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Changes of Photoreceptor Performance Associated with the Daily Turnover of Photoreceptor Membrane in Locusts

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Summary. 1. Intracellular recordings were made of receptor responses in the central region of the compound eyes of the locusts, *Valanga* and *Locusta*. The animals were maintained on their usual daily light cycle, and recordings were made at times known from previous anatomical studies to coincide with changes in ommatidial structure (Tunstall and Horridge 1967; Horridge et al. 1981; Williams 1982a). Anatomical checks were made of the areas of the retina from which recordings had been made (Fig. 1).

2. Angular acceptance at 50% sensitivity in *Valanga* and *Locusta* respectively increased from 1.7° and 1.9° when light-adapted during the day, to 2.7° and 2.8° when dark-adapted for 10–15 min after 'dusk', to 4.7° and 4.9° in a fully night-adapted state. It then decreased to 2.05° and 1.9° when light-adapted for 2 h after 'dawn', and increased to 2.8° and 2.9° after a further 20 min dark adaptation (Table 1). Dark-adapted values were measured by exposing the cells to very dim light and counting the quantum bumps (Fig. 3), thus ensuring that there were no light-adaptive effects from the stimulus.

3. Sensitivity to an extended source increased in both species by at least 1 log unit during the first 1–3 h after 'dusk' before reaching its maximum (Fig. 6). The last 0.6 log units of this increment (3.9-fold increase) is attributed to the enlargement of the field stop and rhabdom. After 2 h light from 'dawn', which induces diminution of the rhabdom and field stop to their day sizes, followed by 20 min of dark adaptation, cells were 3.8 times (data from *Valanga* and *Locusta* grouped) less sensitive to the extended source than they were at night.

4. The increased light capture from the environment, resulting from opening up the rhabdom's acceptance at night, is obtained at the expense of spatial acuity. At night, the angular acceptance at 50% sensitivity becomes more than twice the interommatidial angle ($\Delta\varphi$) (Fig. 8). As a result, discrimination of two points spaced at $2\,\Delta\varphi$, which is possible during the day, becomes impossible at night.

5. No change of spectral sensitivity (Fig. 4), bump latency (Fig. 7), or sensitivity to a point source on-axis (Fig. 5) was detected between the night and day states. The last indicates that the absorption efficiency of the rhabdom is constant, and the Airy disc is smaller than the day-state field stop. Together these findings indicate that the new photoreceptor membrane assembled at dusk does not differ fundamentally from that maintained during the day. Changes in sensitivity are therefore achieved by changing the amount of exposed receptor membrane rather than the nature of the membrane.

Introduction

Physiological changes between day and night in compound eyes were first recorded by electroretinogram measurements, which emphasised changes of sensitivity according to a circadian rhythm (e.g., beetle, Jahn and Wulff 1943; butterflies, Swihart 1963). Intracellular recordings later showed that sensitivity, in particular the angular sensitivity function of single photoreceptors, differed between light and dark adaptation (e.g., Tunstall and Horridge 1967; Walcott 1971). Consideration of an endogenous rhythm led other workers to measure differences, which were often greater, between day

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and night (beetle, Meyer-Rochow and Horridge 1975; crabs, Leggett and Stavenga 1981; Stowe 1980a; mantid, Rossel 1979; mantid and locust, Horridge et al. 1981; *Limulus*, Barlow et al. 1980).

The changes in sensitivity have usually been explained in terms of photomechanical movements of screening pigment, the photoreceptors, or cones. Recently, however, changes associated with the turnover of photoreceptor membrane have been held partly responsible for variations of angular sensitivity (crab, Stowe 1980a; locust and mantis, Horridge et al. 1981). In a number of compound eyes, rhabdom size fluctuates according to the daily cycle of photoreceptor membrane turnover. The locust compound eye is one example (Horridge et al. 1981). In locusts, the cross-sectional area of the distal rhabdom decreases at dawn because of the shedding of photoreceptor membrane by pinocytosis. It increases at dusk as new rhabdomeric membrane is assembled en masse. A change in the cross-sectional area of the ommatidial field stop, which is effected by the primary pigment cells situated immediately distal to the rhabdom (Fig. 2), occurs in tandem with the change of rhabdom size (Williams 1982a).

A previous study measured the angular sensitivity and intensity/response functions of single locust photoreceptors in relation to photoreceptor membrane turnover (Horridge et al. 1981). In this study, however, the daily cycle was not closely followed. Lights out occurred 5 h before the usual time of dusk. 'Dusk' that is premature to this extent induces a new rhabdom size that is only three-fourths of the usual night size (Williams 1982a). Furthermore, measurements were made by stimulating a cell with 500-ms flashes of light at 8-s intervals, and no anatomical check was made to see if the retina was still in the night-state after this exposure. Laughlin et al. (1980) found that after recording submaximal responses to well-spaced 10-ms flashes of light from photoreceptors of the spider, *Dinopis*, the retina did not contain the extra photoreceptor membrane typical of a normal night state. It is possible therefore that the strong amount of light given during the procedure of Horridge et al. would be sufficient to cause the dark-adapted locust cells to undergo some light-adaptive changes.

In locust photoreceptors, dim light elicits small discrete depolarisations, known as bumps (Scholes 1964). Bumps represent the absorption of single photons (Lillywhite 1977). Because spontaneous bumps are extremely rare in darkness, the number of bumps elicited by a stimulus is a direct measure of sensitivity (Lillywhite 1977). Thus, sensitivity can be measured under very low light intensities to ensure that no light-adaptive changes occur. The present study used intracellular recordings of bumps to explore the changes of photoreceptor function associated with the daily shedding and assembly of photoreceptor membrane and the accompanying changes of ommatidial structure. Lighting conditions throughout recording were strictly coincident with the animals' preceding day/night rhythm. Anatomical checks were made of the regions of the retina from which recordings were taken.

Materials and Methods

Apparatus. A 250-W tungsten filament lamp was run from a Hewlett Packard HP6269 B stabilised DC power supply. Light was emitted through a narrow aperture and a 3-mm thick Schott KG 3 heat filter. It was collimated by a glass lens and could pass through one of a range of narrow-band (10-nm half-width) interference filters (413, 438, 452, 474, 494, 513, 533, 574 nm) and gelatin neutral-density filters. A second lens focused the beam on to the end of a light guide. Between this lens and the light guide, a baffle was erected to exclude stray light. A Uniblitz electronic shutter, mounted immediately in front of the tip of the light guide, controlled the duration of the stimuli. A photodiode, used to monitor its operation, showed that the minimum stimulus duration was consistently 4 ms.

