Rhabdom Size and Chromophore Contents under the Daily Light Cycle in the Blowfly, Lucilia cuprina

Rhabdom Size and Chromophore Contents under the Daily Light Cycle in the Blowfly, *Lucilia cuprina*

Tsutomu KAWAUCHI, Takahiko HARIYAMA and Yasuo TSUKAHARA

Graduate School of Information Science, SKK Bldg., Tohoku University, Katahira 2, Sendai 980–8577, Japan

The eyes of the fly have been employed in an extensive range of studies, but the existence of diurnal rhythmicity, which is involved in the adaptation to different light environments between the day and night is still unclear. The rhythmicity of the photoreceptor cells of the blowfly *Lucilia cuprina* was observed over a precise time schedule under a daily light cycle. Both the morphology of the photoreceptive structure called rhabdom and the amount of the visual pigment chromophore were mostly constant. Each photoreceptor area measured from the cross sections and each amount of chromophore was ca. $22.5 \pm 1.85 \, \mu\text{m}^2$ and ca. 1.4 pmol, respectively. The number of the multivesicular bodies, assuming the indicator of the photoreceptive membrane turnover, was small compared with other reports of arthropods. We confirmed that there is no diurnal rhythmicity either in the morphology of rhabdom or in the visual pigment contents under the daily light cycles.

KEYWORDS: membrane turnover, rhabdom, fly, photoreceptor, diurnal rhythm.

Introduction

All animals except those living in the caves, deep sea, or polar regions experience the daily changes in environmental brightness due to the regular exposure to a day/night rhythm. The light intensity normally encountered in nature can vary by a factor of 10^{15} (Fein & Szuts, 1982). One striking aspect of the vision is its ability to function as an information input system during this large daily dynamic range. There are several systems for adapting to the environmental light conditions, the structural change in the photoreceptor cell itself is one solution to adapt to the daily light cycle.

In many arthropods, the structure of the photoreceptor cells changes diurnally. The size of a photoreceptive structure called rhabdom shows diurnal variation, which generally increases at night and decreases during the day (e.g. Blest, 1978: Spider; Nässel and Waterman, 1979: Crab; Williams, 1982a: Locust; Hariyama et al., 1986: *Ligia*). The difference between the speed of degradation and the synthesis of the rhabdomal membrane should be correlated with the rhabdomal size. Generally, the peak of the membrane breakdown occurs at dawn (Blest, 1978; Nässel and Waterman, 1979; Williams, 1982a), and that of the assembly at dusk (Stowe, 1980; Blest, 1988, for review), so that the size of the rhabdom gradually decreases at dawn and increases at dusk. Rhabdomal membrane contains a lot of visual pigments, and the amount of the visual pigment chromophore also changes diurnally, following the changes of the rhabdomal size (Isono et al., 1986; Hariyama and Tsukahara, 1992). In *Ligia exotica*, the sensitivity of the retina measured by an electroretinogram (ERG) method shows a maximum response at midnight and minimum at 10:00 am and almost similar diurnal fluctuations with the rhabdomal size and the rhodopsin content (Hariyama et al., 1986, 1992, 2001). Furthermore, many physiological characteristics, including the wave-guide property of the rhabdom (Kirschfeld and Snyder 1976), spectral sensitivity (Snyder and Miller, 1972, 1973) and the acceptance angle of the photoreceptor cells (Williams, 1982a; Hariyama et al., 2001) are also affected as a result of the changes of the rhabdomal size and rhodopsin content. Therefore, the rhabdomal size and the rhodopsin content have a close relationship in the optical characteristics of the arthropod photoreceptor cell and should be involved in the adaptation to the different light conditions between the day and night.

A fly is an arthropod which has been employed in an extensive range of studies including the visual system. The diurnal rhythmicity in the fly retina has also been investigated, but no diurnal changes were observed in the size of the rhabdom (blowny *Lucilia cuprina*: Williams, 1982b; fruitsfly *Drosophila melanogaster*: Chen et al., 1992). In *Drosophila*, the sensitivity measured by ERG and rhodopsin contents show transient decreases after several hours from the light onset (Chen et al., 1992). Williams (1982b) reported that there is no change in the morphology of the rhabdom and the amount of the rhabdomal membrane breakdown between midday and midnight in the blowfly, *Lucilia cuprina*. The fly photoreceptor therefore seems to be different from the other arthropods in its diurnal changes.

In our present study, the rhythmicity in the photoreceptor cells of *Lucilia cuprina* was observed using a precise time schedule under the daily light cycle. Morphological observations were focused on the size, the structure of

Correspondence: Tsutomu Kawauchi, GSIS, SKK-Bldg., Tohoku Univ., Katahira 2, Sendai 980-8577, Japan (Tel. +81-22-217-5048; Fax +81-22-268-9858; E-mail: kawauchi@biology.is.tohoku.ac.jp)

the rhabdom and the number of the multivesicular bodies, which are assumed to be an indicator of the rhabdomal membrane breakdown. In addition to these morphological aspects, the visual pigment chromophore contents were measured by the high performance liquid chromatography (HPLC).

Materials and methods

Animals and lighting conditions

Blowfly, *Lucilia cuprina* were reared in a wooden cage fitted with a glass window on one side. Water, sugar and beef liver were supplied ad libitum. Illumination was set at 12L:12D (light on at 7:00; off at 19:00). The light sources were regular fluorescent tubes (two sets of 40 W tubes 40–50 cm away from the cage; illuminance at the center of the cage was about 1000 lux). The temperature was set at 24 ± 2°C.

