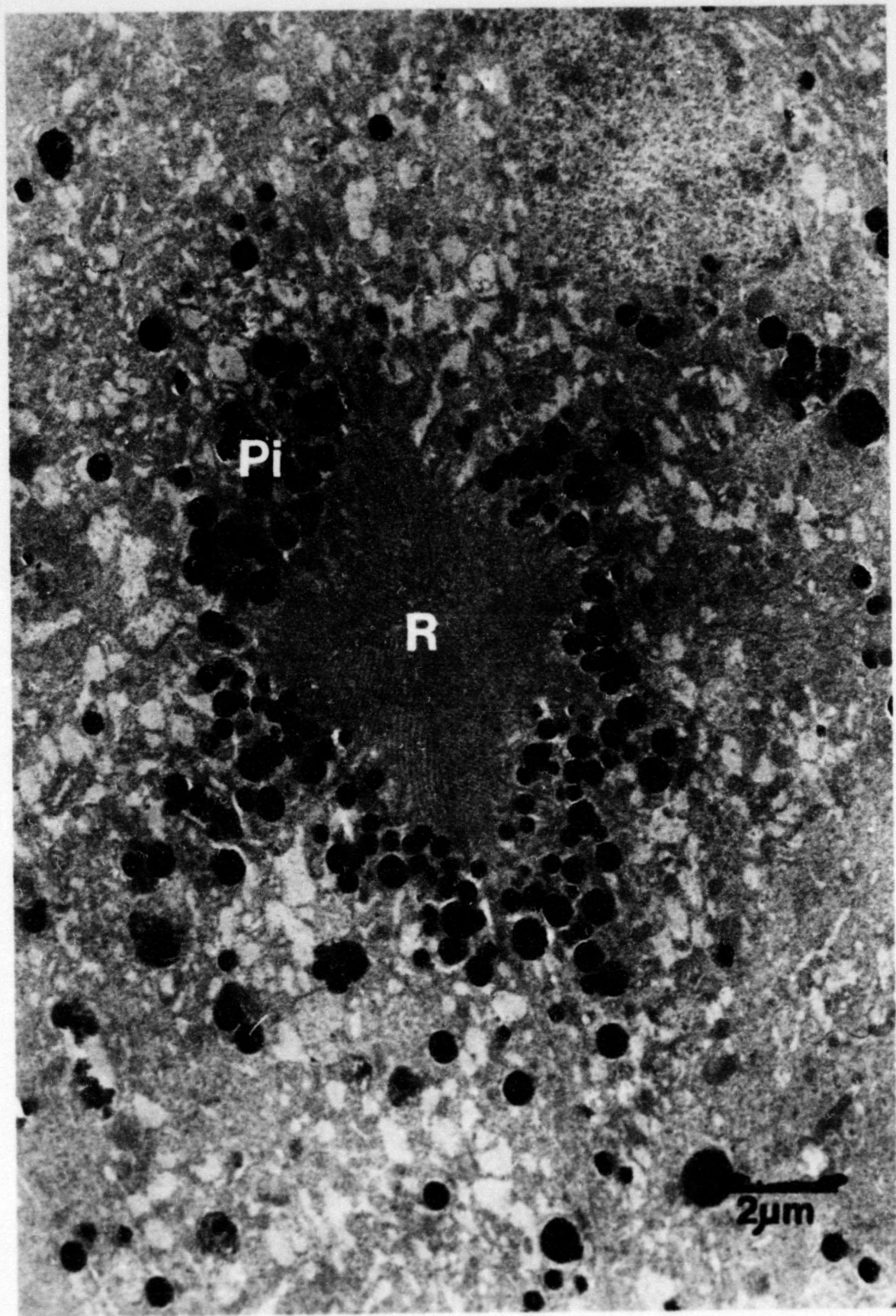
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Figure 2. Electron micrograph of an ommatidial cross-section obtained at midnight from a cockroach maintained under LD12:12 and 25 ± 2 °C (R=rhabdom, Pa=palisade layer, Pi=pigment granules).

Figure 3. Mean rhabdom area of ommatidia determined at four times of day (midday, light offset, midnight, light onset) in cockroaches maintained under LD12:12 and 25 ± 2 °C (SD=standard deviation).

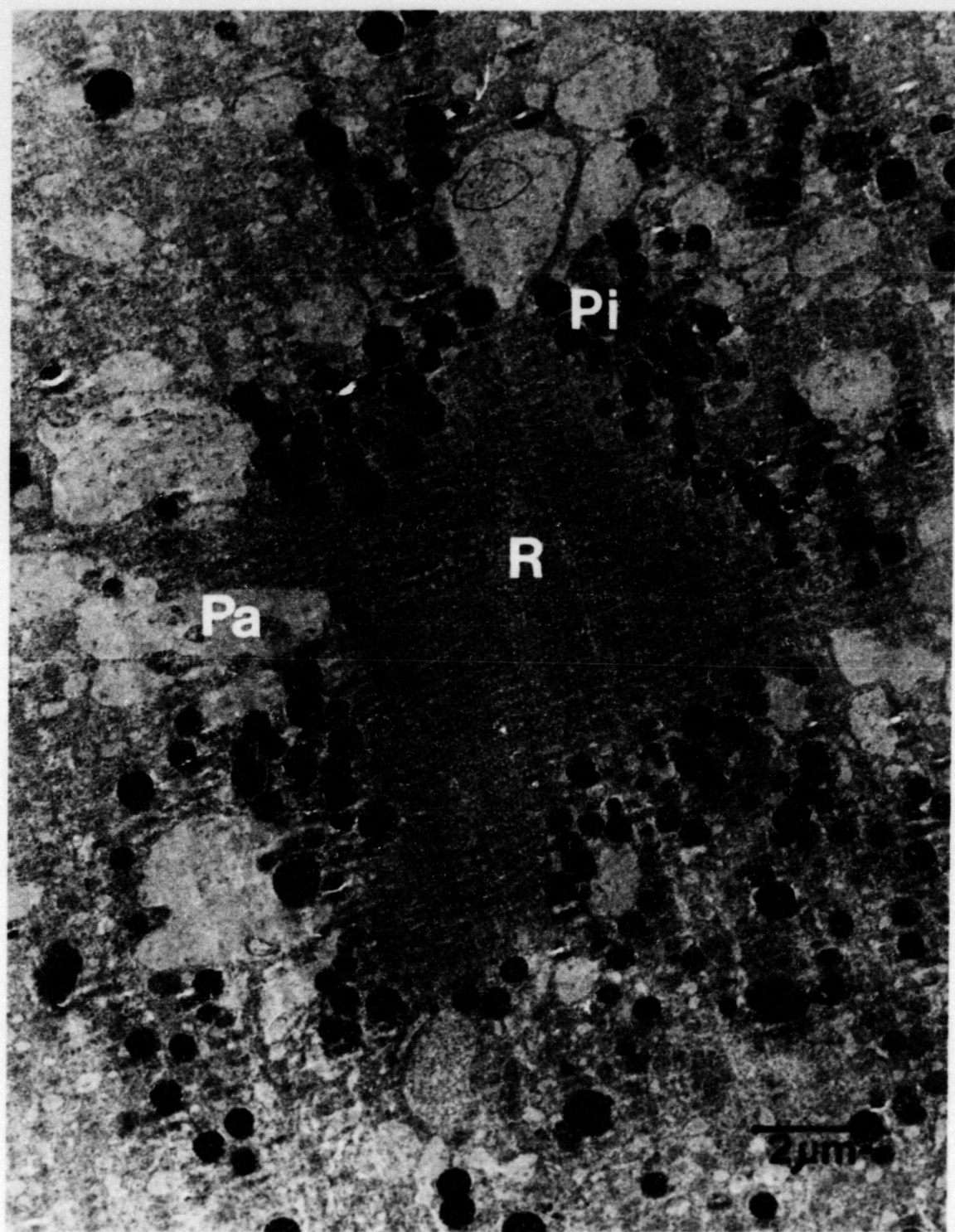Figure 4. Mean number of screening pigment granules clustered within a 10 μm diameter circle centered about rhabdoms of ommatidia determined at four times of day (midday, light offset, midnight, light onset) in cockroaches maintained under LD12:12 and 25 ± 2 °C (SD=standard variation).