Stray light was prevented from reaching the locust during recording by enclosing the preparation and recording equipment in a blackened sheet-metal Faraday cage. The light guide passed through a small sealed hole into the cage. Its stimulating end was mounted on a Cardan arm whose horizontal and vertical axes of rotation passed through the locust's eye. For recordings requiring a point source, a 0.3-mm aperture that subtended 0.1° at the eye was placed over the end of the light guide. Stimulation by a uniform extended source was achieved by placing a ground glass diffuser (Spindler and Hoyer, Göttingen), wrapped in several layers of lens tissue, in front of the eye where it subtended 40°.

Calibrations. Absolute calibration of the transmission of neutral-density and interference filters was done at frequent intervals using an International Light Inc. silicon detector (SEE100) and radiometer (IL700). For very low intensities, such as those used to elicit discrete bumps, measurements were extended by using a photomultiplier tube (PM270D). In practice, however, one light intensity was used for as many different bump measurements as possible, so that any calibration error was avoided; the sample time was altered to obtain a significant number of bumps. The stability of the light source was periodically tested with the photomultiplier and no drift in output was detectable over 2 days; experiments usually lasted about 12 h.

Animals. Initial experiments were performed with adult *Locusta migratoria* L., but when they became unavailable, adult *Valanga irregularis* (Walk.) were used. All animals were taken from laboratory cultures that were fed bran and wheat and maintained on a 16 h light/8 h dark cycle at 20–35 °C. Initially, this cycle coincided with summer day/night, but later experiments were performed with lights out ('dusk') advanced to 1500 h for convenience. Light was provided by a 60 W incandescent bulb in

each cage (36 × 36 × 46 cm), and two 40 W daylight-fluorescent lights shared by three cages; average illuminance in the centre of each cage was 1,000 lux. The day before experimentation, a locust was placed in the Faraday cage and surrounded with white cardboard. At night no light entered through any windows. During the day a 15 W daylight fluorescent light provided the locust with an illuminance of 150–200 lux. Temperature was 25 ± 2° C. Humidity never varied more than 2% from a mean value between 45–55%. The animal was kept under the same conditions of temperature and humidity during preparation and experimentation.

Preparation and Recording. Prior to dusk an unanaesthetised intact locust was mounted in a silver collar. Its head and neck were firmly waxed to the collar, which acted as an indifferent electrode as well as a support. A small hole (less than 10 facets in diameter) was cut in the dorsal cornea with a fresh chip of razor blade. It was sealed with a stiff silicone grease. Care was taken not to disrupt the optics of the eye and to keep its facets clean. After making the hole, the shape of the pseudopupil of the eye was examined for abnormalities. A change in its shape would indicate that the optics had been distorted and the eye could not be used. The locust was mounted on the stage of a micromanipulator with its prepared eye at the centre of a Cardan arm. A glass microelectrode was introduced into the eye through the hole in the cornea. The microelectrodes were filled with 3 mol/l potassium acetate and had resistances of 100–150 MΩ in the retina. Intracellular recordings of potential difference between the microelectrode and the indifferent electrode were amplified by a Grass P16 preamplifier and displayed on an oscilloscope and a chart recorder. The animal remained exposed to 150–200 lux throughout preparation.

Procedure. Although technically more difficult, it was considered that a more valid indication of the effects of different times of day on photoreceptor performance would be obtained if in each experiment recordings were made from one receptor throughout a daily cycle, rather than from different cells, which were possibly of different cell types. Attempts were made to hold each impaled cell from just before dusk until the next day, while measuring its performance at times chosen with respect to known changes of ommatidial structure (Williams 1982a). All recordings used for analysis were from cells that showed stable resting potentials, 'healthy responses', and good optics. Prior to dusk, they gave at least a 50 mV saturated response, and had a symmetrical, narrow light-adapted angular sensitivity function. Throughout the entire duration of an experiment, the overall drift of the dark-adapted resting potential was less than 10 mV. A locust ommatidium has 8 photoreceptor cells, of which 6 are large and 2 small (Wilson et al. 1978). Each recording of the present report was probably made from one of the six larger cells. A light-adapted eye (before dusk and during lights-on after 'dawn') received about 150 lux fluorescent and incandescent lighting reflected from white cardboard around the end of the light guide. Stimuli were superimposed on this background lighting. Sensitivities were determined by referring responses to an intensity/response (V/log I) function obtained at the same time. Sensitivities during dark adaptation were determined directly by counting the number of bumps to an exposure of dim light. The intensity of this exposure was adjusted so that the mean bump rate was never more than about 3/s, and the duration of exposure ensured that a minimum of 100 bumps were recorded for each reading. In order to prevent any light adaptation, a dark-adapted cell was never exposed to light that would elicit a response greater than a train of discrete bumps.

Electron Microscopy. Eyes were fixed in phosphate-buffered glutaraldehyde and paraformaldehyde, followed by OsO_4, as described previously (Williams 1982a).

Results

All results concern the centre of the compound eye of *Locusta* or *Valanga*, so that they are comparable with a previous anatomical study (Williams 1982a). This region of the eye, which views to the side of the locust, has lower resolving power than the forward-looking acute region (Horridge 1978).

Ommatidial Structure

Eyes of *Valanga* were fixed under the conditions defined by the recording procedure; the organisation and size of the rhabdoms of *Valanga* and *Locusta* are similar (Williams 1982a). Two eyes were fixed just before dusk, and two 4–5 h after dusk (Fig. 1). Recordings had been made from all eyes prior to fixation, and the part of the eye examined included about 100 ommatidia in the region from which recordings had been taken. Ommatidial structure was similar to that from eyes not used for electrophysiology. No cells that may have been damaged from the recording procedure were detected. The average cross-sectional area of the rhabdom at the level of the distal nuclear region was 5 μm^2 during the day and 17 μm^2 at night; i.e. a 3- to 4-fold change. These measurements give quick and accurate determinations of the area of the ommatidial field stop, which is always 2.2 times greater (Fig. 2) (Williams 1982a).