Observation of the rhabdomal size and structure

For the scanning electron microscopy, the fly heads were fixed with 4% paraformaldehyde buffered with 0.1 M sodium cacodylate solution (pH 7.2) for 2 h. Following dehydration in a graded series of ethanol, the samples were dried with a critical point drying apparatus (Hitachi HCP-1) and coated with gold vapors (Eico ion coater IB-3), and glued onto aluminum stubs. The observations were carried out with a Hitachi S-310 scanning electron microscope.

For morphological observations of the rhabdomal area, the blowflies were sacrificed at 18:30, 19:30, 1:00, 6:30, 7:30 and 13:00. The flies were decapitated and the compound eyes were surgically removed with a sliver of razor blade under a dim deep red light (> 640 nm). The eyes were immediately immersed in the primary fixative solution (2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, pH7.2), and kept in a refrigerator for overnight. The samples were then rinsed with a 0.1 M cacodylate buffer solution,   and post-fixed for 1 hour in 1% OsO$_4$ buffered with 0.1 M-cacodylate. After rinsing with the cacodylate buffer solution, the samples were dehydrated with an ethanol series and embedded in an Epon-Araldite mixture.

In order to exclude the differences caused by the depth of the retinula cells, a layer about 150 μm from the surface of the cornea where contain the nuclei of R1-6 and R7 was chosen. About 1.0 μm semi-thin cross sections were made prior to the ultra-thin sections, in order to identify the restricted layer containing the nuclei of the photoreceptor cells. Ultra-thin sections were made with an LKB Ultratom Nova microtome. Silver-thin sections were collected on 75A mesh grids and stained with 0.1% uranyl acetate for 20 min and 1% lead citrate for 30 min. All sections were observed with a Hitachi H-300 transmission electron microscope. Electron micrographs of the ommatidia were taken at × 7000 and expanded for × 30000 by the enlarger. The areas of the cell bodies and rhabdom of photoreceptor cells were measured by a transparent grid sheet. The number of the multivesicular bodies (MVBs) was counted from the same photographs. The areas and the number of the MVBs were measured by using twenty-five ommatidia from five flies at each sampling time. Student's t-test was used for the statistical analysis of the number of the MVBs and the size of both rhabdom and cell body (P < 0.01).

The visual pigment chromophore contents

The diurnal fluctuation in the amount of the visual pigment chromophore 3-hydroxyretinal was measured by high performance liquid chromatography (HPLC). Twenty-five blowflies were frozen at each sampling time and stored in deep freezer at − 80°C to minimize the auto isomerization of the visual pigments. All the following procedures were performed under a deep dim red light (> 640 nm). The heads were surgically removed from the frozen animals and homogenized in a micro glass homogenizer on ice. The formation and extraction of oximes in the homogenates were carried out according to the method of Suzuki and Makino-Tasaka (1983). Briefly, homogenates were treated with 0.1 ml 1 M-hydroxylamine and 0.5 ml methanol to obtain chromophore isomers of the pigment in the oxime form. The oximes were then extracted with 0.5 ml dichloromethane and 1 ml n-hexane, and then dried in an evaporator. The extracted oximes were redissolved in the chromatography elution solvent (1% isopropanol and 30% ethyl acetate in n-hexane) for HPLC. The absorbance at 360 nm was monitored using a UV/VIS Detector 870-UV (JASCO, Japan) and the elution patterns were recorded and processed with 807-IT (JASCO, Japan).

The samples were collected at 13:00, 16:00, 18:30, 19:30, 22:00, 1:00, 4:00, 6:30, 7:30, 10:00 and 13:00 the following day. During the daily light cycle, light was turned on at 7:00 and off at 19:00. Mean values and standard deviation in the amounts of 11-*cis* and all-*trans* isomers were calculated from five samples containing five blowfly heads at each sampling time. Student's t-test was used for the statistical analysis (P < 0.01).

Identification of the retinal isomers

A light irradiation experiments were carried out to identify the visual pigment chromophores. The blowflies were transferred to the dark at noon. After a one-hour dark adaptation, a selective light irradiation experiment was performed from 13:00. Because of the screening pigments in the retina, the light exposure is disturbed in an

intact animal. The fly heads were homogenized in 0.1 M phosphate buffer solution. The homogenates of the fly heads were irradiated by a blue or red light for 15 min, and then analyzed by using the HPLC. The control was not irradiated to find out the chromophore level of an intact animal under the dark condition. The peaks of the retinal isomers in the HPLC extraction pattern should be changed by the selective light irradiation, because the 11-*cis* 3-hydroxyretinal in the visual pigment is isomerized by a blue light to the all-*trans* form, whereas a red light induces the opposite reaction (Ranganathan et al, 1995 for review).

Results

Anatomy of the compound eye of blowfly Lucilia cuprina

Scanning electron microscopic observation revealed that the compound eye of the blowny *Lucilia cuprina* consists of about 3500 ommatidia, each ommatidium having a hexagonal corneal facet. No apparent regional differences were observed in the center of the compound eye (Fig. 1a). The structure of the *Lucilia* photoreceptor cell was reconstructed from a series of sections observed by a transmission electronmicroscope. Beneath the cornea, there was a crystalline cone forming an optical path to the rhabdom of the photoreceptor cells. The ommatidium had an open type rhabdom, which had a cavity between each rhabdomere (Fig. 1b). The photoreceptor cells of R1-6 extended continuously from beneath the crystalline cone to the basement membrane. Two smaller photoreceptor cells called R7 and R8 were located on the distal (R7) and proximal sides (R8) in a tiered fashion. The total longitudinal length of the photoreceptor region was ca. 600 μm (Fig. 1c).

