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A 24-HOUR CYCLE IN SINGLE LOCUST AND MANTIS PHOTORECEPTORS

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SUMMARY

1. When fixed during the night the rhabdom of the locust and mantis is much broader than when fixed during the day.

2. Dark-adapted ommatidia of the locust and mantis by day and night have a zone of vacuoles around the rhabdom tip, but when light-adapted this zone is replaced by cytoplasm rich in mitochondria.

3. Illumination of the rhabdom in the night state causes the microvilli to swell and the rhabdom to break down over the course of about 1 h.

4. A diurnal rhythm is apparent in the spontaneous breakdown of the rhabdom in the morning even though the eye has seen no light for 12 h.

5. Intensity/response curves (peak of the response in mV plotted against log intensity of stimulus) show an increase in sensitivity during the night even though the stimulus is a point source on axis.

6. On the other hand, counts of bumps (quantal responses to individual photons) show no change in photon capture efficiency at night when the stimulus is a point source.

7. Strong illumination of the eye in the night state causes a desensitization which continues for 1 h.

8. Measurements of the acceptance angle in the dark-adapted day and night states show that field size is an indicator of the diameter of the rhabdom tip, but actual fields are larger than those calculated from the anatomical dimensions.

INTRODUCTION

On the physiological side, a diurnal rhythm in the sensitivity of an insect compound eye has been known for many years, in particular in Lepidoptera and in Coleoptera. Day and night phases were distinguished in continuous records of the electroretinogram of the beetle *Dytiscus*. Over several days the eye was 1000-fold more sensitive at night than during the day when the animals were in continuous darkness except for the test stimulus (Jahn & Wulff, 1943). As measured by ERG, there is an increase in sensitivity at night in diurnal butterflies apart from the normal effect of dark adaptation (Swihart, 1963). In several nocturnal insects dark-adaptation proceeds faster and further at night than during the day (Mazokhin-Porshnyakov, 1963, 1969). As in the Coleoptera, the results suggested a change in physiological state in addition to the movement of screening pigment.

Intracellular recording from retinula cells of the scarabaeid beetle *Anoplognathus* showed a similar change, measured as the intensity required to give a criterion receptor potential. Also in *Anoplognathus*, miniature potentials (called bumps and attributed to single photon captures) were only recordable in the night state (Meyer-Rochow & Horridge, 1975). Working more recently with single cells, Rossel (1979) described the increased field width in the night state of the eye of the mantis *Tenodera*. From measurements of receptor potential peak, Rossel found similar sensitivity to a point source on the optical axis of the receptor at night and during the day, but reported records of bumps only at night, leaving open the question of response per bump. Since the work of Scholes (1963) we have realized that bumps are more easily recorded in the compound eyes of locusts during the night, but physiological mechanisms of sensitivity changes with time of day have not been sought in insects. In recent accounts the diurnal rhythm in sensitivity has been attributed to movement of screening pigment, as illustrated in the mantis by Stavenga (1979, p. 422), and screening pigment may indeed be the main factor in beetles with clear zone eyes, as inferred in *Anoplognathus* (Meyer-Rochow & Horridge, 1975; fig. 3*a*, *b*). In the locust, however, and even more so in the mantids, it will be shown that changes in field size must also be referred to changes in size of the rhabdom and physiological properties of the retinula cells.

A new outlook was introduced on the morphological side when it was shown that rhabdomeres in mosquito eyes wax and wane on a diurnal cycle (Sato, Kato & Toriumi, 1957). The changes in size, analysed by White (1967), with numerous later papers up to White, Gifford & Michaud (1980), are a result of imbalance between the daily synthesis and the breakdown of rhabdom membrane. The study of rhabdom turnover was introduced into our own laboratory by Blest (1978), who found an almost total daily destruction and renewal of the rhabdom in the eye of the spider *Dinopis*. A feature of *Dinopis* is the rapid but abnormally organized breakdown of the rhabdom when it is illuminated in the night state (Blest, 1980).

In *Limulus* there is also a strong diurnal rhythm of sensitivity and a daily renewal of part of the rhabdom. The onset of light each day triggers a turnover which is for a short period only, and a second exposure on the same day has no further effect. At night the rhabdom microvilli are swollen and irregular in *Limulus*. There are fewer random eccentric cell spikes although sensitivity is greater, as measured by the intensity to give a criterion response (Barlow & Chamberlain, 1980). Nightly growth of the rhabdom, with dramatic breakdown after dawn, has also been described in a crab (Nässel & Waterman, 1979) and in Blest's words (1980) 'doubtless many more will follow'. By no means all insects, however, show marked daily changes in rhabdom size or rapid breakdown on illumination at night. Diptera (for example, the blowfly *Lucilia* and the tipulid *Ptilogyna*) are not spectacular examples (Williams, 1980, and summary in Blest, 1980), although these and many other arthropods show changes in rhabdom fine structure when illuminated in the dark-adapted state, e.g. the bee (Gribakin, 1969) and spider crab (Eguchi & Waterman, 1967). Therefore we looked for insects with large changes in rhabdom size and a catastrophic effect of bright light during the night. It was unexpected that the first examples would be the common laboratory locusts and mantids from which intracellular responses to light are readily obtained from retinula cells.