In addition, two eyes of *Valanga* were fixed 4–5 h after dusk, immediately after exposure to four series of about 20 flashes from an extended source. Each flash ($\lambda = 560$ nm, from a light-emitting diode) was 10 ms long, and was delivered at 15-s intervals. Each series of flashes began at an intensity sufficient to elicit a 35-mV response in an exposed cell, and subsequent flashes decreased in intensity by 0.2 log-unit gradations. Rhabdoms from the exposed area of the retina appeared dark-adapted; each had a palisade of endoplasmic reticular vacuoles around it (cf. Horridge and Barnard 1965). However, they were considerably smaller than normal night rhabdoms – their cross-sectional area averaged only 7 μm^2 – and pinocytotic vesicles were apparent around the bases of the microvilli, indicating that membrane was being shed.

Dark-Adapted Receptor Response

After a few minutes of dark-adaptation, bumps were recorded from a locust photoreceptor in re-

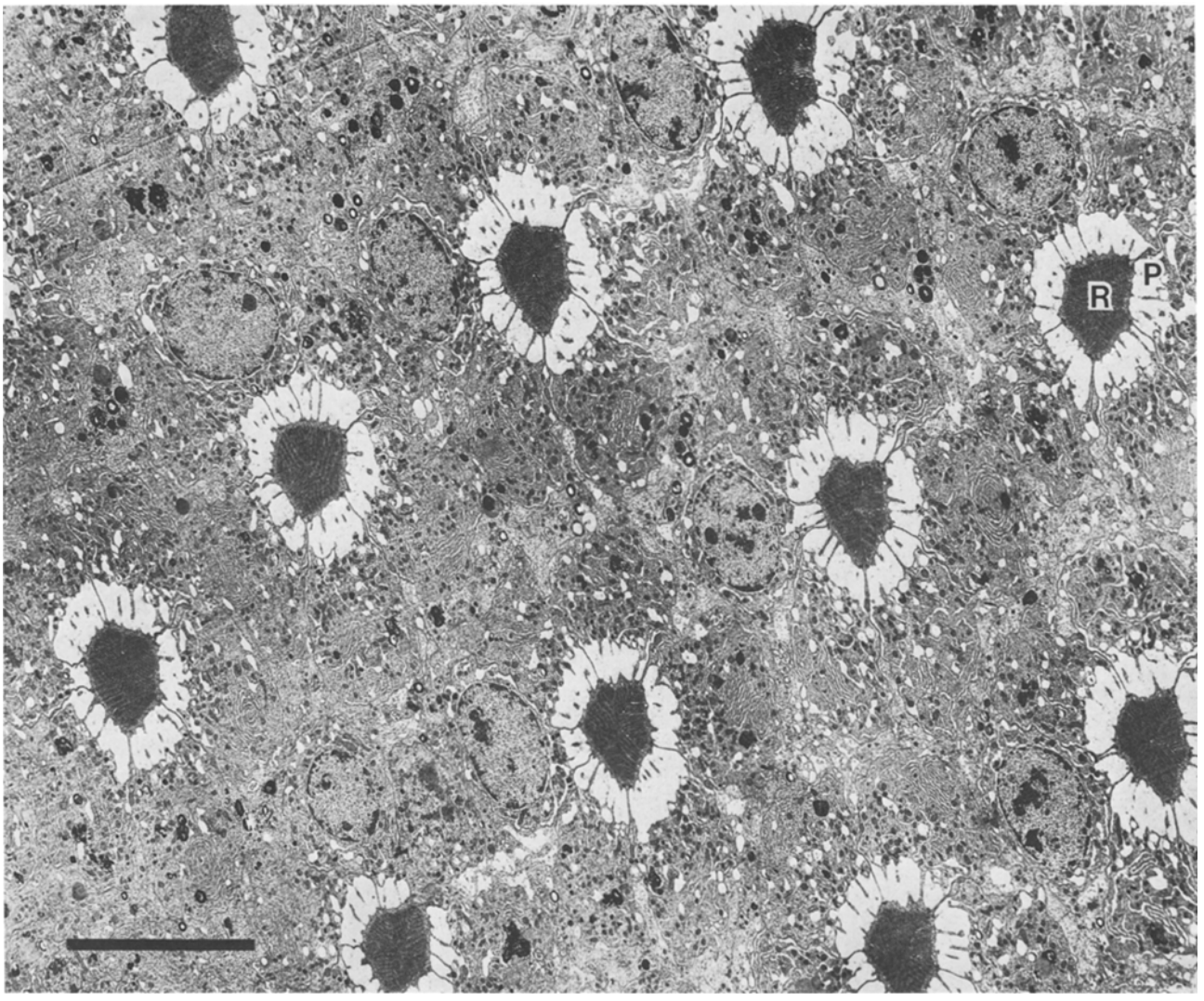

Fig. 1. Electron micrograph of a transverse section of part of a retina of *Valanga*, fixed 5 h after dusk. Prior to fixation, recordings had been made from an unidentified cell from or near the region shown (cell 6 in Table 1). *R* rhabdom; *P* palisade of vacuoles of endoplasmic reticulum. ×2,600; *scale bar:* 10 μm

sponse to dim light (Fig. 3) (Scholes 1964). Bumps were very rare in complete darkness, showing that there were practically no spontaneous bumps (Fig. 3) (Lillywhite 1977). Small bumps of the kind described by Lillywhite (1978) were sometimes observed, but were ignored during analysis. Lillywhite considered them to arise from neighbouring photoreceptors that were naturally electrically coupled to the impaled cell. Although the basis of this conclusion has been questioned (Williams 1982a), it is assumed here that the small bumps at least do not originate from the impaled cell.

Spectral Sensitivity

The first experiments tested if the night and day rhabdoms had different spectral sensitivities, i.e. if the locust eye underwent a 'Purkinje shift'. Any change in spectral sensitivity might be important with respect to the waveguide properties of the rhabdom and would need to be accounted for when comparing the angular and absolute sensitivities of the day and night states.

Measurements were taken from 5 cells. In all cases there was no detectable shift in spectral sensitivity between day and night (e.g. Fig. 4). In agreement with Lillywhite (1978), cells from *Locusta* were maximally sensitive to wavelengths of 474 or 494 nm. The approximate peak wavelength of two cells from *Valanga* was found to be 494 or 513 nm.