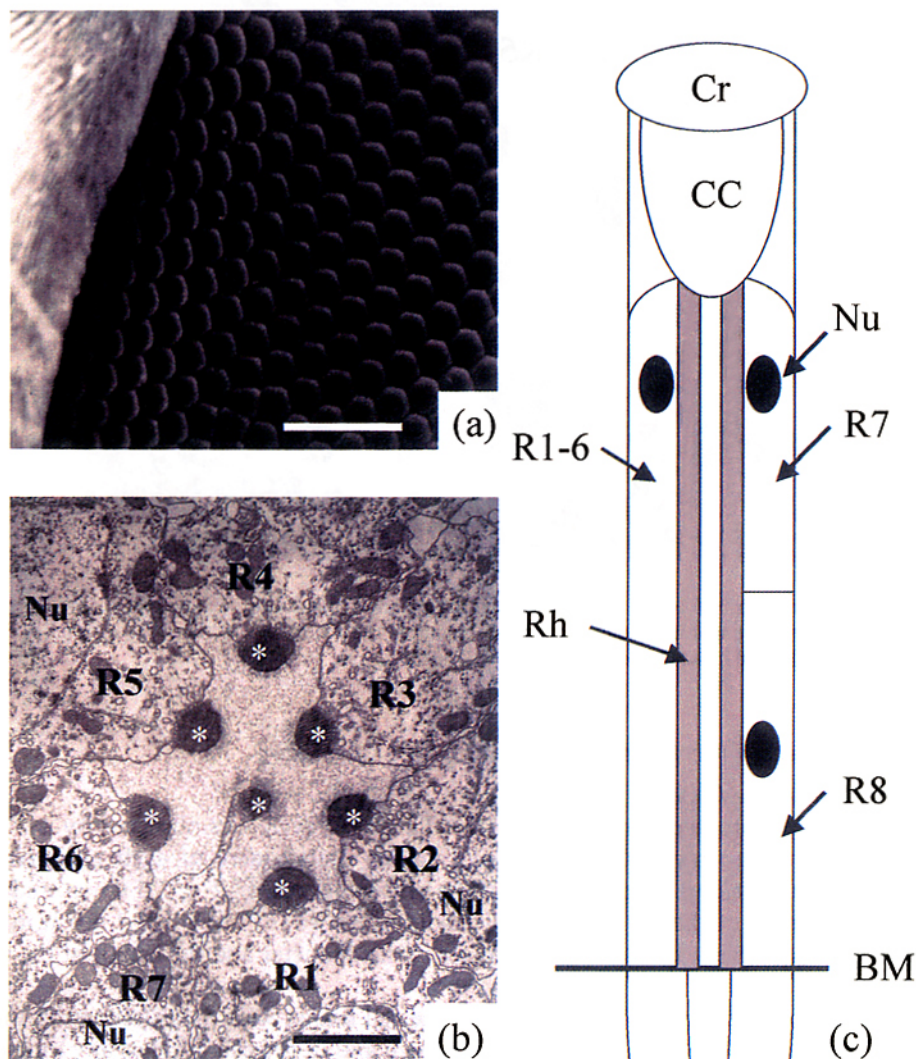
Fig. 1. Ultrastructure of the compound eye of *Lucilia*. (a) Scanning electronmicrograph of the surface of the compound eye. Scale bar, 300 μm. (b) Transmission electron micrograph of a cross-section through an ommatidium at the midday. Seven photoreceptor cells (R1-7) constitute one ommatidium. *:Rhabdomere, Nu:Nucleus. Scale bar, 2 μm. (c) Schematic drawing of a transverse section through an ommatidium of *Lucilia*. Cr, cornea; CC, Crystaline cone; Rh, Rhabdomere; Nu, Nucleus; BM, Basement membrane.
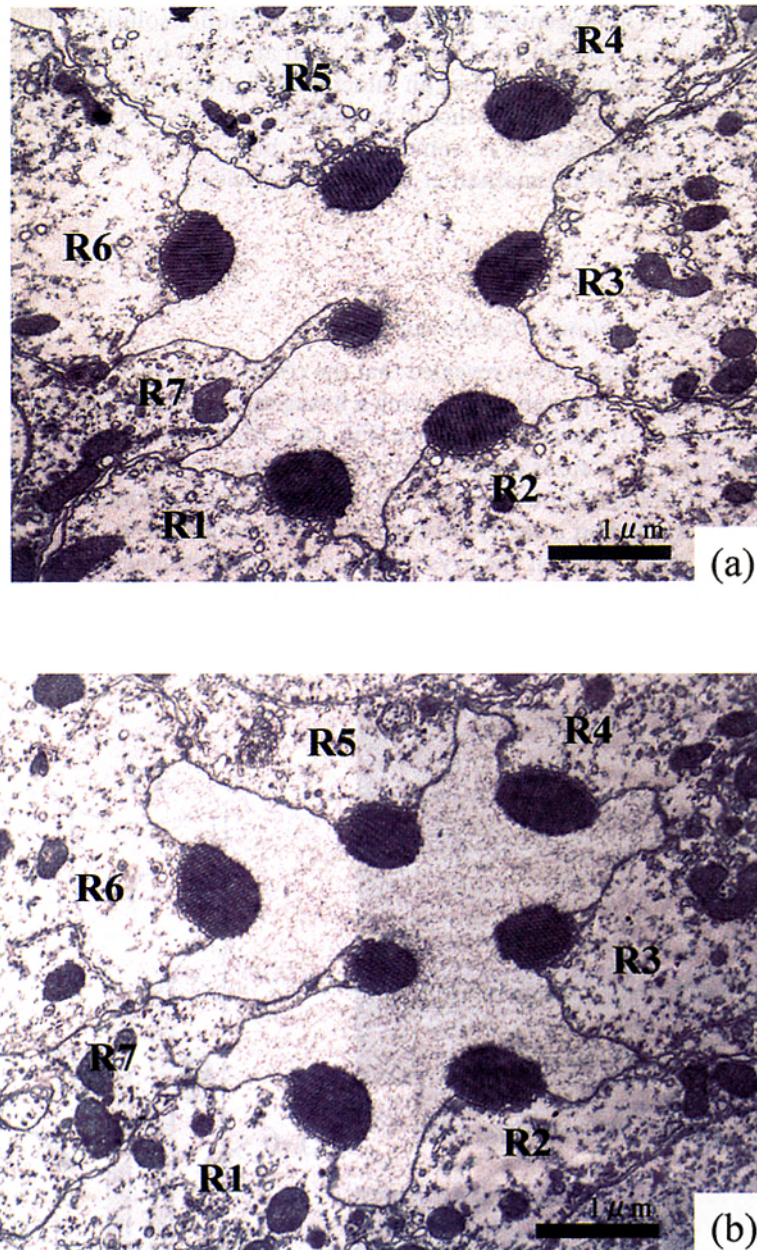
Fig. 2. The cross-sectional transmission electron micrograph of an ommatidium. Rhabdomere composing microvilli
were aligned regularly throughout the day. No obvious change in the microvilli were found at any time of the day. (a)
day (13:00), (b) night (1:00). R1-7, Retinula cells; Nu, Nucleus. Scale bar, 2 μm.