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Figure 5. Mean palisade layer area of ommatidia measured at four times of
day (midday, light offset, midnight, light onset) in cockroaches
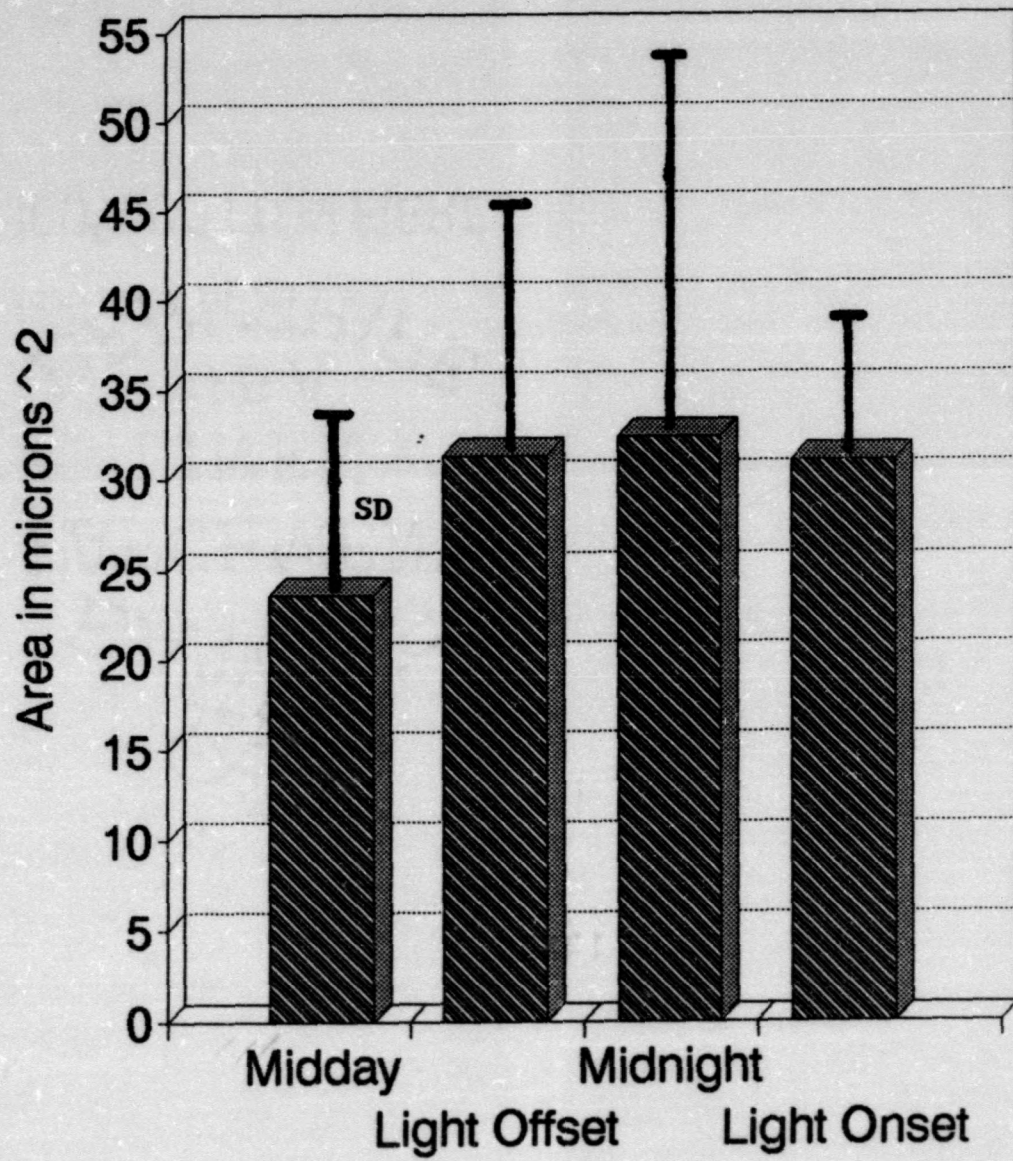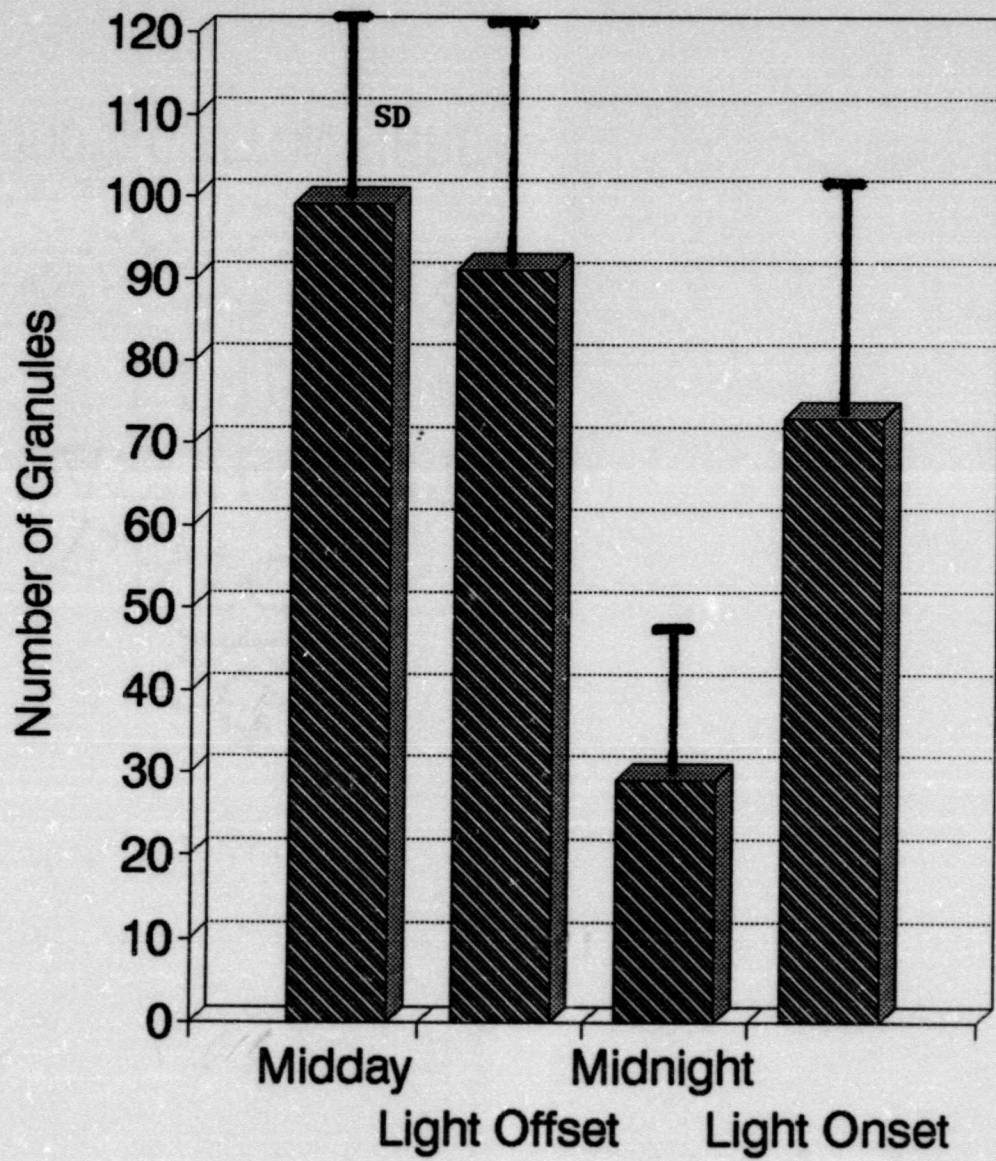maintained under LD12:12 and 25 ± 2 °C (SD=standard deviation).
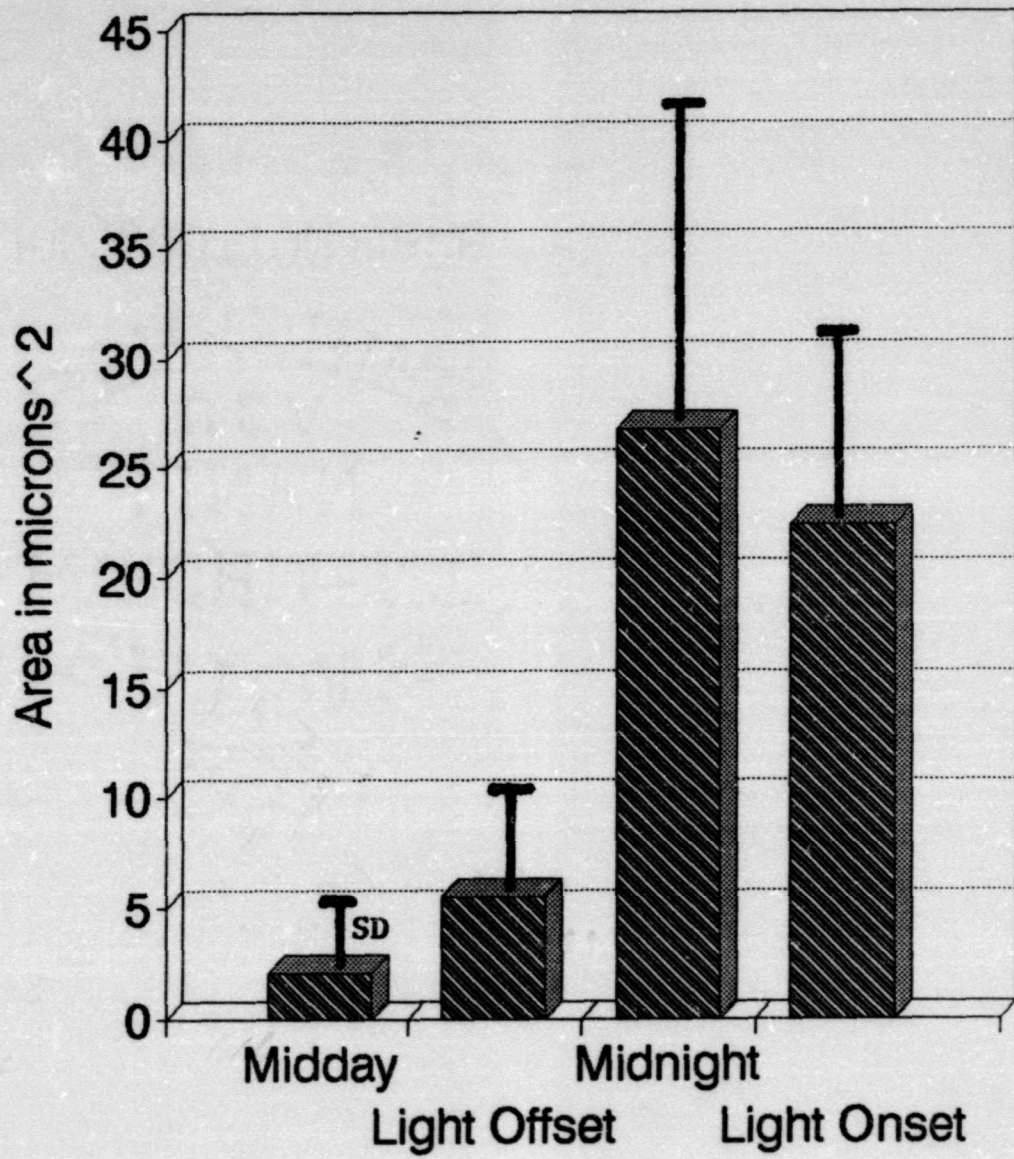
TABLE 1. Mean rhabdom area, number of screening pigment granules clustered within a 10 µm diameter circle centered about the rhabdom and palisade layer area of ommatidia taken at four times of day in cockroaches maintained under LD12:12 and 25 ± 2 °C.

