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Clock Control of Circadian Changes of Ommatidial Structure in the Cockroach, *Leucophaea Maderae* (L.)

Zhuming Zhang
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Zhuming

1993

CLOCK CONTROL OF CIRCADIAN CHANGES OF OMMATIDIAL
STRUCTURE IN THE COCKROACH, *LEUCOPHAEA MADERAE* (L.)

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
Zhuming Zhang
December, 1993

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CLOCK CONTROL OF CIRCADIAN CHANGES IN THE COCKROACH,
LEUCOPHAEA MADERAE (L.)

Date Recommended November 22, 1993

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CLOCK CONTROL OF CIRCADIAN CHANGES OF
OMMATIDIAL STRUCTURE IN THE COCKROACH,
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All eukaryotic organisms display rhythms which persist under constant environmental conditions with periods of approximately, but very seldom exactly, 24 hours. Such rhythms are "circadian" and are driven by an internal "biological clock." Circadian rhythms of locomotor activity, retinal sensitivity to light and ommatidial morphology have been characterized in the cockroach, *Leucophaea maderae* (L.). It is not known whether the same clock(s) controls both circadian rhythms of electroretinogram (ERG) amplitude and morphological changes of the compound eye. In order to determine whether the location of the clock that controls morphological changes is in the same location as the one that controls ERG, brain lesions were made proximal or distal to the region of the putative clock regulating the expression of a circadian rhythm in eye sensitivity to light in anesthetized cockroaches. These and sham operated control animals were held for approximately two weeks under LD 12:12 at $25\pm 2^{\circ}\text{C}$ conditions in environmental chambers. After this time period, conditions of continuous darkness were established in order to allow rhythms to free run. Eye tissue was removed on subjective midday two and subjective midnight two, fixed, embedded, sectioned and the

sections were examined and photographed using a Zeiss transmission electron microscope. Observations were made to establish the presence or absence of a circadian rhythm of submicrovillar cisternae area (SMC), as well as rhabdom area and screening pigment granules organization (SPG). The results indicate that the clock, located in the lobular neuropil region of the optic lobe that controls the rhythm of morphological changes in the eye, is in the same proximity as that which controls the changes in ERG amplitude.

INTRODUCTION

Most organisms must contend with environments that exhibit daily fluctuations in a variety of biotic and abiotic factors. The response of natural selection has been the evolution of regulatory systems whose primary function is to match these cyclical environmental variations with appropriate, periodic alteration in physiology, biochemistry, and behavior. These regulatory systems function as "biological clocks," generating a precise temporal program within the organism that coordinates its activities with the periodic, and thus predictable, environment in which it lives (Page 1990). There are two prominent characteristics about the biological clocks: (1) they are based on endogenously generated oscillations whose periods are "circadian"---- it is usually close to (but is never exactly) 24 hours and (2) the oscillation can be entrained by a limited number of environmental cycles. Among several specific invertebrate models that have been used to address a variety of specific questions about the way in which circadian systems are organized, cockroaches have proven to be particularly suitable models for investigating the physiology of circadian rhythmicity, and it is believed that the anatomical and physiological organization of the circadian system of the cockroach is one of the best understood of any organism (Page 1990).

A variety of daily rhythms have been described for cockroaches including rhythms in oxygen consumption (Richard 1969), feeding (Lipton 1970), sensitivity to insecticides (Beck 1954), cuticular growth (Lukat 1978), electroretinogram (ERG) amplitude (Wills *et al.* 1985) and locomotor activity (Lukat 1979, Page 1985). Recently, circadian changes in ommatidial morphology correlated with changes in eye sensitivity to light were sought (Ferrell and Reitcheck 1993).

The compound eyes of cockroaches (*Leucophaea maderae* and *Periplaneta americana*) were the sole site of phototransduction for entrainment of the circadian rhythm of locomotor activity (Robert 1965, Nishiitsutsuji-Uwo and Pittendrigh 1968). The removal of the optic lobes of the protocerebrum disrupted the circadian rhythm of activity in *L. maderae*. Neural isolation of the optic lobes from the midbrain by bilateral section of the optic tracts also disrupted the locomotor activity rhythm (Nishiitsutsuji-Uwo and Pittendrigh 1968). The recovery of rhythmicity depended on regeneration of the neural connections between the optic lobes and the midbrains (Page 1983). These results pointed toward the central importance of the optic lobes in maintaining the rhythm in locomotor activity and further indicated that their effect was mediated by axons in the optic tract. The loss of rhythmicity following optic-lobe ablation or optic-tract section raised the possibility that the optic lobes were the locus of the endogenous oscillation that controls the temporal distribution of locomotor activity and generate a circadian rhythm in the absence of humoral or neural cues from the rest of the organism (Page 1990). Furthermore, the conclusive evidence that the optic lobe tissue contained a competent circadian pacemaker was provided by an experiment in which the cockroach optic lobe was found to exhibit a circadian rhythm of spontaneous impulse activity when the optic lobe was maintained in organ culture, completely isolated from neural or humoral signals from the rest of the animal under constant environmental conditions (Page 1987, 1988).

The region within the optic lobe which contains the cells that generate the pacemaking oscillation for the locomotor activity rhythm has been localized further. After removal of one optic lobe, surgical or electrolytic lesions distal to the second optic chiasma or dorsal to the lobula have no effect on rhythmicity, whereas lesions

near the lobula in the ventral half of the lobe frequently abolish the activity rhythm (Page 1978, 1983; Sokolove 1975). The results suggest that the cells responsible for generating the circadian signal have their somata and/or processes in this region of the optic lobe (Page 1990). Cytochrome oxidase activity, which is resolvable histochemically, was also used as a marker to locate the pacemaker in *L. maderae*. The largest amplitude in the rhythm of energy metabolism was observed in the lobula plate (Lavialle *et al.* 1989) which supported the results from studies using surgical techniques.

In a series of experiments, severance of the optic lobe proximal to the distal edge of the lobula did not abolish the rhythm of ERG amplitude in most animals, whereas there were no cases of persistent rhythmicity observed after sectioning the optic lobe distal to the lobula. These results indicated that a pacemaker that drives the rhythms in the ERG amplitude was contained within the optic lobe and that the optic lobe is capable of sustaining the ERG rhythms *via* neural connections. The fact that cuts through or distal to the second optic chiasma consistently abolished rhythmicity further suggests that the location of the pacemaker is in the proximal part of the optic lobe, in the lobula region (Wills *et al.* 1985). Therefore, a locus for the oscillator that regulates ERG amplitude is near or identical to the pacemaker locus for the activity rhythm (*i.e.*, the same oscillator controls both rhythms).

For the cockroach, *Leucophaea maderae* (L.), it has been demonstrated that a single pacemaker in the optic lobe drives both the ERG and locomotor activity rhythms (Wills *et al.* 1985) and that the pacemaker that regulates the activity and ERG amplitude rhythm is composed of two oscillators, each located ventrally near the lobular of one of the optic lobes of the protocerebrum. Each oscillator has three output pathways that are functionally and anatomically distinct: one controls the

rhythm in ERG amplitude, a second regulates activity *via* a driven system (possibly a damped oscillator) in the midbrain, and a third couples the oscillator to its companion oscillator in the contralateral optic lobe. There are also two input pathways to each oscillator. In addition to the input from the contralateral optic lobe, there is also a light-entrainment pathway from the photoreceptors in the retina of the ipsilateral compound eye (Page 1990). There is experimental evidence that morphological changes of compound eyes of the cockroach, *L. maderae*, which correlated with the retinal sensitivity to light, are controlled by an internal biological clock (Ferrell and Reitcheck 1993). SMC increased at nighttime and decreased during the daytime. In that both morphology and ERG amplitude of the compound eye showed circadian changes and both are indicative of eye sensitivity to light, an experiment was designed to test whether the clock(s) that controlled the rhythms of ERG amplitude and locomotor activity also controls circadian morphological changes of compound eye, such as changes of rhabdom size, submicrovillar cisternae or SPG. The second objective of the study was to substantiate evidence that the mechanism involved in the control of morphological changes in the compound eyes was circadian, because SMC changed on a circadian basis which was the particular anatomical structure monitored in this study.