Morphology of the photoreceptor cells depending on the time of the day

(1) Area of the rhabdom and cell body

    The ultrastructure of the photoreceptor cells under the daily light cycle was observed by a transmission elec-
tron microscope. The numerous microvilli which make up the rhabdomere of each photoreceptor cell were
aligned regularly throughout the day. The diameter of the microvilli was 47.2 ± 7.5 nm at midday and 48.7 ±
2.7 nm at midnight. The length of the most longest microvilli in R1 was 657.6 ± 106.1 nm at midday and 697.8
± 160.9 nm at midnight. We observed no obvious changes in the diameter and the length of the microvilli at any
time of the day.

    The areas of both the cross-sectional rhabdomeres and the cell bodies were measured under the daily light cy-
cle. There were no significant differences in the rhabdomal size and in the cell body at any time of the day (Fig.
3). The mean value of the area of the rhabdom was 22.5 ± 1.85 μm² and the cellbody 874 ± 72 μm². These data
indicate that the sizes of both the rhabdomere and cell body are constant throughout the day.

(2) Organelles in the cell body

    The size and morphology of the rhabdom were constant throughout the day. No differences were observed in
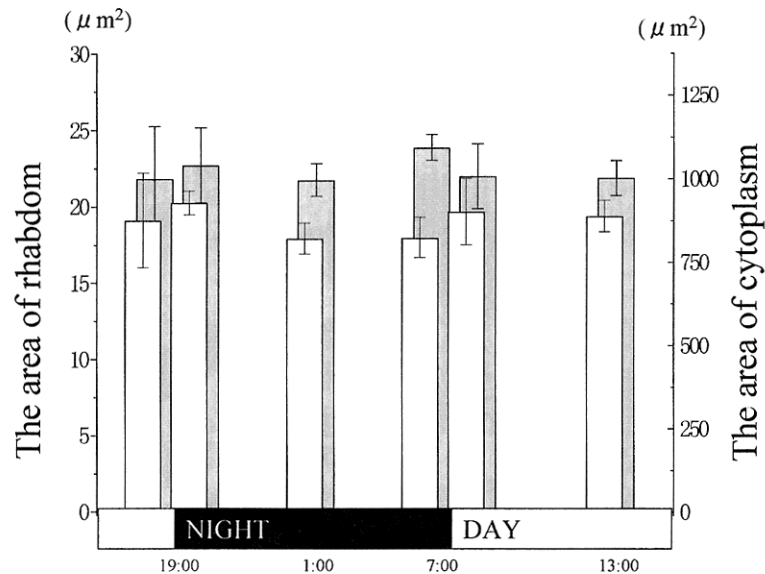
Fig. 3. Diurnal changes in the cross-sectional areas of the rhabdom and cell body under the daily light cycles. Horizontal bar under the graphs indicates the light condition, the white bar is light and black is darkness. A mean value of the area of the rhabdomal size was $22.5 \pm 1.85\ \mu m^2$ and the cell body $874 \pm 72\ \mu m^2$. There were no significant differences in the size of rhabdom and cell body at any time of the day. White vertical bars represent the area of rhabdom, and gray bar is cell bodies. The error bars indicate the standard deviations from the ten ommatidia of each five animals.