TIME OF DAY	N	RHABDOM AREA (µm)[2]	NUMBER OF SCREENING PIGMENT GRANULES	PALISADE LAYER AREA (µm)[2]
MIDDAY	9[1]	23.6 ±8.8[2]	99 ± 21[b]	2.1 ±2.0[c][3]
LIGHT OFFSET	9	31.3 ±12.9	91 ±28[ab]	5.5 ±3.5[c]
MIDNIGHT	9	32.4 ± 20.3	29 ±16[a]	26.8 ± 14.3[d]
LIGHT ONSET	9	31.0 ±6.4	73 ± 26[a]	22.4 ± 8.3[d]
ANOVA TUKEY'S		N.S. -----	P<.01 P<.05	P<.01 P<.05

[1]N is the number of ommatidia examined.

[2]Mean ± the standard deviation about the mean.

[3]Mean values with letters in common are not significantly different at the 95% confidence level according to the Tukey's range test.

Figure 6. Electron micrograph of an ommatidial cross-section obtained at light offset from a cockroach maintained under LD12:12 and 25 ± 2 °C (R=rhabdom, Pa=palisade layer, Pi=pigment granules).

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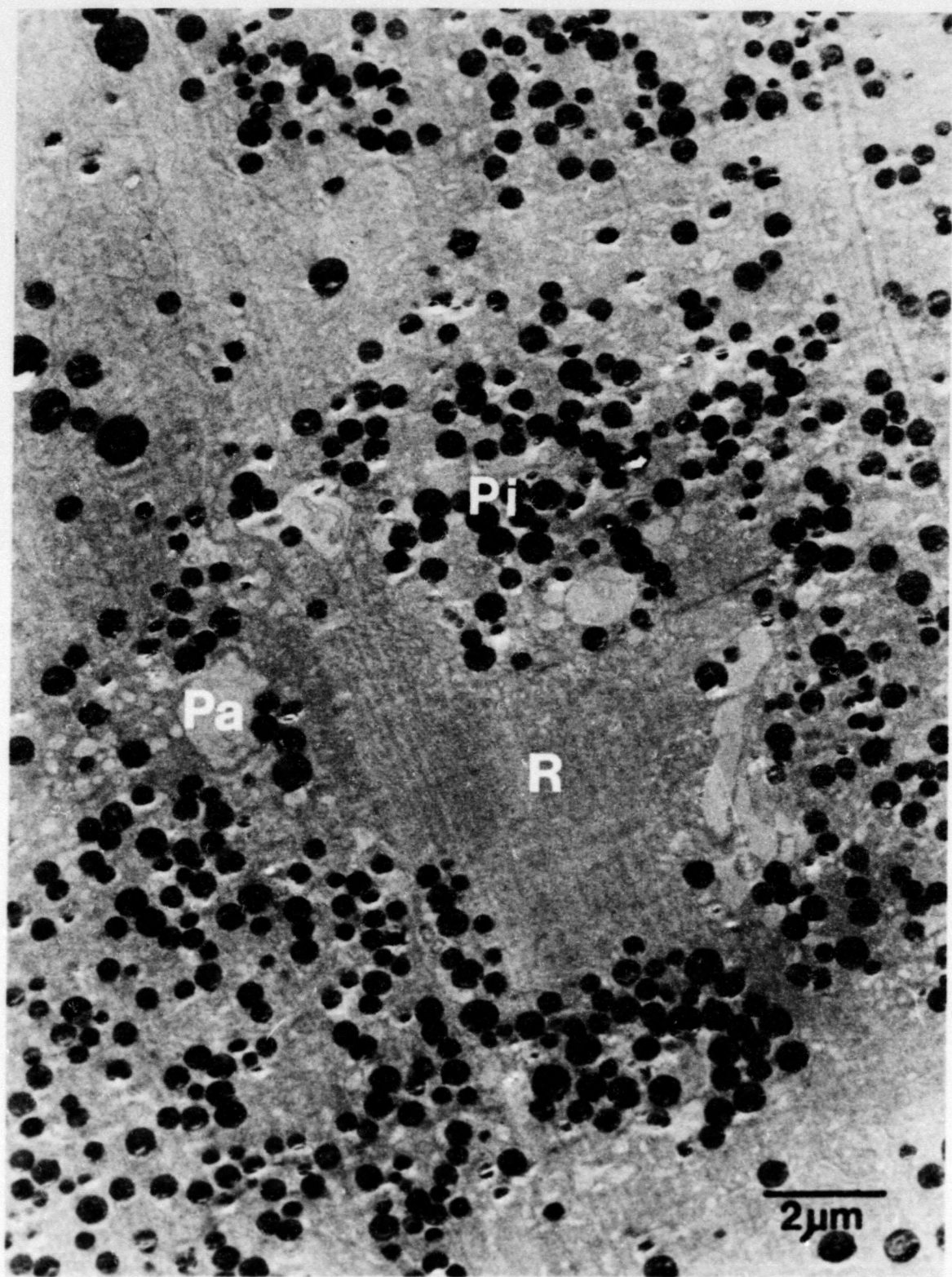
Figure 7. Electron micrograph of an ommatidial cross-section obtained at light onset from a cockroach maintained under LD12:12 and 25 ± 2 °C (R=rhabdom, Pa=palisade layer, Pi=pigment granules).
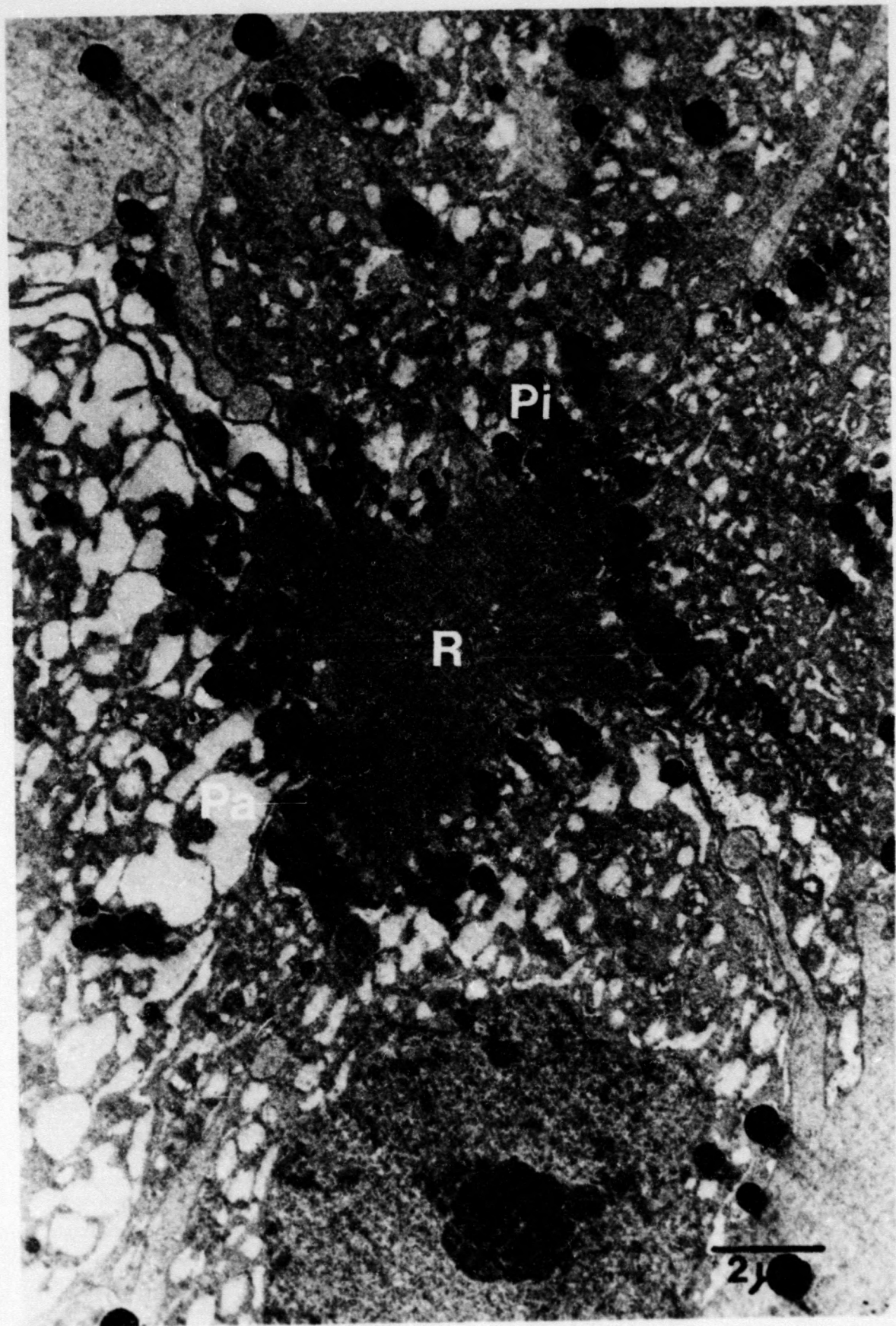
Figure 8. Locomotor activity recordings of two cockroaches, designated by
A and B, entrained to LD12:12 and 25 ± 2°C. Light onset was at
0600 h and light offset at 1800 h.
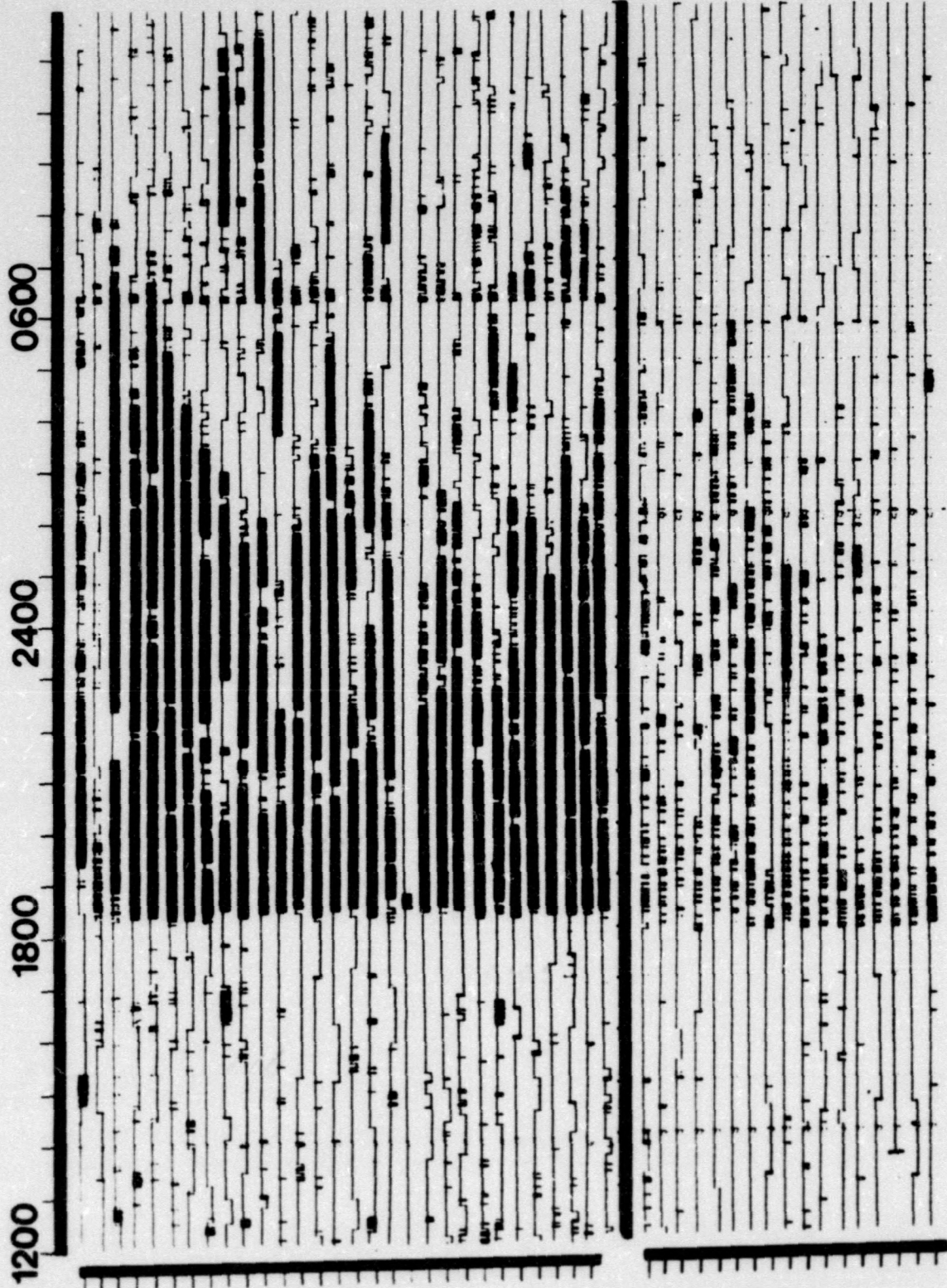
Figure 9. Electron micrograph of an ommatidial cross-section obtained at subjective midday on day three in a cockroach held under constant darkness and 25 ± 2 °C (R=rhabdom, Pa=palisade layer, Pi=pigment granules).