Angular Sensitivity

To determine how much changes in the field stop and rhabdom size influence the angular sensitivity function of photoreceptors, the fields of view ($\Delta\rho$)

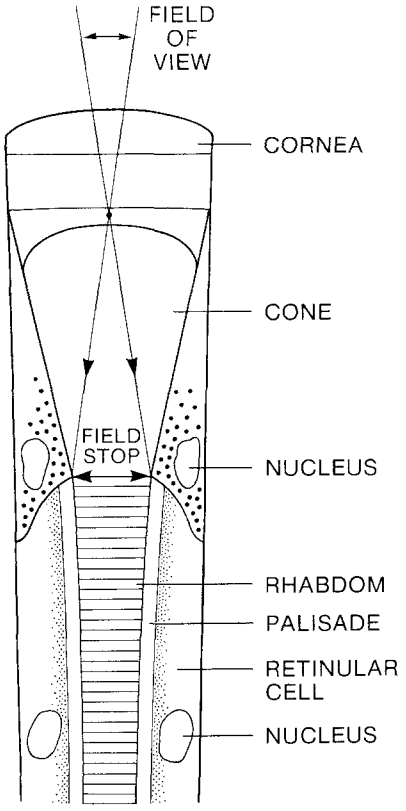

Fig. 2. Illustration of the optics of a locust ommatidium. The field of view is determined by the area of the exposed extremity of the rhabdom and the presence or absence of a palisade of vacuoles of endoplasmic reticulum around the rhabdom. At night, an outward movement of the primary pigment cells increases the area of the field stop, and combined with growth of the rhabdom, increases the field of view. In addition, formation of the palisade increases the light-guiding properties of the rhabdom. The distal end of the rhabdom is penetrated by the proximal end of the cone (not shown) and tapers. Measurements of rhabdom cross-sectional area were made from sections of the nuclear region where rhabdom diameter is nearly uniform along a considerable length. Not drawn to scale

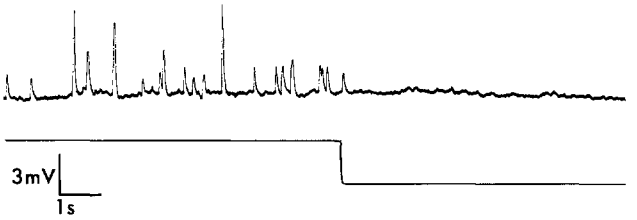

Fig. 3. Intracellular recordings of photoreceptor membrane potential at night in response to dim light. Bumps are of variable amplitude, but each is the response to the absorption of a single photon. Drop in lower trace indicates when stimulus was turned off. Note the absence of bumps in darkness

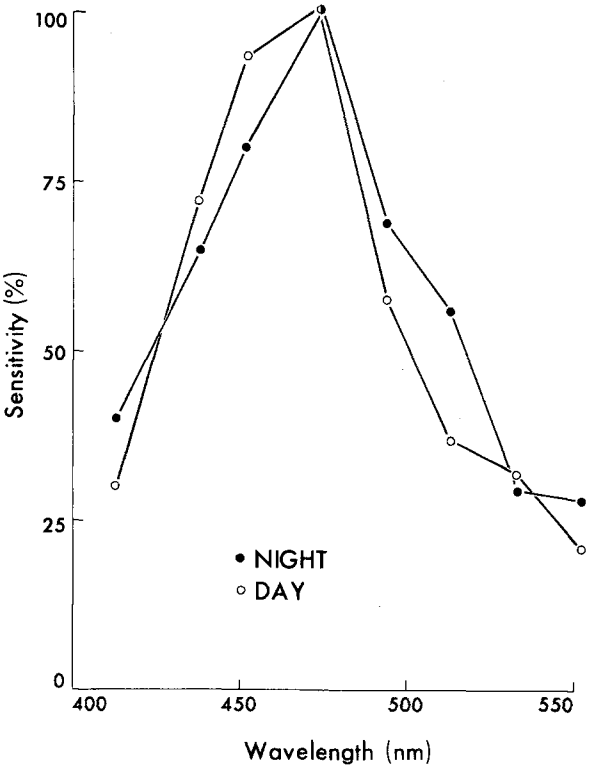

Fig. 4. Spectral sensitivity of a photoreceptor cell of *Locusta* 15 min after 'dusk' (*open circles*) and 4 h after 'dusk' (*closed circles*); i.e., with 'day' and 'night' dark-adapted rhabdom states. Sensitivities to different wavelengths were measured from bump responses and normalised to a percentage of that for peak wavelength

of 3 *Locusta* cells and 7 *Valanga* cells were measured through periods of the daily cycle, known from previous studies (Williams 1982a) to be coincident with changes of ommatidial structure (Table 1). The size of $\Delta\rho$ was determined by the region where sensitivity to the point source was at least 50% of that on-axis. Responses were measured only along the vertical axis; fields of view were assumed to be circular as shown by Wilson (1975).

Measurements of light-adapted cells were made as a final verification that their optical axes had been located by the point source. The average value for $\Delta\rho$ was 1.9° (S.D. = 0.3°; $n=3$) for *Locusta* and 1.7° (S.D. = 0.1°; $n=7$) for *Valanga*.

Removal of the adapting lamp simulated a sudden nightfall (written as 'dusk'). It elicited a drop in a cell's potential of 7–15 mV. Ten to fifteen min after 'dusk', $\Delta\rho$ was determined by measuring sensitivity in terms of bumps. By this time, the palisade would have formed around the rhabdom, for it is fully effective after 6 min of darkness (Tunstall and Horridge 1967), but no changes in the size of the field stop or rhabdom should have begun. The average value for $\Delta\rho$ was 2.8° (S.D. = 0.4; $n=3$) for *Locusta*, and 2.7° (S.D. = 0.3; $n=7$) for *Valanga*.

The next measurement of $\Delta\rho$ was made from the same cell and after at least 4 h had elapsed

Table 1. Angular sensitivity measurements (in degrees) of photoreceptor cells according to time of day. Cells were tested with either 'white' light (from a tungsten source), or (cells 1, 5, 9) monochromatic light (413 nm)

Cell[a]	Pre-dusk	'Dusk' +		'Dawn' +					
	(LA)	15 min (DA)	4–8 h (DA)	10 min (LA)	60 min (LA)	120 min (LA)	140 min[b] (DA)	160 min[c] (LA)	9 h[d] (DA)
1	1.9	2.9	5.0	2.6		1.9	3.2		
2	1.7	2.4	4.5	2.5	2.1	2.0	2.9		
3	2.2	3.1	5.1	3.6		1.9	2.5		
4	1.6	2.6	5.4	2.7	1.9	2.0	3.0	1.8	
5	1.9	2.5	4.0			2.1	2.6		
6	1.8	2.5	4.9	Fixed for anatomy					
7	1.7	3.3	4.3	Fixed for anatomy					
8	1.7	2.4	4.7	No light					3.2
9	1.5	2.6	4.8						
10	1.6	2.7	2.6	2.0					