the other intracellular organelles, such as the mitochondria, rough endoplasmic reticulum and nuclei (Fig. 2), except some tiny vacuoles. More vacuoles were observed during the day compared with the night (Fig. 4). In the arthropod photoreceptor cell, the microvilli composing the rhabdomal membrane degrades to small vesicles, which have the same diameter as the microvilli. However, the observed vacuoles showed several sizes ranging from 80 nm to $0.4\ \mu m$. We chose the multivesicular bodies (MVBs) as the indicator of the amount of rhabdomal membrane degradation. MVBs (Fig. 5a) were observed from all the samples of the blowfly photoreceptor cells (Fig. 5b). The number of the MVBs was relatively higher during the day (0.88 ± 0.95 MVBs per ommatidium) than during the night (0.42 ± 0.65 MVBs per ommatidium), but there was no significant difference (P = 0.084). A significant difference was found in the light onset (Fig. 5b). 30 min before the dawn, the number of the MVBs was 0.33 ± 0.65 MVBs per one ommatidium, however, which increased to 1.08 ± 1.24 MVBs 30 min after the dawn (P = 0.006) (Fig. 5b). These data indicate that the rhabdomal membrane breakdown was transiently enhanced after the light onset in the blowfly photoreceptor cell.

Visual pigment chromophore contents under the daily light cycle and under the continuous darkness

(1) Characterization of the retinal isomers

A light irradiation experiment was performed to identify the retinal isomers in *Lucilia* using HPLC. The HPLC pattern obtained from the blowfly under a dark conditions showed six peaks (Fig. 6a). To identify the peaks, each homogenate sample was irradiated with either a blue or red light for 15 min respectively, and then analyzed by HPLC. After the blue light irradiation, peaks 3 and 4 were increased, and peaks 2 and 6 were decreased (Fig. 6b). An opposite reaction was observed after the red light irradiation; peaks 3 and 4 were decreased, and peaks 2 and 6 were increased (Fig. 6c). Peak 1 was the solvent front. The light irradiation did not affect the height of the peak 5. The substance of peak 5 is unknown.

The four eluent peaks were identified as follows: peak 2; 11-*cis* 3-hydroxyretinal (syn), peak 3; all-*trans* 3-hydroxyretinal (syn), peak 4; all-*trans* 3-hydroxyretinal (anti), and peak 6: 11-*cis* 3-hydroxyretinal (anti), syn and anti are structural isomers caused by hydroxylamine in the extraction.

(2) Rhythmicity of the chromophore amount

The diurnal rhythmicities in the amounts of both the 11-*cis* (syn) and all-*trans* 3-hydroxyretinal (syn) isomers were observed (Fig. 7a). The amount of 11-*cis* (syn) isomer showed a slight increase after the dusk, but there was no significant difference between 18:30 and 19:30 (P = 0.03). The amount of 11-*cis* (syn) isomer at each sampling time showed a random fluctuation, so that there were no significant differences. The average contents of 11-*cis* isomers was about 1.4 pmol per head throughout the day All-*trans* 3-hydroxyretinal (syn) isomer content increased shortly after dawn (6:30 vs. 7:30, P = 0.0027), but decreased soon again (7:30 vs. 10:00, P = 0.0001). The 11-*cis* isomers content was constant, but the all-*trans* isomers content changed after the light onset. We also
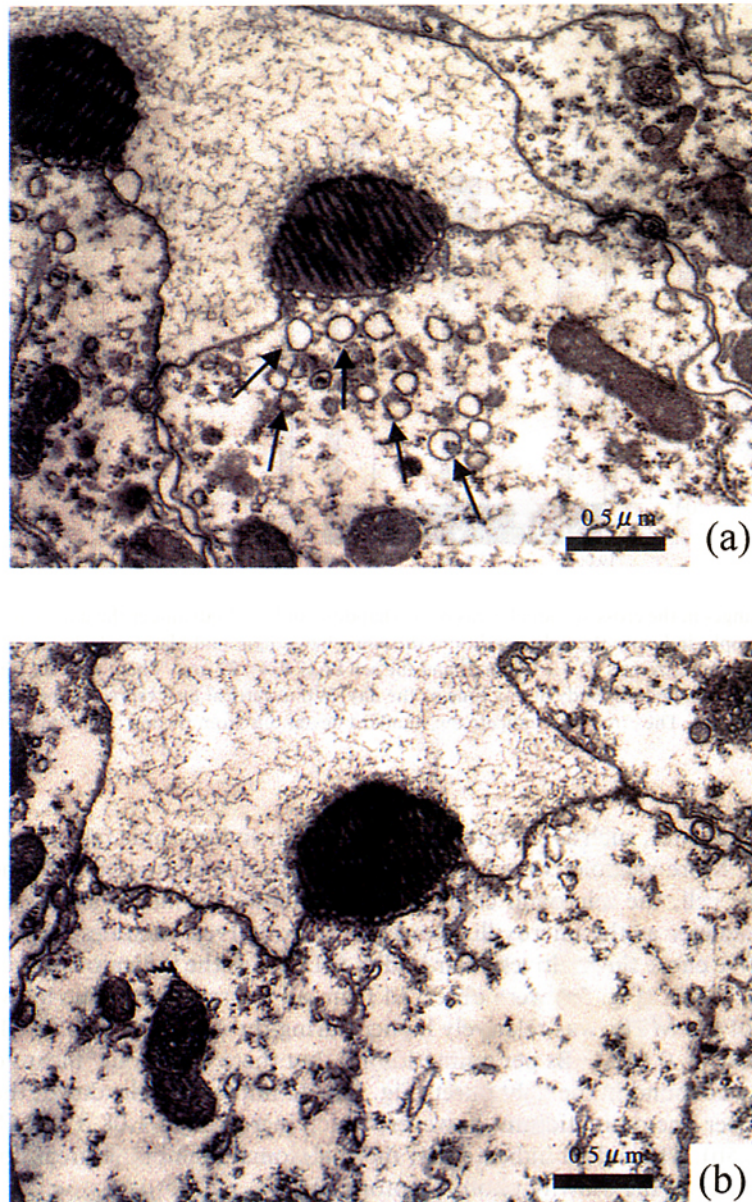
Fig. 4.   Cross-sectional electron micrographs of an ommatidium at midday (13:00) and in midnight (1:00). No difference was observed in other intracellular organelles except some certain tiny vacuoles. Many vacuoles were observed during the day (a) compared with the night (b). Note the numerous vacuoles and vesicles (arrows) during the day. Scale bar, 1 μm.