MATERIALS AND METHODS

Cockroaches, *L. maderae*, were reared in a colony maintained under 12 hours of light and 12 hours of dark (LD 12:12) and provided with food (*i.e.*, Puppy Chow) and water *ad libitum*. At least one week prior to initiating constant darkness (DD), males were placed in environmental chambers at $25 \pm 2^\circ\text{C}$ with the same photoperiodic schedule as the rearing colony. Each of six of these animals was placed in a running wheel equipped with a magnetic reed switch wired to an Esterline-Angus event recorder. Two magnets mounted on the wheel closed a switch with each revolution of the wheel and a dash mark was made on a paper strip chart moving at a constant rate. With this system, the locomotor activity was continuously recorded and the activity patterns of the 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 vial fitted with a cotton wick that protruded through another hole in the face plate. All other roaches transferred to environmental chambers at the same time as those housed in running wheels were housed in small groups in ventilated plastic cages in which food and water were provided as in the rearing colony.

After the animals in running wheels had become entrained to LD 12:12, as determined by analysis of activity recordings, selected animals from plastic cages and running wheels received brain lesions either proximal or distal to the lobula neuropil. The purpose of the distal cut was to remove the connection between the clock and the compound eye, whereas proximal cuts left the connection between the clock and compound eye intact, but the clock was separated neurally from the other parts of the brain. The sham-operated group was used to determine operation

effects, and only the cuticle of the head capsule was cut, lifted, and replaced in its original position without touching brain tissue.

Prior to an operation, each animal was transferred from a plastic cage or a running wheel within an environmental chamber to a petri dish for CO₂ anesthetization. The head of an anesthetized roach was positioned through a hole in the petri dish lid and tape placed behind the neck to prevent the head from slipping back through the hole. A second piece of tape was placed across the abdomen of the animal on the underneath side of the lid to secure the animal. CO₂ was provided throughout the operation *via* a second hole in the petri dish lid and the operation was carried out under a dissecting microscope. The brain dissections were carried out only to affect the right eye of each animal. Using a fractured razor blade scapel, scissors and a pair of forceps, a flap of cuticle was cut, lifted and folded toward the anterior portion of the eye. The orientation of the cut helped ensure a constant air supply *via* the trachea to the compound eye. After exposing the midbrain, a cut distal or proximal to the lobula neuropil was made using an iridectomy scissors. The cuticle was replaced, the wound was sealed by melting a piece of parafilm over it and animals were put back in the plastic cages. Care was taken during the operational procedure, to ensure that cockroaches did not lose too much body fluid.

About two weeks later when activity recordings of the animals in running wheel indicated the animals had recovered from surgery and were still entrained, conditions of constant darkness (DD) were initiated in order to provide free running conditions. Tissue samples were obtained surgically from each of three roaches from each treatment group at the second subjective midday or midnight. Two strips of eye tissue were removed from the operated side, and two strips from the opposite

eye as a control. Anesthetization and animal restraint procedures were the same as those for performing brain lesions. Removal of eye tissue was carried out under a dissecting microscope using far red light emitted through a bandpass filter mounted on a shielded intensifier lamp. The wavelength emitted (*i.e.*, >630nm) is outside the range of spectral sensitivity of cockroach (Mote and Goldsmith 1983). Eye tissue was removed from the anterior one-third of the compound eyes using a fractured razor blade scalpel, iridectomy scissors and forceps and placed immediately into a vial containing 4% glutaraldehyde for fixation. These vials were placed in a dark cabinet and left there for 2.5 hours. The tissue samples were then rinsed twice for 15 minutes each time in Sorensen's phosphate buffer adjusted to pH 7.2. The buffer was changed to a 1% osmium tetroxide solution and the eye tissue postfixed for 1.5 hours. After rinsing twice with buffer for 15 minutes each time, the tissue samples were carried through a series of alcohol dehydrations: 20%, 35%, 50%, and 70%. The samples remained in the 70% alcohol for several hours and the series of alcohol dehydrations was continued: 85%, 95%, and 100% alcohol twice. Each alcohol dehydration step was for 10 minutes. The tissue samples were then subjected to a series of 100% alcohol : Spurr resin mixtures, from 3:1 to 1:1 to 1:3 to 0:1. The tissue was left in each mixture for 30 minutes. The sample was then placed into a Beam capsule containing some Spurr resin and the capsule was appropriately labeled. The samples were placed in the bottom of the Beam capsule with the convex side facing down and air bubbles were removed from the tip of the Beam capsule. The capsules were placed in preheated capsule holders which were placed in an oven set at 60°C for less than 10 hours. The cured resins were allowed to cool for about 24 hours and then were removed from the Beam capsule using a razor blade.

A Reichert OM U2 ultramicrotome was used to cut tissue sample sections using glass knives. 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 1950X. Photomicrographs were made of ommatidial cross sections taken in the vicinity of the proximal tip of the crystalline cone. The electron micrographs were printed at an enlargement of 3.4 (*i.e.*, total magnification of 6630X) to ensure that all data were uniform.

Photomicrographs of ommatidia, three from each of three roaches at each sampling time of four groups, were analyzed for the following morphological features: 1) organization of screening pigment granules (SPG) about the rhabdom, 2) rhabdom area and, 3) submicrovillar cisternae (SMC) area. The organization of screening pigment granules was determined by centering a circle with a diameter representing 10 μm about the rhabdom and counting the number of pigment granules present within the circle boundary. The areas of the rhabdom and SMC were determined using a Jandel 3-D computer program. The photomicrographs were traced into a CompuAdd 286 system computer equipped with a math coprocessor using a digitizer and the area was calculated using the program. The reliability of the program was checked using polygons of known area. The data were subjected to one-way analysis of variance and Student-Newman-Keul's range test in order to determine significance in temporal differences in these parameters at the 95% confidence level.

RESULTS

Rhabdom area did not vary temporally based on comparisons between control and sham operated eye tissue samples collected at subjective midday and midnight. Also, rhabdom area was not effected by brain lesion treatments. The values for all treatment groups at both sampling times ranged between $27 \pm 7.1 \mu\text{m}^2$ in the midnight group that received a lesion distal to the clock and $41.8 \pm 8.0 \mu\text{m}^2$ (Table 1 and Figures 1, 2, 3, and 4). SPG organization also remained constant through time (see Figures 1, 2, 3 and 4) with a large variation within treatment groups (Table 1). Standard deviation values within treatment groups ranged from ± 6 pigment granules in eye tissue sampled at midnight from roaches receiving a lesion distal to the clock to ± 23 pigment granules in tissue sampled at midday from roaches receiving lesion distal to the clock (Table 1).