Figure 10. Electron micrograph of an ommatidial cross-section of a
cockroach obtained at subjective midnight on day three from a
cockroach held under constant darkness and 25 ± 2 °C
(R=rhabdom, Pa=palisade layer, Pi=pigment granules).

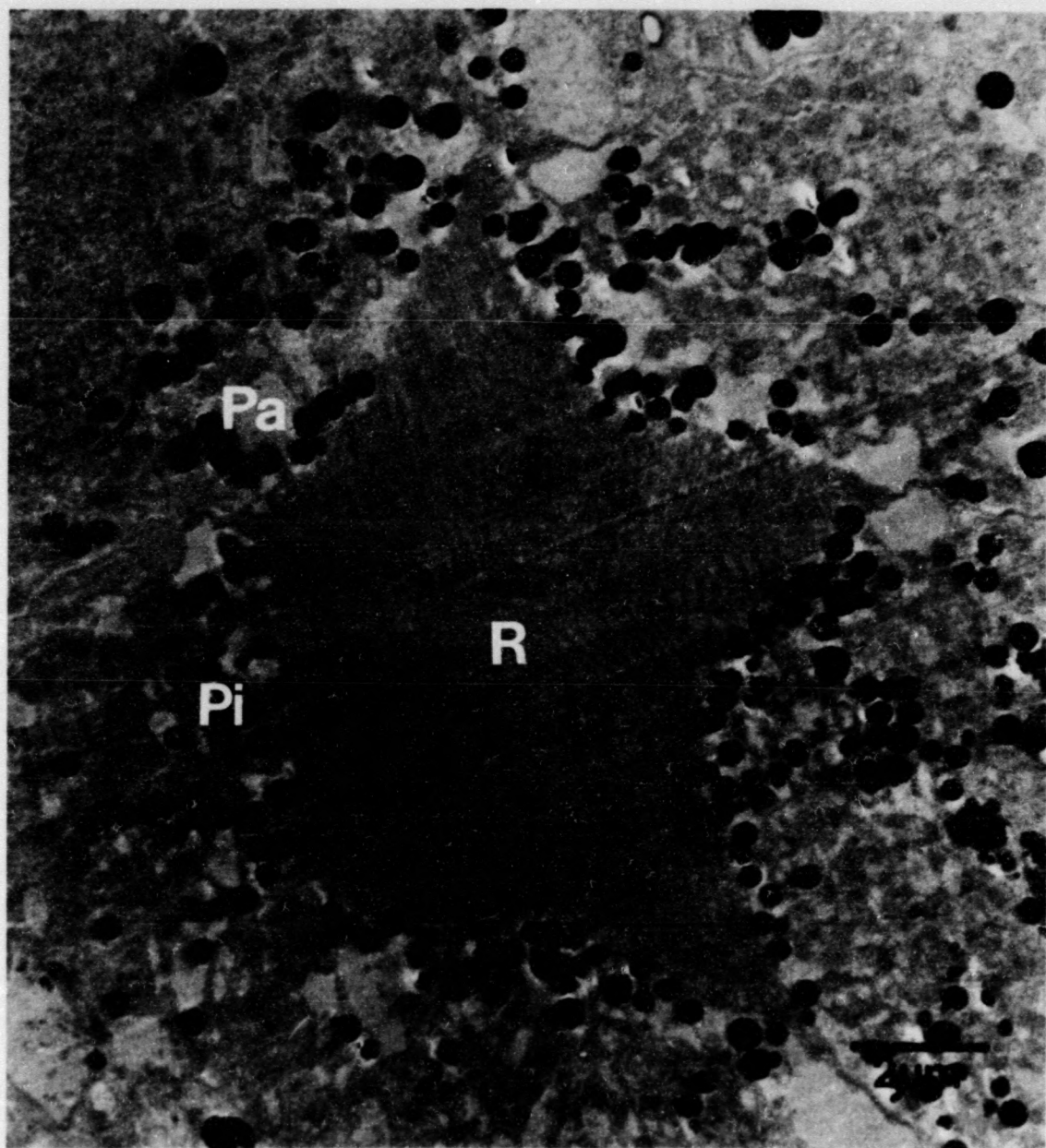22

Figure 11. Mean rhabdom area of ommatidia determined at two subjective times of day (midday, midnight) over a three day period (1=day 1, 2=day 2, 3=day 2) in cockroaches maintained under constant darkness and 25 ± 2 °C (SD=standard deviation).
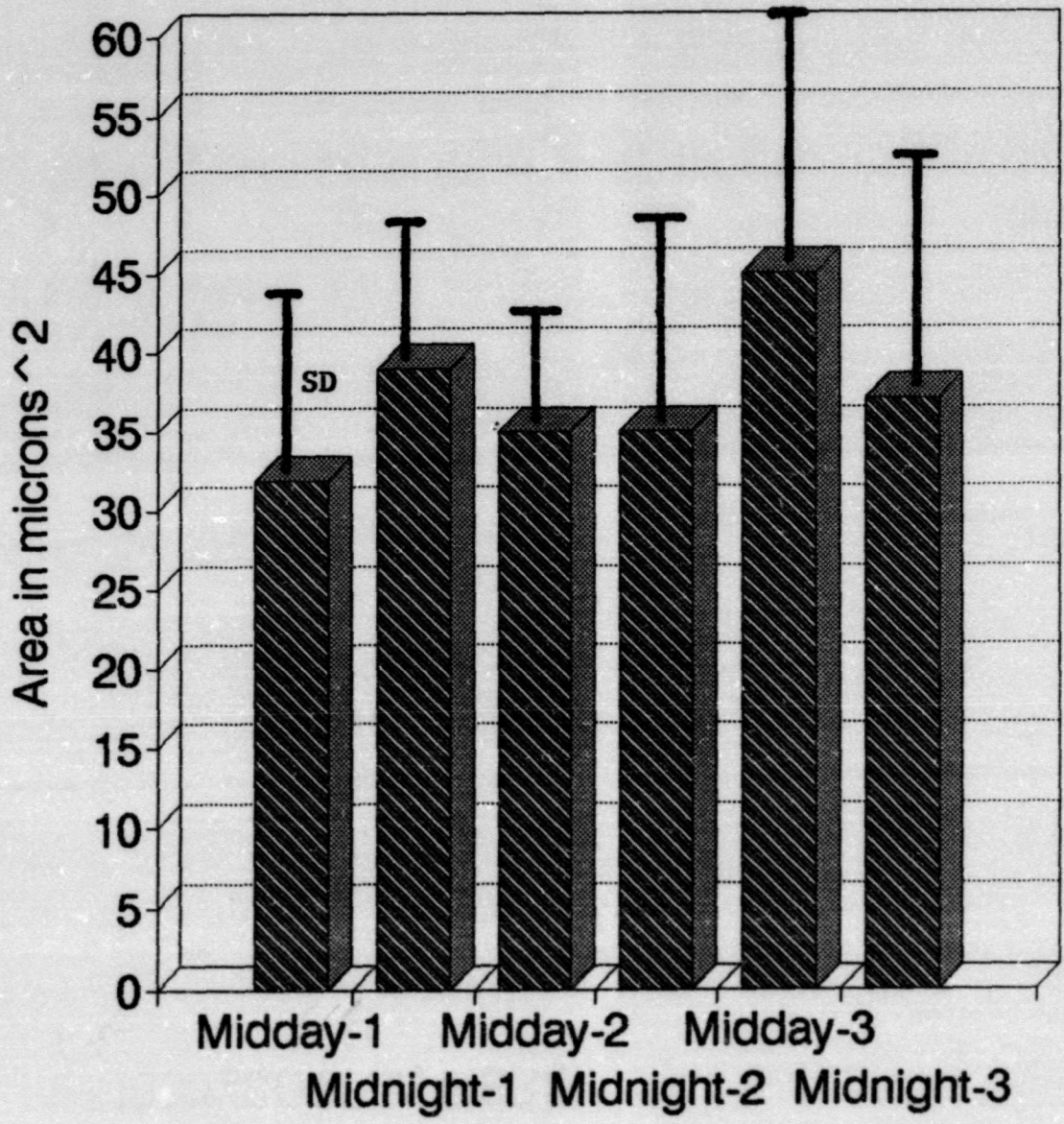
Table 2. Mean rhabdom area, number of pigment granules clustered within a 10 μm diameter circle centered about the rhabdom and palisade layer area of ommatidia taken at two times of day over a three day period in cockroaches maintained under constant darkness and $25 \pm 2°C$.