[a] Cells 1–3 from *Locusta*, cells 4–10 from *Valanga*
[b] Last 20 min in darkness
[c] Darkness for 20 min, 120–140 min after 'dawn'
[d] No exposure to light at time of 'dawn'

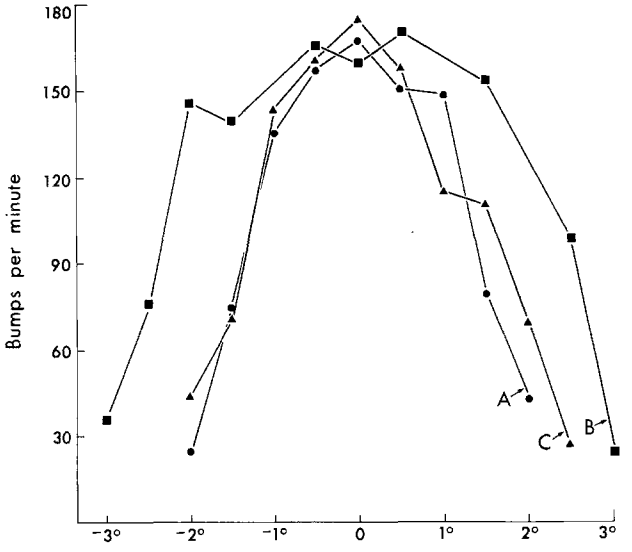

Fig. 5. Plots of the angular sensitivity function measured from a single photoreceptor (cell 1 in Table 1) just after 'dusk' (*A*), 5 h after 'dusk' (*B*), and after 30 min dark-adaptation that followed 2 h exposure to light at 'dawn' (*C*). Stimulus was a point source (subtending 0.1° at the eye) of monochromatic light (413 nm) at the one intensity for all measurements

since 'dusk'. By this time the larger night rhabdom was assumed to be completely assembled so that the eye was in its fully night-adapted state. This assumption was supported by anatomical monitoring, and measurements of sensitivity to an extended source (see below) which showed that sensitivity reached a plateau in less than 4 h (Fig. 6). The average $\Delta\rho$ at night is 4.9° (S.D. = 0.3°; $n=3$) for *Locusta*, and 4.7° (S.D. = 0.5°; $n=6$) for *Valanga*. The curve of the angular sensitivity function has a flat top (Fig. 5), which has not been described previously for locust photoreceptors (cf. Tunstall and Horridge 1967; Wilson 1975; Horridge et al. 1981); angular sensitivity functions have been commonly approximated to a Gaussian function (Götz 1964; Snyder 1977). An anomalous value for $\Delta\rho$ was measured from one cell (cell 10 in Table 1). Unfortunately, sensitivity to an extended source was not measured from this cell, nor was the eye fixed for anatomical examination. It seems that for some unknown reason the night rhabdom did not form. This value was not considered for calculation of the mean $\Delta\rho$.

At the usual time of 'dawn' the locust was exposed again to illumination of 150 lux. After 1 h, coinciding with a reduction in rhabdom size because of shedding (Williams 1982a), $\Delta\rho$ had returned to a value near that of the previous day. When returned to darkness for 20 min after a further 1 h of light, $\Delta\rho$ was comparable to that found just after the onset of 'dusk' (*Locusta*: mean = 2.9°, S.D. = 0.4°; $n=3$. *Valanga*: mean = 2.8°, S.D. = 0.3°; $n=2$). One cell (cell 8) was not exposed to light at the usual time of dawn. Nine hours later its $\Delta\rho$ was 3.2°. This value is less than $\Delta\rho$ at night, and consistent with anatomical data, showing that shedding of photoreceptor membrane and movement of the primary pigment cells is under some degree of endogenous control (Williams 1982a).

A comparison of $\Delta\rho$ for dark-adapted cells (a) before the assembly of new photoreceptor membrane, (b) after its assembly, and (c) after the shedding of photoreceptor membrane at dawn (e.g. Fig. 5) shows that the enlarged field stop and rhabdom at night effect an increase of $\Delta\rho$ from 2.8° (overall mean; $n=15$) to 4.9° in *Locusta*, and 2.7° ($n=9$) to 4.7° in *Valanga*, or an increase in solid angle, $(\Delta\rho)^2$, of 3.1-fold in both species of locust.