SMC area did vary on a temporal basis under free running conditions based on comparisons between eye tissue samples collected from control and sham operated animals. SMC area was reduced in both midday control and midday sham operated groups (*i.e.*, $14.8 \pm 6.9 \mu\text{m}^2$ and $16.0 \pm 5.7 \mu\text{m}^2$, respectively) compared with the SMC area in midnight control and midnight sham operated groups (*i.e.*, $33.3 \pm 15.1 \mu\text{m}^2$ and $36.5 \pm 12.1 \mu\text{m}^2$, respectively) (Table 1 and Figures 1 and 2). Isolating the eye from the clock with a lesion distal to the clock or leaving the connection between the clock and eye intact with a proxiaml cut did not appear to effect submicrovillar area in eye samples taken on subjective midnight two (Table 1). SMC area at midnight for animals receiving lesions distal (*i.e.*, $35.2 \pm 14.9 \mu\text{m}^2$) or proximal (*i.e.*, $34.8 \pm 8.2 \mu\text{m}^2$) to the clock were similar to the SMC areas determined for controls and sham operated groups. In contrast, SMC areas in eye tissue removed at midday

from roaches receiving lesions distal (*i.e.*, $42.4 \pm 6.6 \mu\text{m}^2$) or proximal (*i.e.*, $29.0 \pm 8.9 \mu\text{m}^2$) to the clock were larger than SMC areas in midday controls or sham operated animals (Table 1 and Figures 3A and 3B). Although SMC areas in eye tissue removed at midday from animals receiving lesion with the clock and eye still connected (*i.e.*, proximal lesions) were unexpectedly similar to the midnight state, ommatidia from one animal had SMC similar to the subjective midday state.

Table 1. Rhabdom and submicrovillar cisternae area (μm^2), and the number of retinula cell screening pigment granules within a $10\mu\text{m}$ diameter circle centered about the rhabdom determined from electron photomicrographs of nine ommatidia.

	RHABDOM AREA	SUBMICROVILLAR CISTERNAE AREA	SCREENING PIGMENT GRANULE NUMBER
MDP	39.0 ± 9.3 ¹	29.0 ± 8.9 ^{b2}	30 ± 10
MDD	35.4 ± 11.3	42.4 ± 6.6 ^b	29 ± 23
MDS	32.4 ± 11.4	16.0 ± 5.7 ^a	46 ± 21
MDC	37.8 ± 8.1	14.8 ± 6.9 ^a	35 ± 7
MNP	38.0 ± 2.9	34.8 ± 8.2 ^b	27 ± 20
MND	27.4 ± 7.1	35.2 ± 14.9 ^b	34 ± 6
MNS	32.8 ± 8.7 ³	6.5 ± 12.1 ^b	31 ± 22
MNC	41.8 ± 8.0	33.3 ± 15.1 ^b	36 ± 9
ANOVA ₃	N.S.	$P < 0.05$	N.S.

1. Mean \pm Standard error.

2. Mean values that do not share letters in common were found to be significantly different at the 95% confidence interval ($P < 0.05$) using Student-Newman-Keul's range test.

3. Data were analyzed using an one-way analysis of variance (ANOVA). N.S. = no significant differences were found at the 95% confidence interval.

MDP = midday proximal cut

MNP = midnight proximal cut

MDD = midday distal cut

MND = midnight distal cut

MDS = midday sham operated

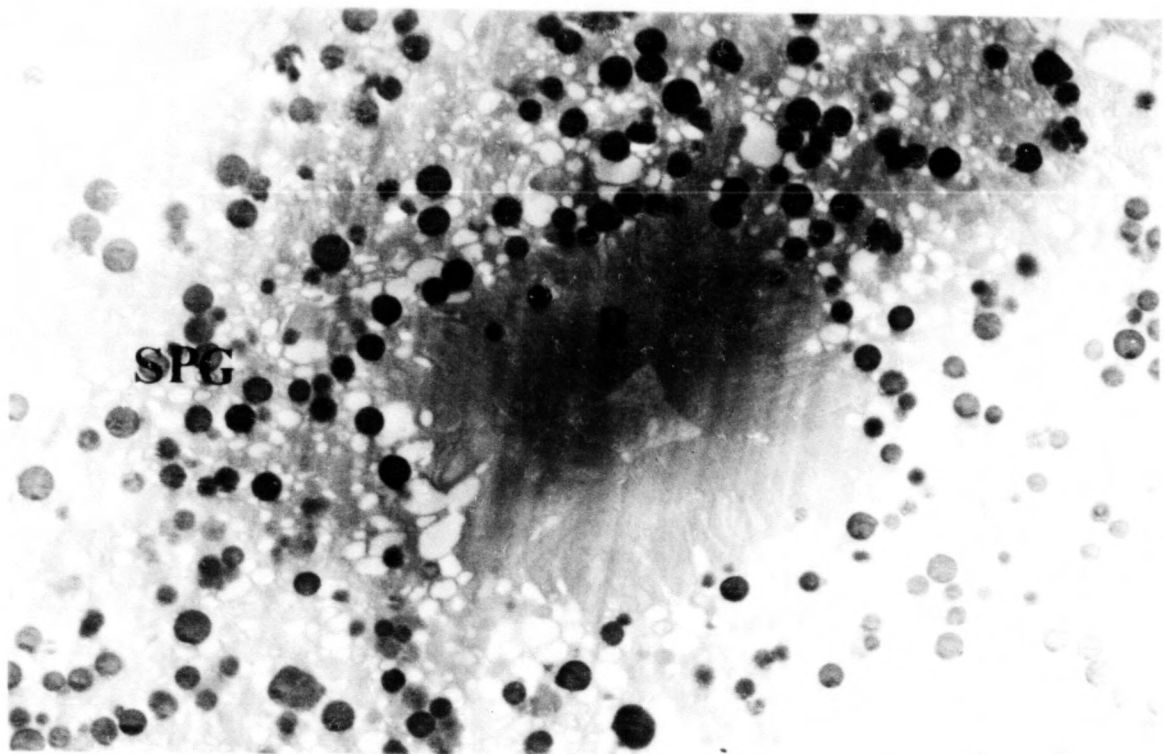
MNS = midnight sham operated

MDC = midday control

MNC = midnight control

Figure 1. Electron photomicrographs of ommatidial cross-sections sampled at (A) subjective midday (MDC) and (B) subjective midnight (MNC) from roaches in control groups held under constant darkness and $25\pm 2^{\circ}\text{C}$. The submicrovillar cisternae (SMC), rhabdom area (R) and screening pigment granules (SPG) are indicated.