TIME OF DAY	N	RHABDOM AREA $(\mu m)^2$	NUMBER OF SCREENING PIGMENT GRANULES	PALISADE LAYER AREA $(\mu m)^2$
MIDDAY-1[1]	9[2]	32 ± 11[3]	61 ± 35[3]	14 ± 6[a4]
MIDNIGHT-1	9	39 ± 8	51 ± 16	24 ± 10[ab]
MIDDAY-2	9	35 ± 7	62 ± 10	21 ± 8[ab]
MIDNIGHT-2	9	35 ± 12	58 ± 15	30 ± 7[bc]
MIDDAY-3	9	45 ± 15	63 ± 10	17 ± 6[ab]
MIDNIGHT-3	9	37 ± 14	40 ± 11	39 ± 16[c]
ANOVA		N.S.	N.S.	P<.01
TUKEY'S		-----	-----	P<.05

[1]Days after the initiation of continuous darkness.

[2]N is the number of ommatidia examined.

[3]Mean ± the standard deviation about the mean.

[4]Mean values with letters in common are not significantly different at the 95% confidence level according to the Tukey's range test.

Figure 12. Mean number of screening pigment granules clustered within a
10 μm diameter circle centered about rhabdoms of ommatidia
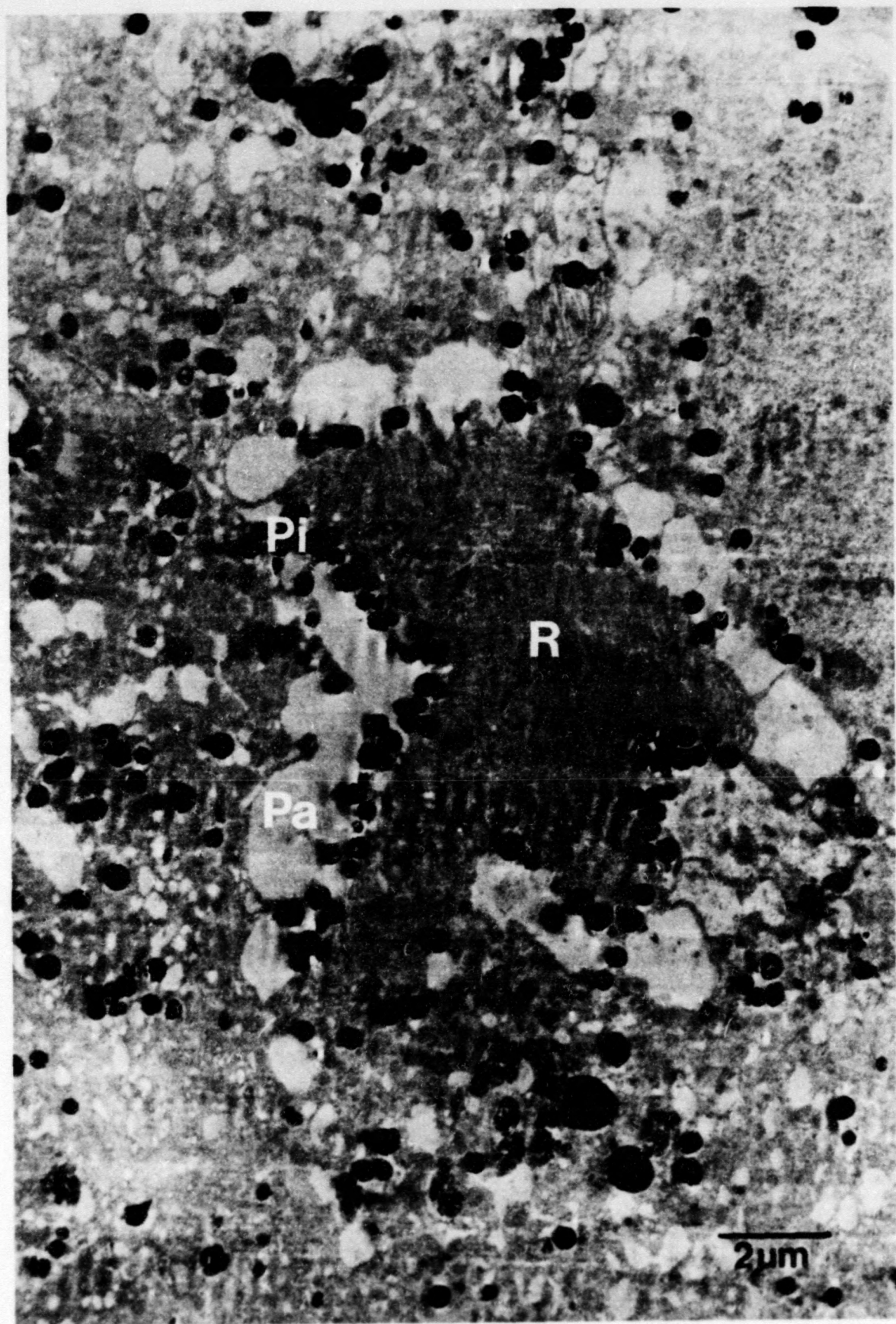determined at two subjective times of day (midday, midnight)
over a three day period (1=day 1, 2=day 2, 3=day 3) in cockroaches
maintained under constant darkness and 25 ± 2 °C
(SD=standard deviation).