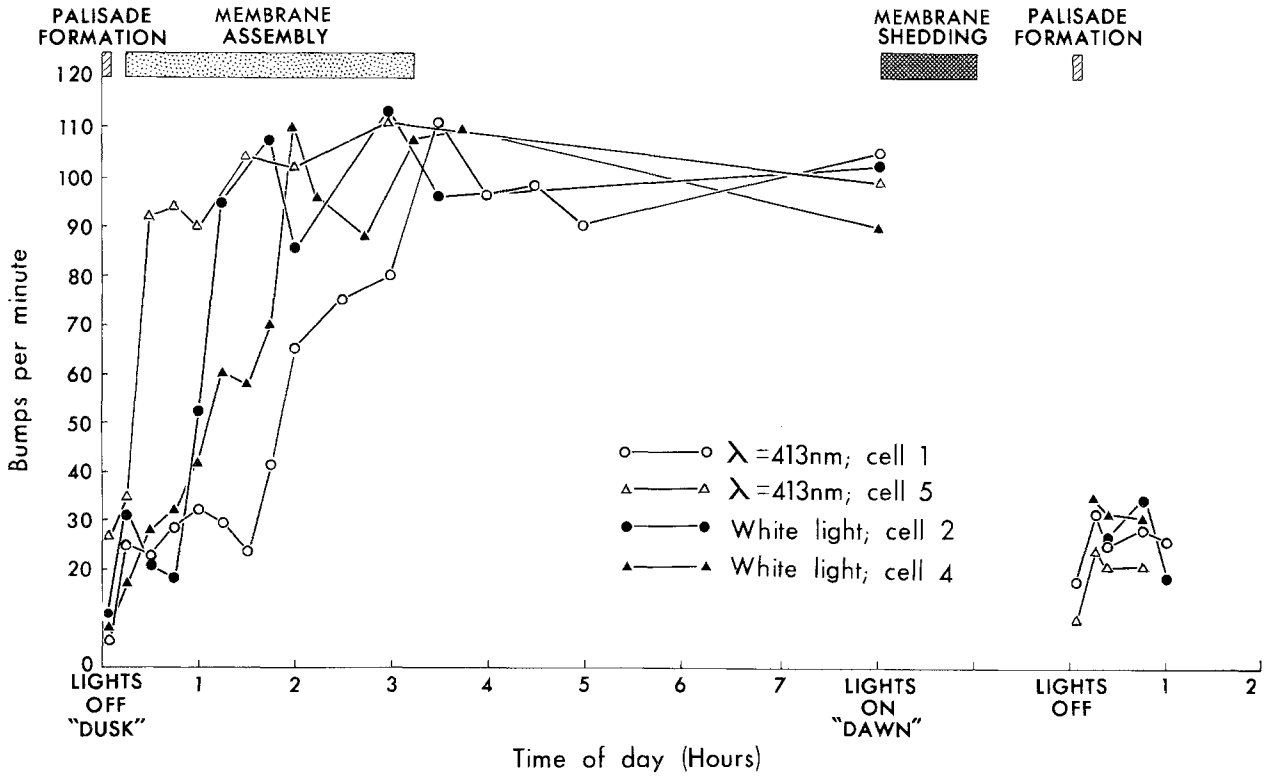

Fig. 6. Sensitivity of single photoreceptors to an extended source. All recordings, except for those obtained during the first 15 min of darkness, were made with one light intensity, which was chosen so that the maximal response rate would be about 100 bumps/min. Sensitivities have been normalised so that the mean sensitivity of the fully night-adapted state is exactly 100 bumps/min

Absolute Sensitivity

The sensitivity to a point source on the optical axis of a dark-adapted cell was independent of the different field stop and rhabdom sizes (e.g. Fig. 5). This constancy was found whether the stimulus was white light (tungsten source) or monochromatic light of wavelength 413 nm. The latter is absorbed at only 10–40% of the efficiency of the peak wavelength (Lillywhite 1978; present results, e.g. Fig. 3), so that its use provides a more sensitive test for any change in absorption efficiency of the rhabdom. We already know that a very high proportion of peak wavelength light incident on the rhabdom is absorbed (Lillywhite 1977). If a means to change absolute sensitivity between the day and night states existed, it would be more likely to be detected from a change in the absorption of an off-peak wavelength.

Sensitivity to an Extended Source

Sensitivity to an extended source, subtending 40° at the eye, was monitored throughout the night from 4 cells (Fig. 6), beginning as soon as bumps were distinctive after 'dusk'; the first measurement was usually completed by 3–5 min after the onset of darkness. For the first 1–3 h, an increase in sensitivity of about 1 log unit was detected. However, it was not feasible to determine a baseline sensitivity, for sensitivity might have begun to increase prior to the first measurement. The photomechanical changes that produce the palisade probably begin immediately after the onset of darkness. An optically equivalent effect, achieved by moving pigment granules within the photoreceptor cells and known as the longitudinal pupil mechanism, is *completed* within 10–60 s in many insects (Stavenga 1979). In 2 out of the 4 cells, sensitivity noticeably plateaued for a short period subsequent to the second measurement, made 15 min after 'dusk' (Fig. 5). The magnitude of the rise that followed this plateau averaged 3.9-fold, and was comparable to the drop in sensitivity found after 2 h light at 'dawn', followed by 20 min dark adaptation (mean = 3.8-fold; S.D. = 0.6; $n = 4$, data grouped from *Locusta* and *Valanga*) (Fig. 6).

Latency of Bumps

A fundamental parameter of phototransduction is the time taken for the production of a bump after photon absorption; i.e. bump latency. To determine if phototransduction is affected by rhabdom size or the age of the photoreceptor membrane, the bump latencies of day and night rhabdomeres

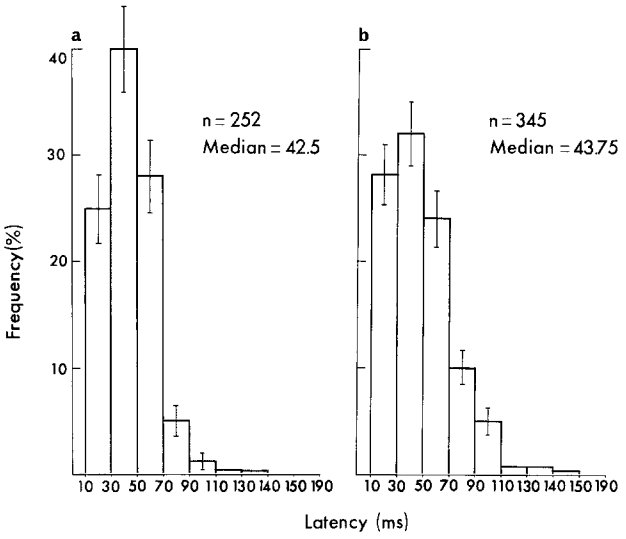

Fig. 7. Histogram of bump latencies of a single cell of *Locusta* recorded (**a**) 10–15 min after 'dusk' and (**b**) 5 h after 'dusk'. Error bars extend 1 S.D. Temperature 25° C, at both times

were compared. Short flashes (4-ms duration) of white light were delivered at 1–2 s intervals from an extended source. The intensity of the light was constant, and sufficient to elicit a bump from an average of about every 2 flashes. For bumps of at least 1.5 mV, the time interval between the onset of the flash and initial rise of the bump was noted to the nearest 20-ms 'bin', directly from the oscilloscope sweep that was triggered by the stimulus.

The latency was measured from 4 cells (1) in their night state, and (2) in their day state – either just after 'dusk' or dark-adapted after 2 h light following 'dawn'. In all cells, no difference was apparent in the bump latency between the day and night rhabdom states (e.g. Fig. 7); the distribution of latency was invariably similar to that found by Lillywhite (1977) and, in a more detailed analysis (Howard, in preparation).

Discussion

Spectral Sensitivity

The spectral sensitivity functions of cells were unchanged between day and night, and were similar to those found by Lillywhite (1978). The constancy of spectral sensitivity indicates that the new photoreceptor membrane assembled at dusk has the same spectral absorption as that which makes up the day rhabdoms. Moreover, it means that no adjustment of the angular and absolute sensitivities was required in order to make comparisons of these functions between the day and night states.