A.MDC



B.MNC

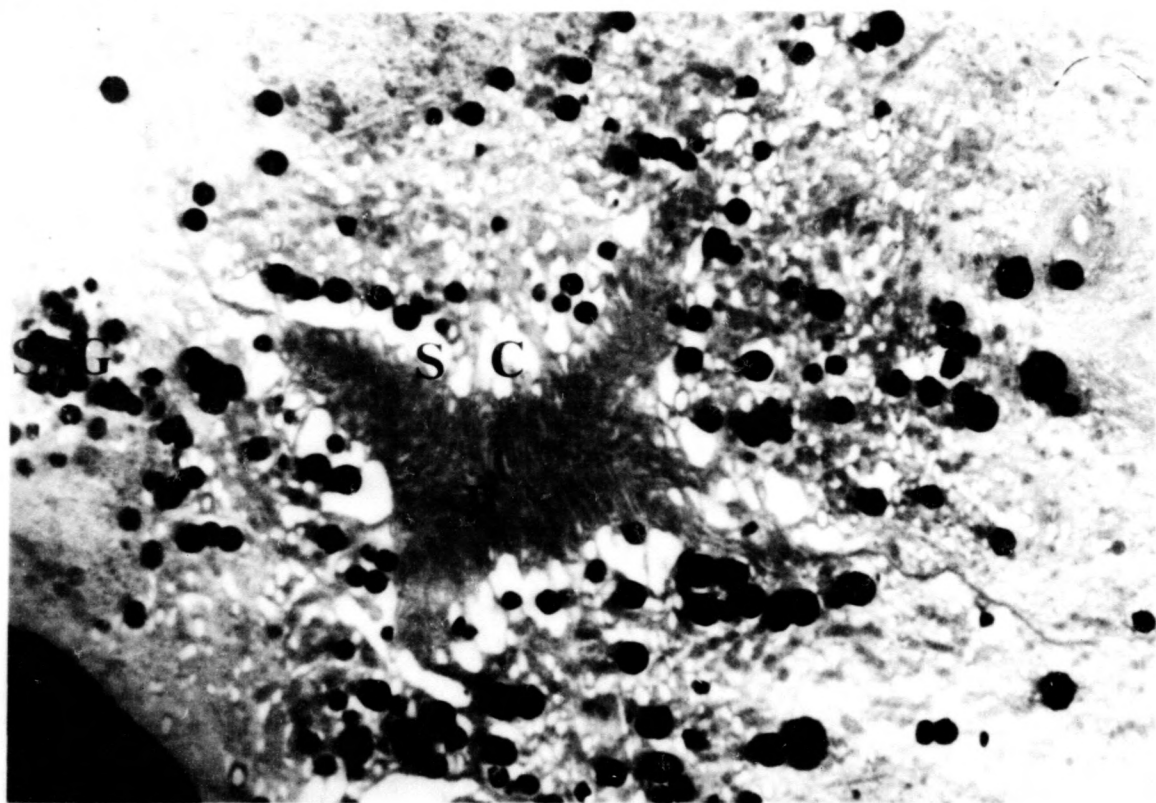


Figure 2. Electron photomicrographs of ommatidial cross-sections sampled at (A) subjective midday (MDS) and (B) subjective midnight (MNS) from roaches in sham operated groups held under constant darkness and $25\pm 2^{\circ}\text{C}$. The submicrovillar cisternae (SMC), rhabdom area (R) and screening pigment granules (SPG) are indicated.

A. MDS



B. MNS

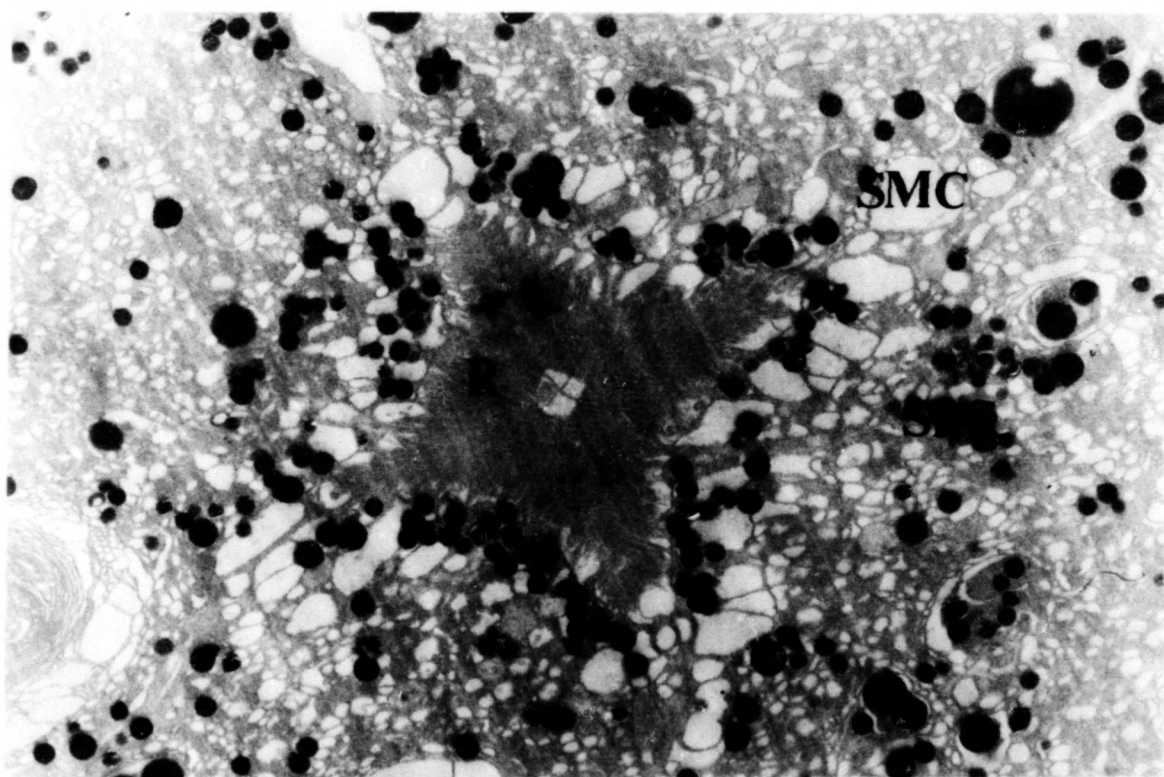
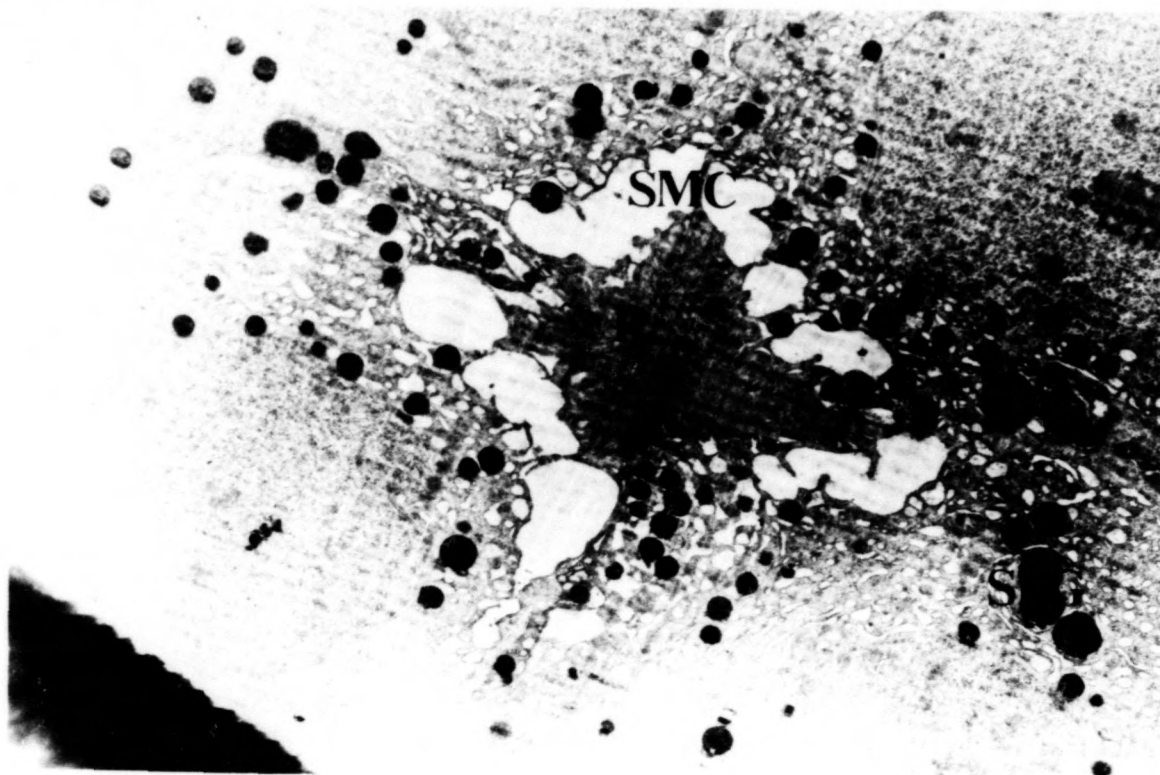