Figure 13. Mean palisade layer area of ommatidia determined at two subjective times of day (midday, midnight) over a three day period (1=day 1, 2=day 2, 3=day 3) in cockroaches maintained under constant darkness and 25 ± 2 °C (SD=standard deviation).
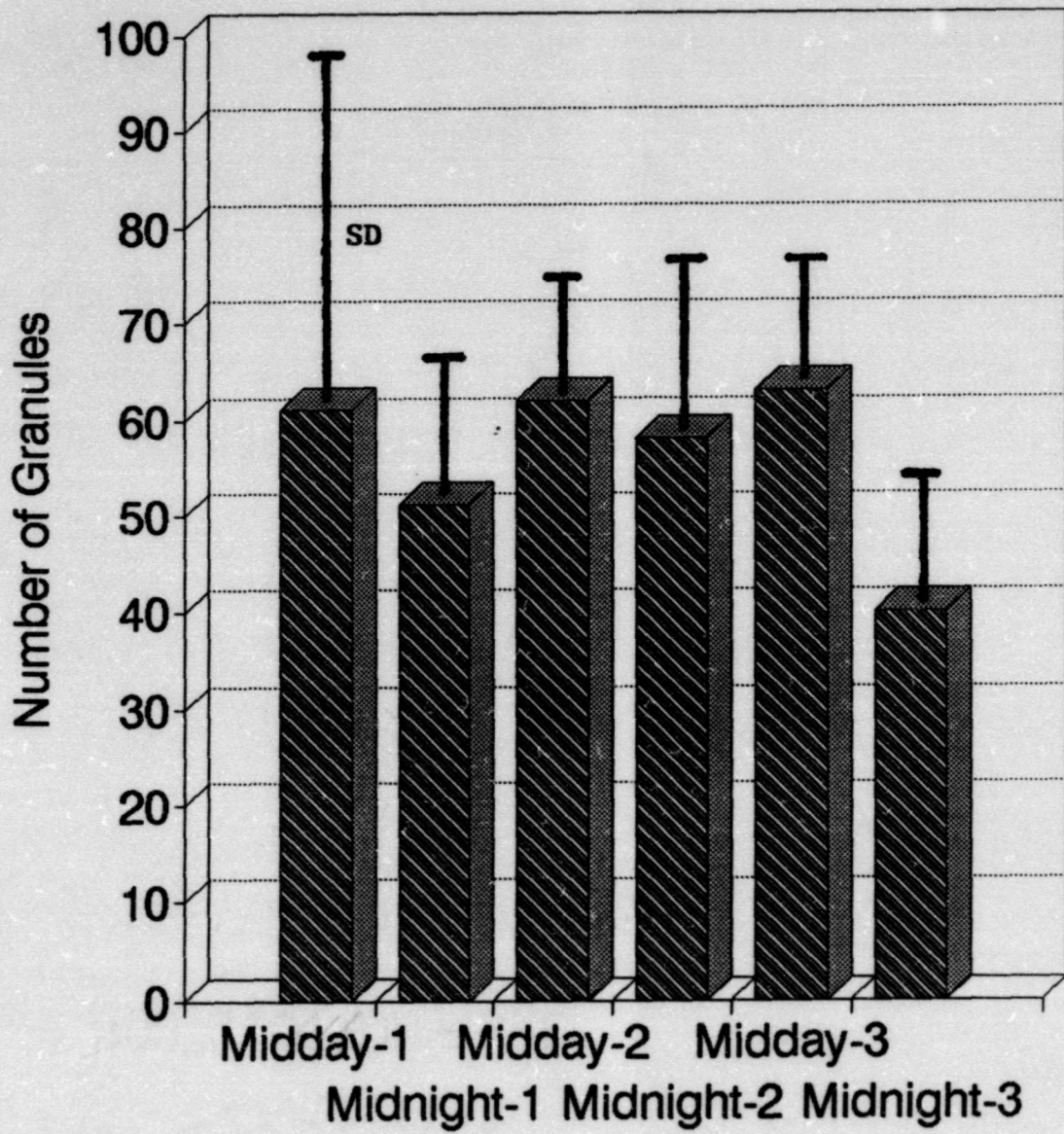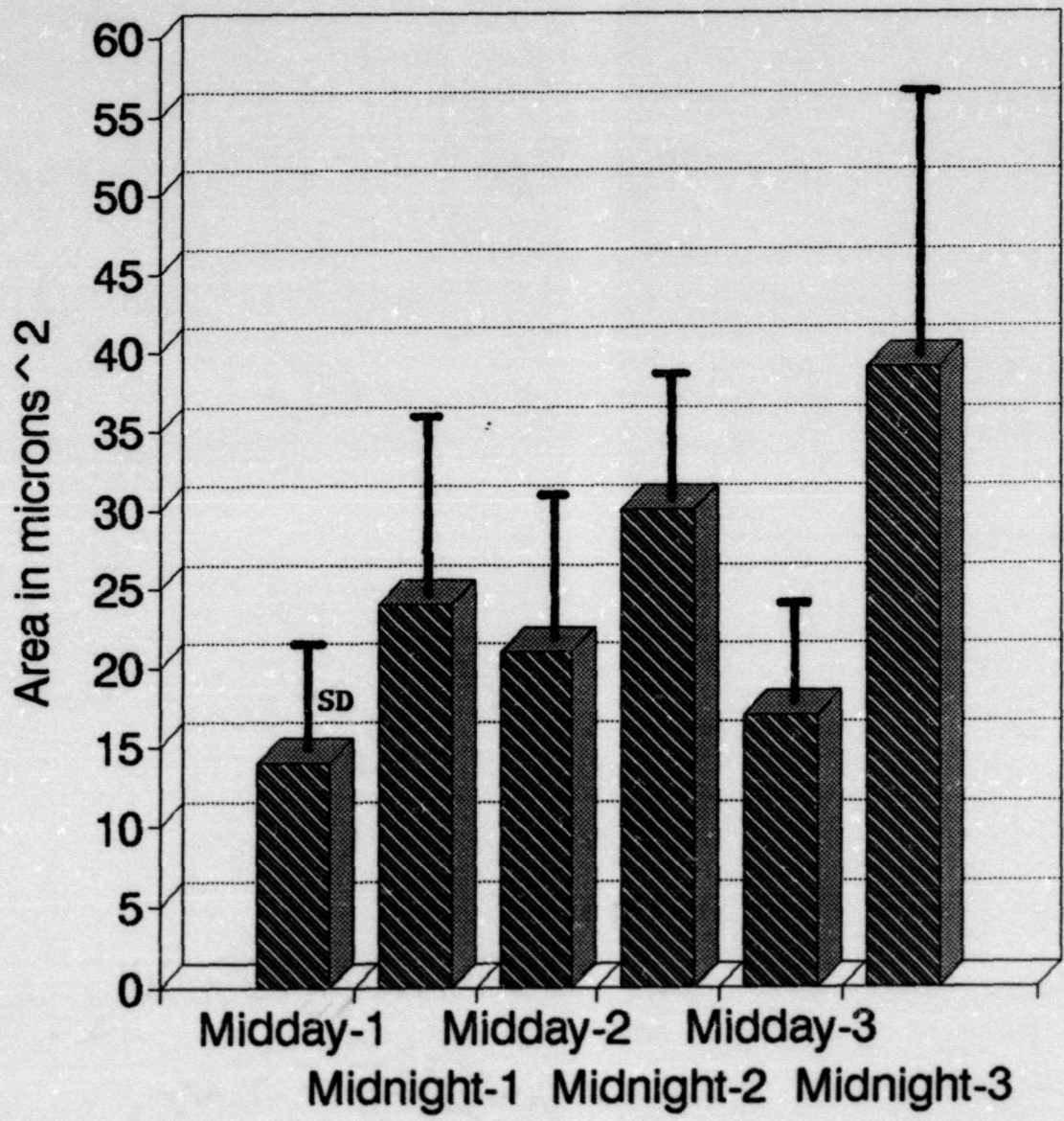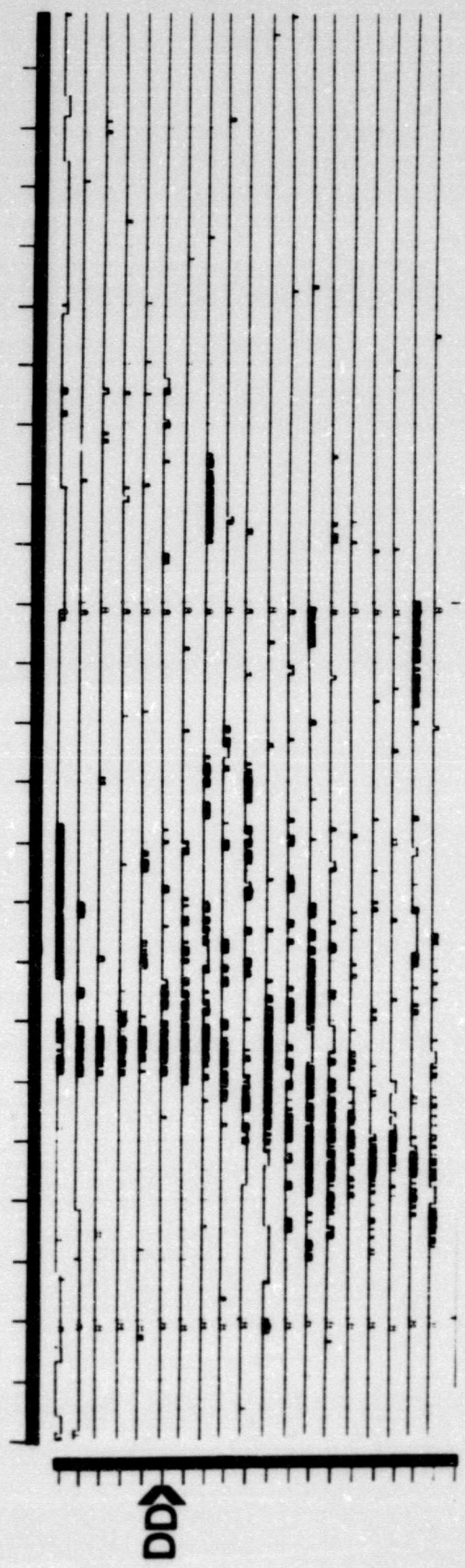
Figure 14. Locomotor activity recording of two cockroaches maintained
under LD12:12 and 25 ± 2 °C and subsequently placed under
constant darkness (DD) on the day indicated by <. Light onset
was at 0600 h and light offset was at 1800 h.