Angular Sensitivity

Horridge et al. (1981) report dark-adapted angular sensitivity measurements of day (1.25°–2.0°) and night (up to twice as large an angle) rhabdoms in the acute region of the compound eye of *Valanga*. However, these workers introduced their animals to darkness 5 h before the usual time of dusk. Moreover, sensitivities were measured by subjecting a cell to many long flashes of light delivered at brief intervals and referring the responses to an intensity/response function obtained at the same time. After exposing an area of the retina to a similar number of flashes at comparable intensities (but shorter exposure times) from an extended source, retinulae were found in the present study to have palisades around their rhabdoms, but rhabdom size (and therefore also field-stop size) was considerably reduced. The comparison between many rhabdoms affected by an extended source (cf. the present study) and one rhabdom affected by a point source (cf. Horridge et al.) is valid because light and darkness affect each ommatidium independently of adjacent ommatidia (Williams 1982b).

In an attempt to maintain cells in their natural light condition, the night-adapted eyes in the present experiments were exposed only to dim light which elicited trains of discrete bumps. Electron microscopy showed that this exposure was not sufficient to affect the structure of the night rhabdom (Fig. 1). The values of $\Delta\rho$ obtained from the night-state eye of *Valanga* in the present experiments (mean = 4.7°) are larger than the values of Horridge et al. partly because they are from a less acute region of the eye. Part of the reason also appears to be that they were determined from a deeper state of dark adaptation.

Still wider acceptance angles were found in *Locusta* by Tunstall and Horridge (1967). They measured 6.6° for dark-adapted cells and 3.4° for light-adapted cells. However, such wide angles were probably a result of optical damage inflicted during preparation of the eye. Wilson (1975) took great care to preserve the optics and measured 2.5° and 1.4° for dark- and light-adapted cells respectively. In this study, before recordings were made, eyes were dark-adapted for 30 min, then light-adapted, dark-adapted for a further 20 min, and finally light-adapted again by flashes during alignment of the stimulus with the cell. Measurements obtained under these conditions should probably be compared to those from the dark-adapted day-state eyes (just after 'dusk' or dark-adapted after light at 'dawn') of the present study. Wilson's values for $\Delta\rho$ are then slightly less than those presented here (Table 1), but not inconsistent with them. This difference, although possibly not significant, could result from several factors. Wilson does not specify the region of the eye he examined; he may have recorded from a more acute region.

Moreover, his dark-adapted measurements were obtained from responses to 70-ms flashes of light, 8–10 s apart, thus possibly inducing some light-adaptive changes that were avoided in the present experiments. Lastly, the background illumination used by Wilson to light adapt cells was considerably stronger than that used in the present study, so that a greater degree of light adaptation may be responsible for his narrower light-adapted value for $\Delta\rho$. Like Wilson's results, those of the present study are considered to be unaffected by optical damage.

The advantage of increasing $\Delta\rho$ when ambient luminance decreases has been reviewed recently (Snyder 1979). Briefly, it provides a means of increasing the number of captured photons at a time when a scarcity of photons lowers the signal-to-noise ratio of the photoreceptors (Laughlin 1975). However, the increase of $\Delta\rho$ 'trades-off' with a decrease in spatial acuity. From this point of view it is instructive to compare $\Delta\rho$ with the interommatidial angle ($\Delta\varphi$).

For two points to be separately resolved, they can be no closer together than $2\,\Delta\varphi$, so that one ommatidium corresponds to each point and a central ommatidium detects the space between. In this limiting situation $\Delta\rho$ must be sufficiently less than $2\,\Delta\varphi$ for the central ommatidium to receive significantly less light than that reaching the other ommatidia; otherwise to two points blur into one (cf. Fig. 23 in Laughlin 1980). In some animals $\Delta\rho$ is very much less than $2\,\Delta\varphi$; for example, $\Delta\rho$ is approximately equal to $\Delta\varphi$ over the light-adapted fly and mantis eyes (Kirschfeld 1976; Rossel 1979). Such undersampling is argued to increase contrast resolution, or permit a high spatial acuity with fewer ommatidia by scanning (cf. discussions by Laughlin 1980; Kirschfeld, in preparation).

The locust eye has an oval shape. Using the pseudopupil of the eye, Burtt and Catton (1954) calculated $\Delta\varphi$ along the vertical axis to be 1.1°, and 2.4° on the horizontal axis in the central region of the eye of *Locusta*. Measured values of $\Delta\rho$ are compared to these values of $\Delta\varphi$ in Fig. 8. During the day $\Delta\rho$ is lowered so that $\Delta\rho/\Delta\varphi$ equals 1.7 and 0.8 along the vertical and horizontal axes respectively, thus providing increased contrast resolution (Fig. 8a). At night Δp increases (Fig. 8b, c), so that $\Delta\rho/\Delta\varphi$ becomes greater than 2 and it becomes impossible to resolve two points spaced at $2\,\Delta\varphi$ (Fig. 8c).

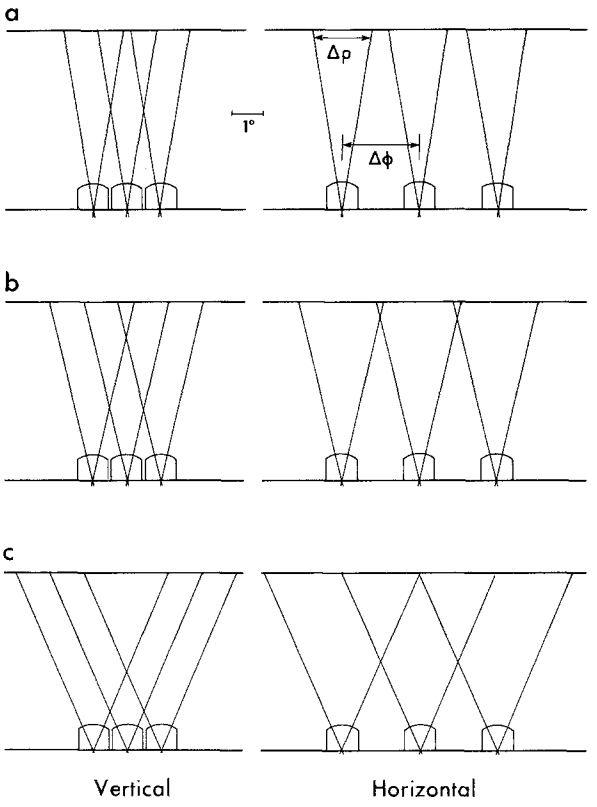

Fig. 8a–c. Schematic representation of the spatial acuity of the eye of *Locusta* in its different adaptive states. Angular measure has been transformed to linear measure, however the diagram is designed such that the measured fields of view ($\Delta\rho$) are shown at a magnification of 10×. Interommatidial angles ($\Delta\varphi$) in the vertical and horizontal axes of the eye are taken from Burtt and Catton (1965). Values for $\Delta\rho$ are means found in the present study. **a** Light-adapted during the day ($\Delta\rho/\Delta\varphi = 0.8, 1.7$); **b** 15 min after 'dusk' ($\Delta\rho/\Delta\varphi = 1.2, 2.6$); **c** 4–8 h after 'dusk' ($\Delta\rho/\Delta\varphi = 2.1, 4.5$)