Figure 3. Electron photomicrographs of ommatidial cross-sections sampled at subjective midnight from roaches with a cut ..(A) distal (MND) or (B) proximal (MNP) to the putative clock. Roaches were held under constant darkness and $25\pm 2^{\circ}\text{C}$. The submicrovillar cisternae (SMC), rhabdom area R) and screening pigment granules (SPG) are indicated.

A.MND



B.MNP

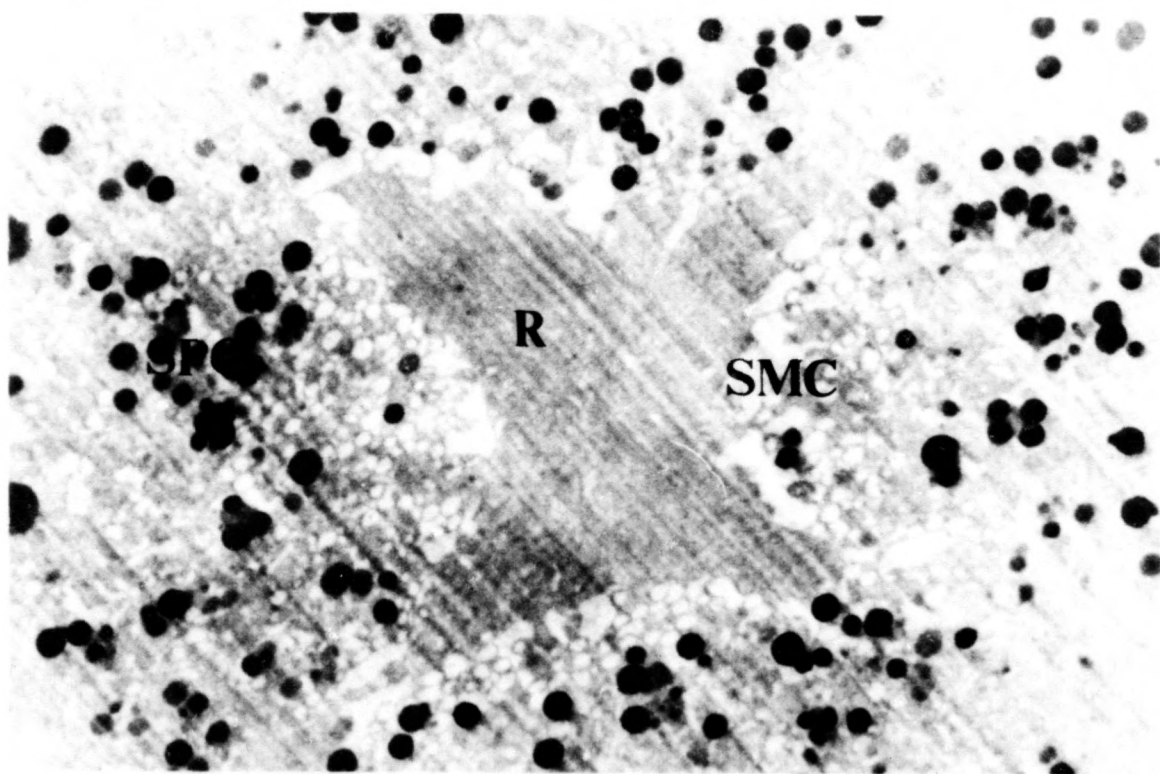
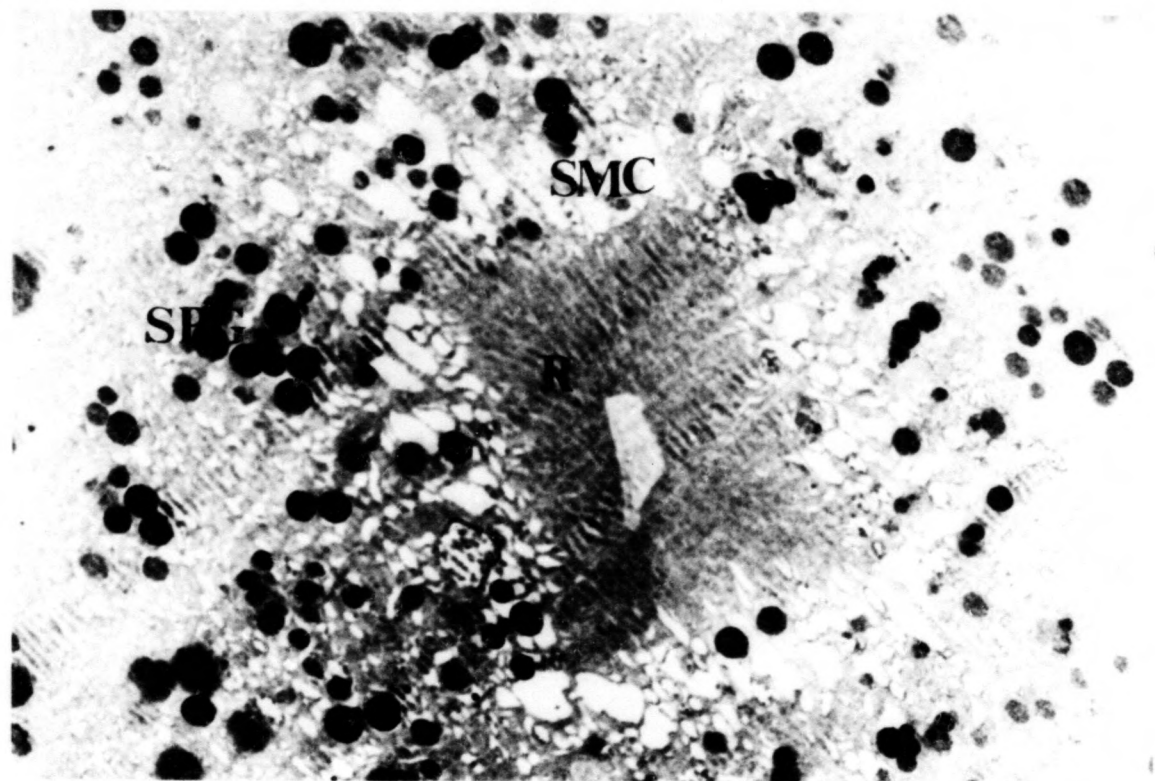


Figure 4. Electron photomicrographs of ommatidial cross-sections sampled at subjective midday from roaches with a cut (A) distal (MND) or (B) proximal (MNP) to the putative clock. Roaches were held under constant darkness and $25\pm 2^{\circ}\text{C}$. The submicrovillar cisternae (SMC), rhabdom area (R) and screening pigment granules (SPG) are indicated.