measured chromophore content in a continuous darkness following the dusk to the next midday (Fig. 7b). The transient increase of all-*trans* isomers at dawn disappeared in the continuous darkness, suggesting that the brief increase in the amount of the all-*trans* isomers was caused by the light rather than by the endogenous rhythm.

Discussion

The diurnal changes in the structure and chromophore content

The eyes of many animals have functions to cope with the changes in the light environment. The change of the structure in the photoreceptor cell is one solution to adapt to the light intensity changes. In many arthropods, the size of the rhabdom shows diurnal variation under the daily light cycle (e.g. Blest, 1978: Spider; Nässel and Waterman, 1979: Crab; Williams, 1982a: Locust; Hariyama et al., 1986: *Ligia*). In contrast, the constancy of the rhabdomal structure and size throughout the day has been reported in *Drosophila* (Chen et al., 1992) and in the blowfly *Lucilia* with regard to the two-point observations at midday and midnight (Williams, 1982b). And in our present study, the constancy of the rhabdomal size and structure in *Lucilia* was confirmed throughout
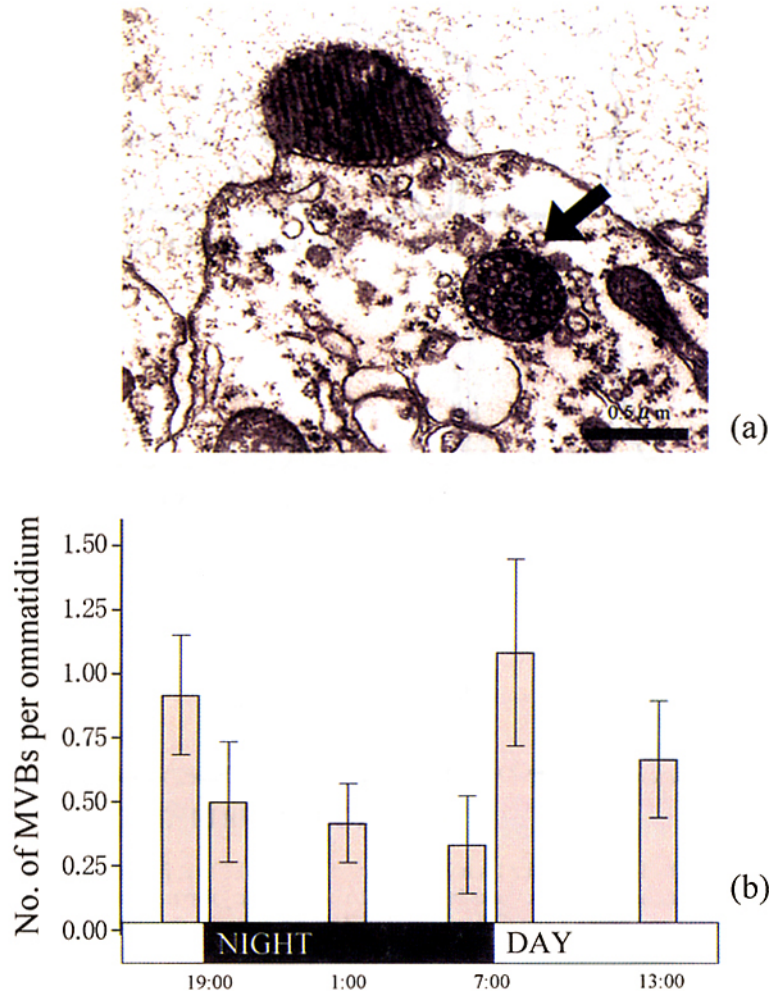
Fig. 5. (a) The MVBs were observed in the blowfly photoreceptor cells at all times under the daily light cycle. Appearance of the MVBs in the cell body (arrow). Scale bar, 1 μm. (b) The number of the MVBs between the day and night is not different significantly (P = 0.084). A significant difference was found only at the light onset, at dawn. (b) The error bars are standard errors for the ten ommatidia of each five animals.

day (Fig. 3).

The amount of 11-*cis* retinal changes diurnally following the rhabdomal size in the locust (Isono et al., 1986) and *Ligia exotica* (Hariyama and Tsukahara, 1992). The amount of 11-*cis* retinal isomer fluctuates diurnally with a several-fold difference between the day and night in those arthropods. In white-eyed *Drosophila*, the amount of the visual pigment content measured by microspectrophotometry shows unique circadian rhythmic changes, which have a transient decrease within several hours after the light onset (Chen et al., 1992). We did not observe any circadian rhythmic change in the visual pigment contents in *Lucilia*. The amount of 11-*cis* 3-hydroxyretinal was constant (Fig. 7a, b), which corresponded with the lack of the rhabdomal size changes. The fly photoreceptor seems to have no apparent diurnal rhythmic changes compared with the other arthropods.