Absolute Sensitivity

The constant sensitivity to a point source on-axis between dark-adapted day and night states, indicates, firstly, that most of the image of a point source that is produced by the lenslet falls within the area of the day-size field stop (Horridge et al. 1981). When the field stop enlarges at night no further significant amount of light from a point source is captured by the rhabdom. The flat top of the angular sensitivity function at night (Fig. 5) is consistent with this explanation. When the point source is moved slightly off-axis, the full Airy disc is still captured by the night rhabdom. It thus follows that rhabdom acceptance is essentially governed by geometrical optics, so that funnelling of light by the cone must be negligible.

Secondly, the quantum capture efficiency of the night and day rhabdoms must be the same. This conclusion is consistent with freeze-fracture evidence that shows that the microvillar intramembrane particle density, which is believed to represent rhodopsin concentration, is constant between rhabdoms in the two states (Williams 1982a). Con-

sequently, Lillywhite's (1977) finding that one absorbed photon produces one bump in locust photoreceptors (that were dark-adapted for 'at least 2 h', and therefore probably possessed a newly assembled 'night' rhabdom: cf. discussion by Williams 1982a) can be extended to include rhabdoms of both the day and night states. The bump characteristics of the *Limulus* lateral eye appear to be quite different: the frequency of light-induced bumps in response to a given light intensity increases at night, while the spontaneous bump rate, which is quite significant during the day, diminishes (Kaplan and Barlow 1980).

Sensitivity to an Extended Source

The effective gain in the number of photons captured by the larger night rhabdom and field stop is shown by the photoreceptors' increase in sensitivity to a uniform extended source subsequent to the first 15 min of darkness after 'dusk'; that is, after the palisade is fully formed (Tunstall and Horridge 1967). Because the distal end of the locust rhabdom remains at a set distance from the lenslet (Williams 1982a), and the absorption efficiency of the rhabdom is constant (above), this gain is directly attributable to, and should be of the same magnitude as the increase in cross-sectional area of the field stop. The extent of changes in cross-sectional area of the field stop is similar in *Locusta* and *Valanga* (Williams 1982a), so that data from the two locusts have been grouped.

The cross-sectional area of the field stop was found (from measurements of rhabdomal cross-sectional area) to increase by 3- to 4-fold between day and night. This gain is consonant with the measured increase in sensitivity to an extended source: the night rhabdom was 3.8 times (overall mean; S.D. = 0.5; $n = 6$), or 0.6 log units, more sensitive than the dark-adapted day rhabdom. Previously, a 4.7-fold difference in the cross-sectional areas of the rhabdom and field stop was found between day and night (Williams 1982a), but in that study illumination during the day was 1 log unit higher than in the present study, and the day-state rhabdom and field stop were slightly smaller: at the level of the distal nuclear region, the cross-sectional area of the rhabdom was 3.6 μm^2, compared to 5 μm^2 in the present study. It seems that as in the mosquito (White and Lord 1975), rhabdom size in the locust may be modulated by intermediate light intensities.

During the assembly of new photoreceptor membrane, rhabdomeres appear disordered in electron micrographs (Williams 1982a; Stowe 1980b for a similar event in a crab). Although it is not known if a whole rhabdomere is in disarray at a given time, it appears that segments up to 100 μm long are disordered for up to 20–30 min. It was therefore suggested that the disarray might affect photoreceptor sensitivity. However, no evidence of such an effect was apparent from regular sampling of sensitivity to an extended source of white light or monochromatic light of an off-peak wavelength during the assembly period (cf. Fig. 6). No significant drop in sensitivity was apparent at any time between 'dusk' and 'dawn', and the only disturbance (found in 2 out of 4 cells) in an otherwise steady rise to maximal sensitivity from 'dusk' can be assumed to have occurred after the completion of the palisade and before the rhabdom and field stop had begun to enlarge. As for the test of constancy of absorption efficiency, use of an off-peak wavelength (413 nm) provided a more sensitive test than using white light. Because of the exponential nature of light absorption down a receptive segment, a substantial proportion of an efficiently-absorbed wavelength will be absorbed by a small proportion of the rhabdom, usually the distal part. Absorption of an off-peak wavelength is distributed more evenly down the rhabdom, so that abnormal functioning of a segment of the rhabdom should be more readily apparent. This negative result poses the question of whether or not the disarray is an artefact of electron microscopical preparation. Alternatively, and more likely, the disarray may not significantly affect the absorption efficiency of the rhabdomere. After a damaging exposure to incandescent lighting, the ERG sensitivity of rat eyes returns to normal well before the outer segment discs recover their regularity (Kuwabara 1970). If a parameter of sensitivity is affected, then it may be polarisation sensitivity, which is, in any case, quite low in locusts (Shaw 1969; Lillywhite 1978; own observations).

Latency of Bumps

Comparison of bump latencies between day and night showed that this parameter of phototransduction is not affected by the dramatic changes in rhabdomeric structure at dusk and dawn. This finding has some relevance to current ideas about phototransduction. Phototransduction is considered to be mediated by the local release of an unknown transmitter substance from the site of photon absorption. This diffusable transmitter interacts with membrane receptors that control sodium conductance channels. If the conductance

channels are restricted to the bases of the microvilli, as suggested by Hamdorf and Kirschfeld (1980), then the same bump latency found for long (night) and short (day) microvilli indicates that any time taken for the transmitter to diffuse down the microvillus to the channels is not a major part of the phototransductive latency. Alternatively, of course, if the diffusion time is significant, the present result indicates that conductance channels must be located along the length of the microvilli.

Conclusion. Changes in the size of the field stop and rhabdom, which occur during the daily shedding and assembly of photoreceptor membrane, alter the angular sensitivity of the ommatidium, and thus, its photon capture from an extended field. No changes were detected in the performance of the photoreceptor membrane per se, indicating that the functional difference between the newly assembled night rhabdom and the older day rhabdom is achieved entirely by a difference in the amount, and not the nature, of the membrane comprising each.

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