A. MDD



B. MDP

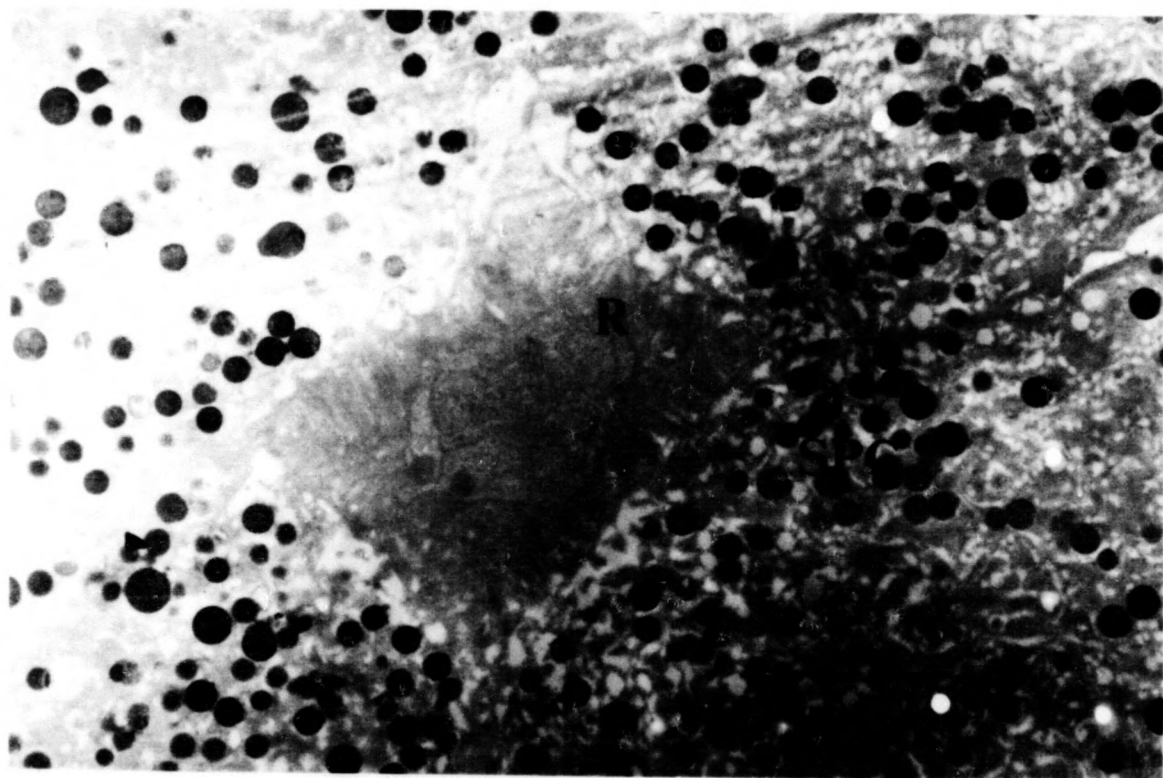


Figure 5. Mean rhabdom area of ommatidia determined in different treatments groups at subjective midday and subjective midnight in cockroaches maintained under constant darkness and $25\pm 2^{\circ}\text{C}$ (SEM = Standard error about the mean).

For abbreviations refer to Table 1.

RHABDOM AREA

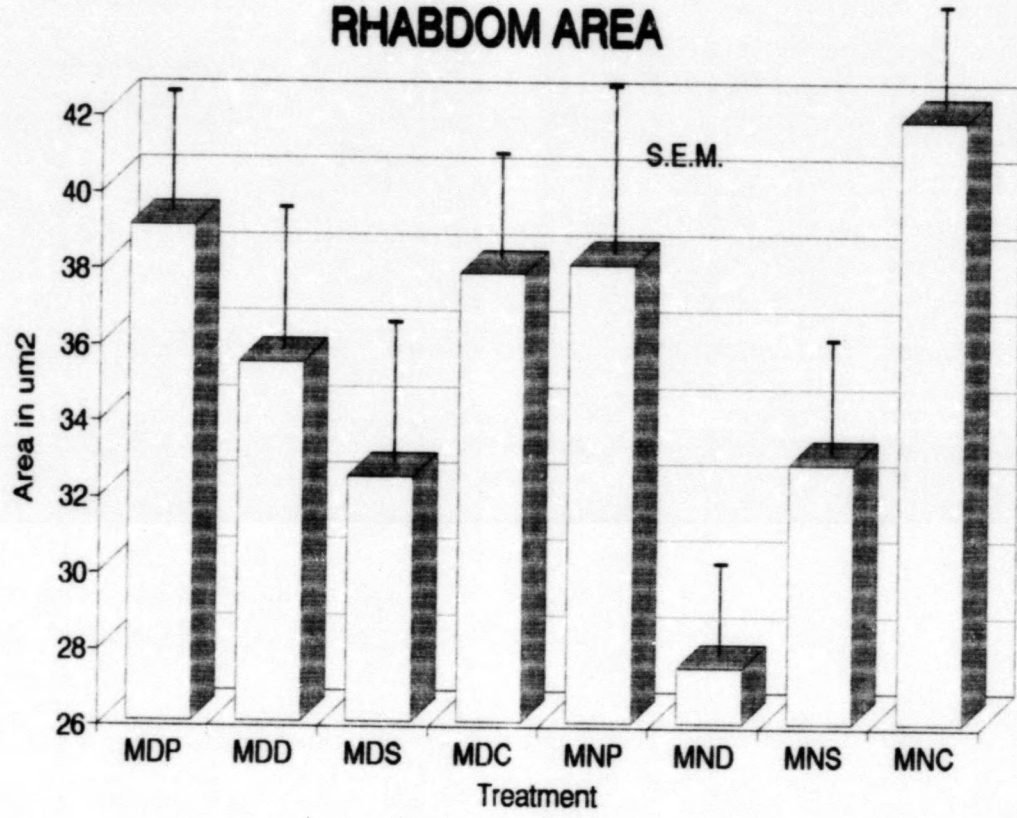


Figure 6. Mean submicrovillar cisternae area of ommatidia determined with different treatments at subjective midday and subjective midnight in cockroaches maintained under constant darkness and $25\pm 2^{\circ}\text{C}$ (SEM = Standard error about the mean).

For abbreviations refer to Table 1.