All-*trans* 3-hydroxyretinal showed a transient increase 30 min after the dawn (Fig. 7a). This increment of all-*trans* retinal should be a light dependent phenomenon since it vanished in the continuous darkness. All-*trans* retinal is a the chromophore of metarhodopsin, which is derived from the rhodopsin photo-isomerization. Therefore, the increment of all-*trans* retinal probably indicated the increment of the metarhodopsin contents. The number of the MVBs also showed a transient increase at 30 min after the light onset. The relationship between the increments all-*trans* retinal and MVBs could remind of the possibility, that the isomerization of the rhodopsin is concerned with the producing processes of the MVBs.

Rhabdomal membrane turnover

The turnover in the rhabdom composing cell membrane is correlated with the rhabdomal size. Generally, the peak of the rhabdomal membrane breakdown occurs at dawn (Blest, 1978; Nässel and Waterman, 1979; Williams, 1982a) and that of the assembly at dusk (Stowe, 1980; Blest, 1988, for review), so that the size of the rhabdom gradually decreases at dawn and increases at dusk. The rhabdomal size and structure of the *Lucilia*
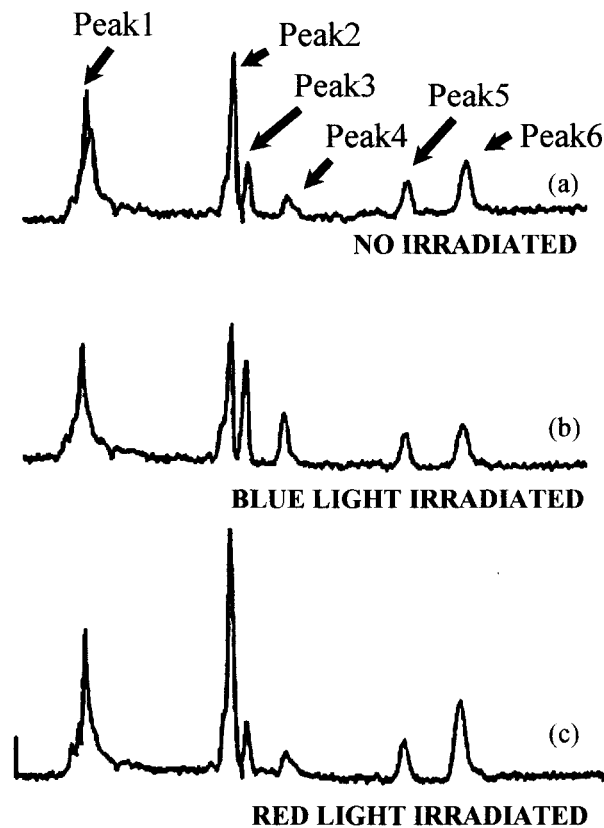
Fig. 6.   Chromatogram of the 3-hydroxyretinal isomers in *Lucilia* heads. (a) The HPLC pattern obtained from the blow-
fly under the dark conditions showed six peaks including the peak of the solvent front. After a blue light irradiation,
peaks 3 and 4 increased and peaks 2 and 6 decreased (Fig. 6b). An opposite reaction was observed after a red light
irradiation. The four eluent peaks were identified as follows: peak 2; 11-*cis* 3-hydroxyretinal (syn), peak 3: all-*trans* 3-
hydroxyretinal (syn), peak 4; all-*trans* 3-hydroxyretinal (anti), and peak 6; 11-*cis* 3-hydroxyretinal (anti).

photoreceptor cells were constant under the daily light cycle. In spite of the constancy, we found changes in the
number of vacuoles/vesicles in the cytoplasm, which were more numerous during the day than night (Fig. 4). Be-
cause the sizes of these membranous structures in the blowfly photoreceptor cells were deviated, it was difficult to
distinguish whether these membranous structures were vacuoles or vesicles. In the arthropod photoreceptor cell,
the microvillus composing rhabdomal membrane degrades to small vesicles and incorporates to the MVBs
(Stowe et al., 1990). Therefore the MVBs involve these small vesicles, which have the same diameter as the
microvilli. The change in the number of MVBs is a putative indicator of the rhabdomal membrane turnover in
the *Lucilia* photoreceptor cell (Fig. 5). The number of the MVBs significantly increased 30 min after the light on-
set. This data suggested that the increase of the MVBs was related with changes in the light environments. The
transient increase in the number of the MVBs at the light onset has been reported in many other arthropods as
well. Generally the membrane shedding peak was observed at dawn, and the rhabdomal size gradually decreased
(Blest, 1978; Williams, 1982a). The timing of the peak of the shedding is similar to that of the other arthropods.
However, the rhabdomal size in *Lucilia* was not changed (Fig. 2, 3). Changes in the number of the MVBs and
other lysosome related bodies at the light onset have been precisely reported in the crayfish photoreceptors: small
MVBs are present at a constant level throughout most of the diurnal cycle but show a sharp transient increase 30
min after the light onset (Hafner et al., 1980). The increase in the number of the MVBs 30 min after the light on-
set in the crayfish is much larger than that of *Lucilia*. A small amount of MVBs is one aspect of a fly photorecep-
tor cell. It seems that the lack of the vigorous morphological changes is correlated with a small number of the
MVBs.