DISCUSSION

Rhabdom cross-section area did not appear to change with time of day in *Leucophaea maderae*, unlike in other arthropods such as *Limulus*, in which changes in the length and the thickness of the rhabdom occurred on a daily basis (Barlow et al. 1980) and the grapsid crab, *Hemigrapsus sanguineus*, which had a rhabdom volume greater at night than during the day (Arikawa et al. 1988). Due to dramatic differences in rhabdom structure among these arthropods, direct comparisons among anatomical features could not be made. The number of screening pigment granules clustered within a 10μm diameter circle centered about the rhabdom changed temporally in roaches held under LD12:12 conditions. In the light-adapted state, screening pigment granules clustered about the rhabdom, whereas in the dark-adapted state, screening pigment granules dispersed into the cytoplasm of the retinula cells and became separated from the rhabdom by a palisade layer. These changes are similar to those described for another cockroach species, *Periplaneta americana* (Butler 1973) and for other arthropods such as the crayfish *Procambarus* (Olivo and Larsen 1978) and the horseshoe crab, *Limulus* (Miller and Cawthon 1974). Daily changes in rhabdom area and screening pigment organization did not continue on a circadian basis. Therefore, it seems unlikely that the expression of daily differences observed in these parameters are regulated endogenously by a pacemaker.

Temporal changes in the area of the palisade layer that occurred on a daily basis continued to be partially expressed on a circadian basis. The temporal pattern of these changes in entrained animals (i.e. under LD12:12)

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was similar to that described for *P. americana* in which the palisade layer increased in the dark-adapted eye and diminished in the light adapted eye (Butler 1973). In *L. maderae* removed from entrainment (i.e., held under DD), the palisade layer reached the dark-adapted state but returned only partially to the light-adapted state. It seems likely that the palisade layer increased as a result of efferent output from a pacemaker and diminished slowly in the absence of efferent output toward the day-adapted state. These results are consistent with a hypothesis involving a push-pull mechanism that has been proposed to explain a similar circadian rhythm in morphological changes in the eye of *Limulus* (Barlow 1990). According to this hypothesis, the push toward the nighttime state is produced by a signal from a pacemaker within the brain via an efferent neural pathway. The neurotransmitter octopamine released from neurons of this pathway push the retina toward the nighttime state (Barlow 1983). A circulating hormone that reduces sensitivity of the eye to light may pull the retina back to the daytime state in order to anticipate the coming dawn (Barlow 1990). In *L. maderae* it appears that the push toward the nighttime state results from efferent output originating from a pacemaker. In contrast the eye passively changes toward the daytime state. The presence of light may be necessary to accelerate this process and produce the daytime condition observed in roaches held under LD12:12..

The temporal change in the palisade layer area observed in this study might partially account for the temporal changes in eye sensitivity to light measured by electroretinograms (ERG) in *L. maderae* (Wills et al. 1985). The ERG amplitude changes temporally on a circadian basis in *L. maderae* (Wills et al. 1985). Since the ERG is a measure of the sensitivity of the eye to light, then some morphological changes might be expected to take place

correlated with changes in retinal sensitivity. The pattern of changes in ERG amplitude parallel temporal changes in palisade layer morphology. The palisade layer increased at a time when ERG amplitude was highest and decreased at a time when ERG amplitude was lowest.

Three hypotheses have been advanced to account for temporal changes in eye sensitivity to light that could involve anatomical changes. The first hypothesis is that there could be changes which would increase the number of photoreceptor molecules in the rhabdom available to catch photons (Colwell and Page 1989), perhaps resulting from a change in the number of microvilli in the rhabdom between night and day. The fact that the rhabdom area did not change with time of day in *L. maderae* does not support this concept. However, it is still possible that the number of microvilli changed without producing changes in the rhabdom diameter. Changes in microvilli length and packing have been observed in other arthropods (Waterman 1982). This possibility could not be addressed in this study because of resolution limitations. The second hypothesis involves a change in the organization of screening pigments within retinula cells that could be expected to lead to a change in the probability that a photon would effectively reach the photoreceptive region, the rhabdom (Wills et al. 1985). The fact that the organization of screening pigments about the rhabdom did not vary on a circadian basis indicates that their light screening function is not a major factor in producing changes in eye sensitivity observed on a circadian basis (Wills et al. 1985). The third hypothesis is that the strength of the signal in response to each photon is altered through a process of amplification. The change in the palisade layer area between subjective day and subjective night observed in this study is correlated with the change in eye sensitivity to light. Perhaps a regulatory ion that enhances the signal's response to light could be

sequestered within the palisade layer in close proximity to the microvilli at night, when the eye is most sensitive to light. An increased calcium pool is maintained within the palisade layer adjacent to the microvilli during the dark-adapted state in another arthropod, the honeybee drone (Baumann and Walz 1989). When the photoreceptor was subjected to light, Ca^{++} is released in close proximity to the microvilli and may have enhanced the response to light . In the presence of increased light intensity, Ca^{++} within the palisade layer was released into the cytoplasm and may have helped facilitate the changes in screening pigment organization that take place when the eye goes from a dark-adapted state to a light-adapted state (Baumann and Walz 1989). The mechanism whereby the screening pigment granules are distributed into the cytoplasm of the retinula cells is not known. It has been hypothesized that a transport mechanism involving a microtubule system that may be activated by an increase in cytoplasmic calcium ions results in the movement of screening pigment granules, such as in the arthropod *Procambarus* (Olivo and Larsen 1978). Increased light could cause Ca^{++} stored in the palisade layer to be released into the cytoplasm by diffusion. This would diminish photoreceptor sensitivity and move screening pigment granules closer to the rhabdom.

The exact location(s) of the cells that make up the circadian pacemaker(s) in the brain of *L. maderae* is not known. Many studies have been performed to identify the general location of this pacemaker (Nishiitsutsuji-Uwo and Pittendrigh 1968, Page 1983, Wills et al. 1985 and Lavialle et al. 1989). The circadian clock that controls the circadian rhythmicity of the ERG is in the same area of the brain as the clock controlling the locomotor activity rhythm (Roberts 1974 and Page 1983). The location of a pacemaker has already been narrowed to the lobula plate of the lobula neuropil in the optic lobes through

studies of metabolic activity rhythms (Lavialle et al. 1989). Whether these biological clocks are anatomically close or identical to one another (Colwell and Page 1989) can only be established by determining the exact anatomical location(s) of cells that regulate the expression of these rhythms. Circadian changes observed in the palisade layer area within a single retinula cell can be used to assay for the location of the pacemaker that regulates this rhythm through lesion studies similar to those used to determine the location of the pacemaker controlling the ERG rhythm (Colwell and Page 1989). If the pacemaker that regulates the expression of the rhythm of the palisade layer area can be localized, it should be possible to trace efferent pathways from their connection with retinula cells back toward the pacemaker. Thus, it should be possible to identify the individual cells of the pacemaker. If the location of cells of the pacemaker can be determined, then neural pathways between pacemakers and the behavioral and physiological rhythms they regulate can be mapped. This should lead to an understanding of circadian organization with multicellular organisms.

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