SMC AREA

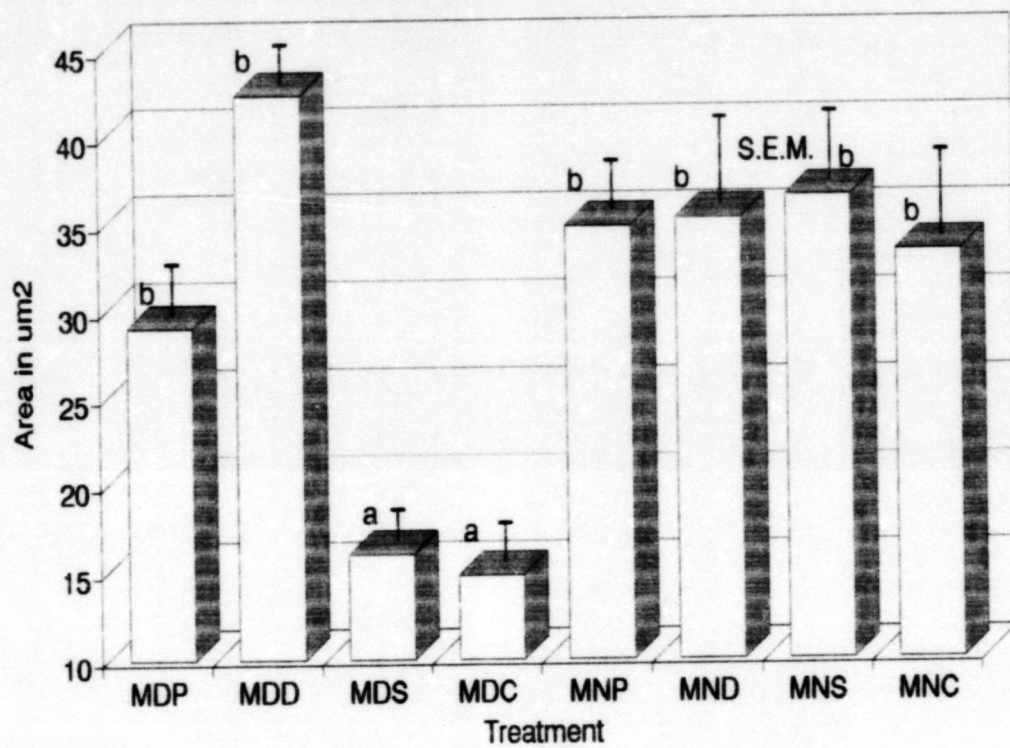


Figure 7. Mean screening pigment granules clustered within a 10 μm diameter circle centered about rhabdom of ommatidia determined with different cockroaches maintained under constant darkness and $25\pm 2^\circ\text{C}$ (SEM = Standard error about the mean).

For abbreviations refer to Table 1.

SPG NUMBER

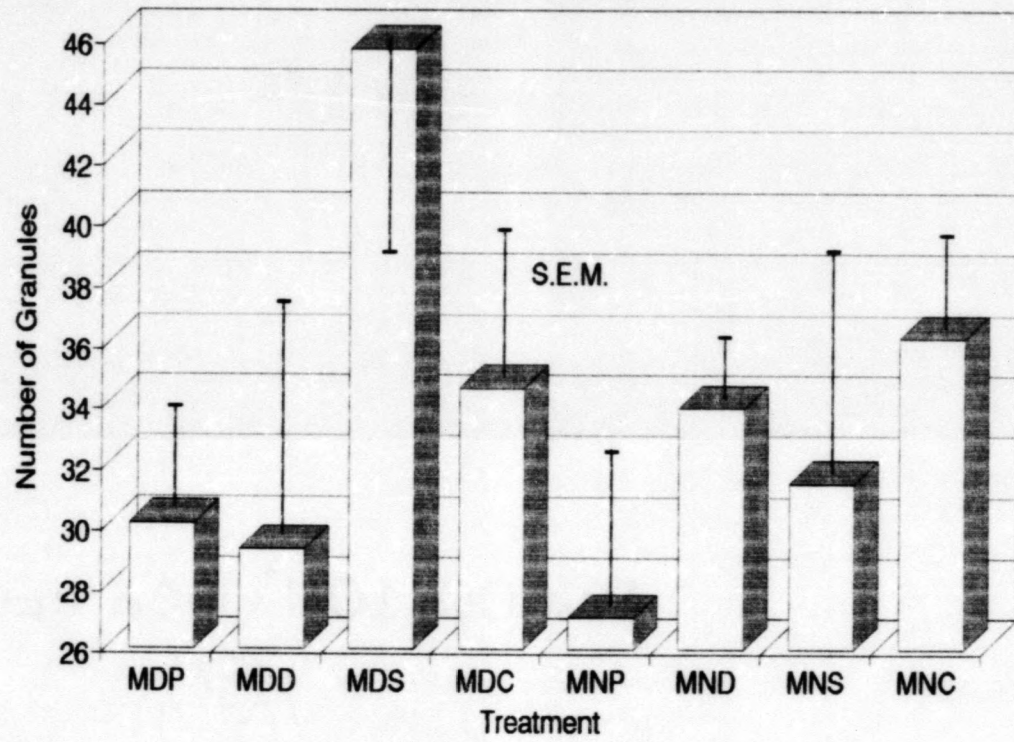
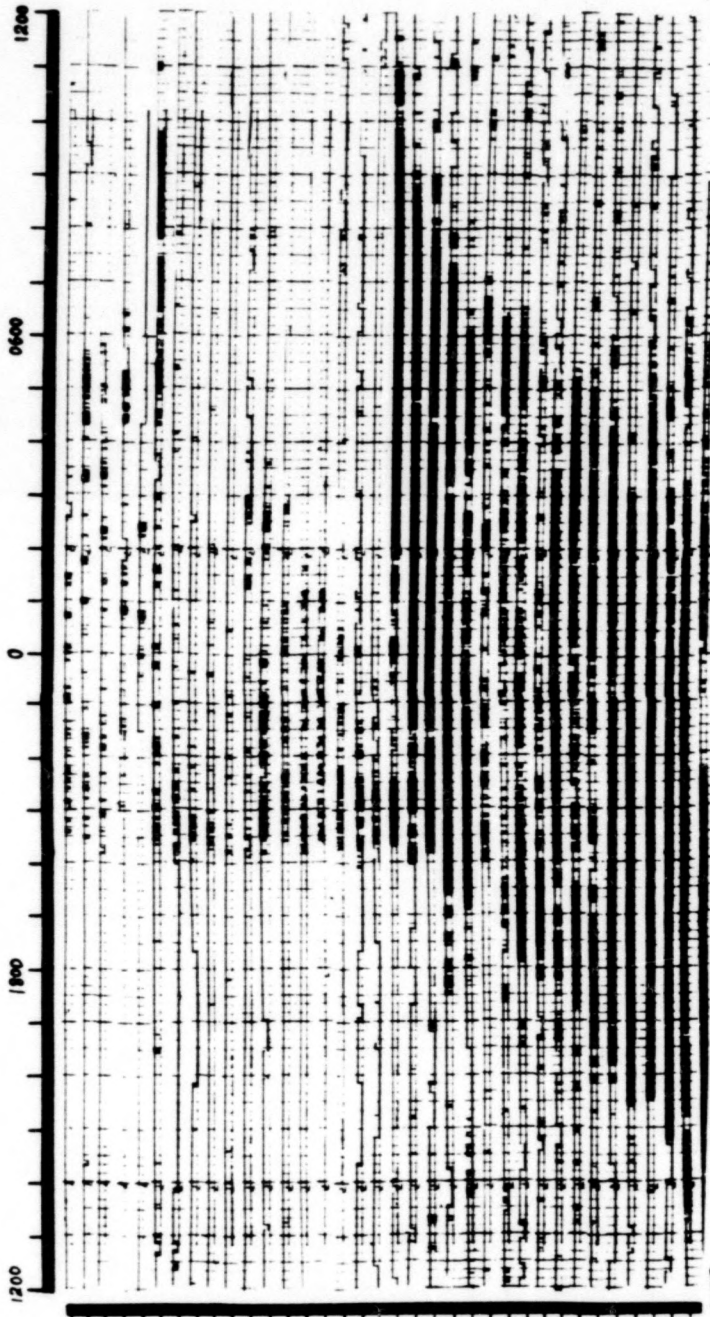
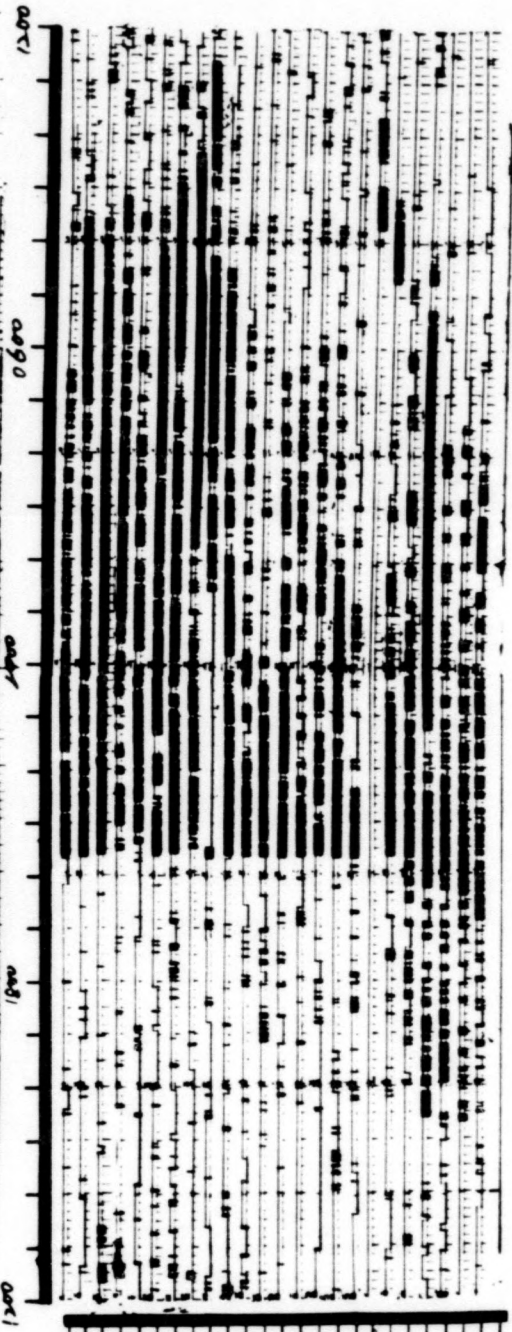