Diurnal rhythmicity in the arthropod photoreceptor cell

The diurnal changes in the rhabdomal size affect many physiological characteristics, including the wave guide
property of the rhabdom (Kirschfeld and Snyder, 1976), spectral sensitivity (Snyder and Miller, 1972, 1973) and
acceptance angle of the photoreceptor. The changes in the size of the rhabdom should affect these characteristics
of the photoreceptor cells. Absence of diurnal rhythmicity in the rhabdomal size and structure in the fly pho-
toreceptor cell should not affect these functions, in other words, the fly photoreceptor has the same optical char-
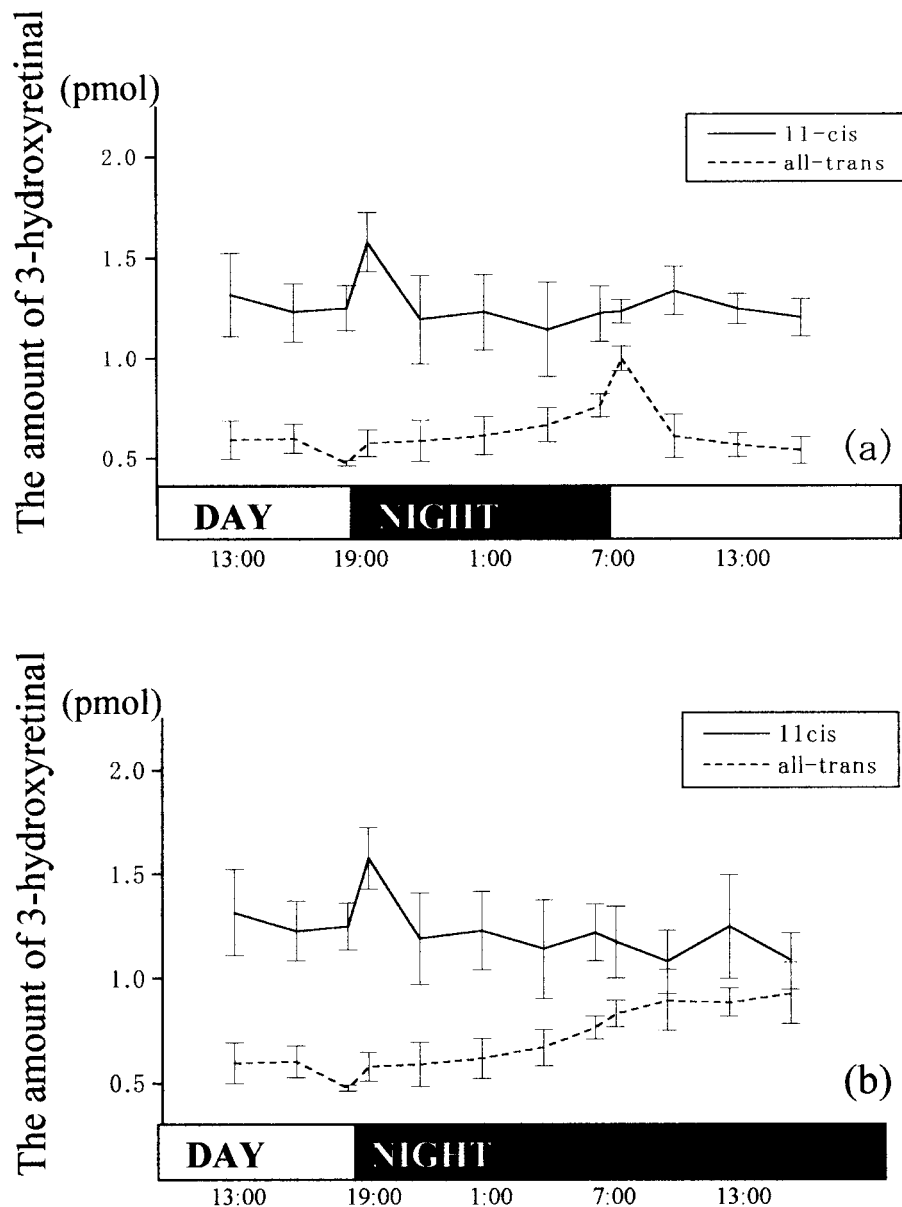acteristics throughout the day.

Fig. 7. Diurnal changes in the amounts of (syn) 11-*cis* and all-*trans* 3-hydroxyretinal during the L:D cycle (a) and in continuous darkness (b). The 11-*cis* 3-hydroxyretinal isomers remained constant, while the all-*trans* isomers changed after the light onset. The transient increase of all-*trans* isomers at dawn disappeared in the continuous darkness. Each point represents the mean value of the twenty-five samples with a standard deviation.

In spite of no obvious changes in the retina, some circadian changes in the secondary optic neurons lamina have been reported. The number of the synapses and size of the neurons in the housefly lamina show circadian rhythmicity (Pyza and Meinertzhagen, 1993, 1995).

It is still unclear how these morphological changes affect the fly vision, but there is a possibility that the adaptation to the different light intensities between the day and night is mainly controlled by changes in the secondary optic neurons.

The fly photoreceptor therefore seems to be different from the other arthropods in the adaptational system concering the light environments under the daily cycle.

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