Figure 8. Locomotor activity recording of two cockroaches maintained under LD 12:12 and $25\pm 2^{\circ}\text{C}$ and subsequently placed under constant darkness (DD) on the day indicated. Light onset was at 0800 h and light offset was at 2000 h.



DD >



DD >

DISCUSSION

Studies on the physiological basis of circadian rhythms in light sensitivity indicated that a variety of mechanisms might be involved in the modulation of the ERG response in compound eyes. The movement of screening pigments, as well as changes in rhabdom size and/or shape, would be expected to lead to a change in the probability that a photon will be effectively absorbed in the retinula cells, which, in turn, would lead to a shift in the intensity-response function similar to that observed between the subjective day and night in *L. maderae* (Wills *et al.* 1985).

Similar to results from previous studies in *L. maderae* (Ferrell and Beitcheck 1993) and *P. americana* (Butler 1973), rhabdom area did not vary temporally. Although changes in membrane surface area can not be excluded by results of this study, it seems unlikely that changes in rhabdom structure account for the temporal difference in ERG amplitude observed in *L. maderae* by Wills *et al.* (1985). Likewise, brain lesion treatments did not appear to alter rhabdom area. This fact was taken as an indication that the operations themselves did not have a deleterious effect on eye morphology, thus strengthening the value of data concerning other parameters.

Although a daily rhythm in SPG organization about the rhabdom has been observed in *L. maderae* (Ferrel and Reitcheck 1990) and another cockroach species (*P. americana*, Butler 1973), where in SPG organization did not change through time in free running animals. Therefore, it seems unlikely that the SPG organizational rhythm is under endogenous clock control. Because of this fact, it seems unlikely that changes in SPG organization can account for the temporal variation in ERG amplitude measured by Wills *et al.* (1985) in *L. maderae* held under free running conditions.

Results of the present study are consistent with those of previous studies in *L. maderae* (Reitcheck and Ferrell 1990, Ferrell and Reitcheck 1993) in that the SMC area was the only morphological feature of ommatidia examined that continued to vary temporally on a circadian basis. The SMC areas measured at subjective midnight in this study were not different to a light-dark cycle (Ferrell and Reitcheck 1990, Ferrell and Reitcheck 1993). Although the SMC reached the nighttime state fully, it diminished partially toward the daytime state at subjective midday. Thus, consistent with previous results (Ferrell and Reitcheck, 1993), the temporal difference measured under DD was less than that observed under LD 12:12 conditions. The mechanism controlling circadian changes in structure associated with light/dark adaptations in *L. maderae* and another extensively studied arthropod, *Limulus polyphemus* (Kieran and Chamberlain 1990), seems to be analogous (Ferrell and Reitcheck 1993) despite the dissimilarities in eye morphology. The circadian changes in ommatidial morphology in *Limulus* are generated through an interplay between clock output *via* an efferent neural pathway and direct photic input to the eye. Clock output results in the formation of a more extensive SMC associated with the nighttime state in *L. maderae*, whereas in the absence of the clock output SMC passively decreased toward the daytime state (Ferrell and Reitcheck 1993). These findings are consistent with findings from electrical recordings of efferent neural activity from cultured optic lobes isolated from *L. maderae*. Neural output from the putative clock in the lobula neuropil controlling the circadian rhythms of locomotor activity and eye sensitivity to light was greatest during subjective day and lowest during subjective night (Colwell and Page 1990). The inverse phase relationship of this circadian output from the clock and the driven portion of the circadian cycle of eye morphology (*i.e.*, increased SMC area), correlated with

increased ERG amplitude (Wills *et al.* 1985) is suggestive that clock output has an inhibitory influence on morphological changes toward the nighttime state. The shallow rhythm in SMC area changes also mimics the shallow rhythm in ERG amplitude (Wills *et al.* 1985).

The clock that controls the SMC area rhythm appears to be located in the lobular neuropil region similar to the location of the clock that controls the rhythm of ERG amplitude. SMC areas at nighttime were large in control animals and animals in all surgical treatment groups, perhaps due to the lack of an inhibitory output of the clock. SMC areas of sham operated and control groups at subjective middays were small. SMC areas similar to those at midnight occurred in animals that received cuts distal to the clock. In that this treatment cut off the nerve connection between the clock and the compound eyes, the SMC area may have lost inhibitory output from the clock, such that it showed nighttime morphological characteristics. Cuts made proximal to the clock were expected to produce SMC areas at subjective midday similar to the SMC areas in midday sham and control groups. This expectation was not met except perhaps in one animal. Possibly the operation disrupted the clock or the trachea which supplies neural control between the clock and the eye, such that clock could not send negative control output to the compound eye. Inhibitory clock output at midday apparently results in the decrease of SMC area associated with light-adapted state in *L. maderae*, whereas, the absence of inhibitory clock output at night might release a neural output to drive SMC areas to nighttime extent. Conversely, the decrease of SMC area seems to be a passive process permitted to occur when some pathway is inhibited by clock output, unless affected by light.

Since the clock location of the morphological circadian changes of the compound eye of cockroach *L. maderae* had been determined to be at the same location as the one which controls ERG amplitude, it should be possible to begin tracing the neural pathway between its origin and termination, (*i.e.*, the neural pathway between the the clock and retinula cells).

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