MATERIALS AND METHODS

The locusts were taken from a culture of *Valanga irregularis* kept, to encourage breeding, on a summer cycle of 16 h light and 8 h dark. The light was on from 06.00 to 22.00 h in a greenhouse lined with foam plastic insulation so that natural daylight is added to that of the fluorescent lamps but direct sunlight is excluded. Measured through a 544 nm interference filter, the daytime intensity was 1×10^{15} photons cm^{-2} s^{-1} on a typical day. All measurements of photon flux given below for the experimental conditions were made through the same filter, which had a peak transmission near that of the spectral sensitivity peak for locust and mantis eyes.

Morphology

To make the anatomical comparison between night and day states as valid as possible, the left eye of an animal was fixed for electron microscopy during the day then the right eye of the same animal at night. As a control, tests were made by fixing first one eye in the night then the other during the day. Facets to be fixed in the light-adapted state were exposed on axis to an ambient intensity of 4×10^{14} green (544 nm) photons cm^{-2} s^{-1} from fluorescent lamps. Fixation was by the method given in detail by Williams & Blest (1980). The eye is cut beneath the surface of the fixative and all subsequent times and temperatures were constant for each specimen. To avoid differences between eye regions, we used a particular group of ommatidia located by counting facets from the edge of the eye adjacent to the ocellus on left and right eyes. All sections were cut transversely through the distal region of the rhabdom, adjacent to the cone tip, as identified by the centre of the cone tip in the centre of the rhabdom, or by the close proximity and large size of the five cone-cell extensions. The gross structure of the eye in the day state has been recently described (Wilson, Garrard & McGinness, 1978).

Electrophysiology

Methods were as previously outlined from this laboratory (Lillywhite, 1977; Hardie, 1979; Rossel, 1979). The animal is held in a perspex slot around the neck and fixed firmly with wax/resin mixture in a way that allows it to breathe normally. Important points are to make a tiny hole for the entry of the electrode and check that the optics are not damaged, as shown by the form of the pseudopupil. Also, the position of the optical axis of the cell is checked at every measurement. Electrodes were filled with 3 M potassium acetate and had a resistance of about 100 M Ω that could be monitored at any time. Recording over long periods requires that the electrode current be adjusted to zero, for which purpose the Grass P16 preamplifier was most convenient.

To make the stimulus diameter small compared to the angular sensitivity curve a pinhole in a metal screen subtending 0·1 ° at the eye was placed over the end of the flexible light guide, which moved around the eye on a perimeter arm. Flashes were 500 ms duration, repeated at 3 s intervals during the recording of responses. The tests were made under the following regime. The locust was set up in the morning, left until after lunch and kept in the day state with an ambient light of $4{\cdot}0 \times 10^{14}$ photons cm^{-2} s^{-1} (measured through the interference filter peaking at 544 nm)

shining on the eye region of interest. This is more than sufficient to saturate the receptor potential of the dark-adapted eye. First the optical axis of a retinula cell is found by moving the stimulus relative to the eye, with repeated *weak* flashes until the maximum response is found. Then responses were measured with the flash intensity progressively increased in steps of 0·2 log unit. Tests during the day were made after a period of 15 min in darkness to make the eye temporarily dark-adapted (DA). This is not to be confused with the night state. The ambient light which maintained the day state was turned out at 5 p.m., and turned on again at the same intensity after the first measurements had been made on the following morning. The maximum available stimulus (—log intensity = 0 on the diagrams) was $1{\cdot}3 \times 10^{14}$ photons cm^{-2} s^{-1} at 544 nm, which was adequate to saturate dark-adapted ommatidia. The same intensity was used to illuminate the eye in the night state, causing a long-lasting desensitization. Measurements of the response are to the peak of the receptor potential, plotted as V. All V/log I curves and angular sensitivity curves were measured with green light at 544 nm that had passed through the interference filter used for the calibrations. Intensities were calibrated with an International Light Inc. SE 400 flux meter with minimum detectable intensity 6×10^8 photons cm^{-2} s^{-1}.

RESULTS

Morphology at the rhabdom tip

Day state

When eyes were fixed during the normal working day, the rhabdom tip in the selected eye region had a diameter of 1·7–2·1 μm. In both species kept in the light (4×10^{14} photons cm^{-2} s^{-1} at 544 nm) and fixed in the light, the rhabdom is surrounded by cytoplasm rich in mitochondria (Fig. 1A). These ommatidia are defined as being in the light-adapted (LA) day state. Alternatively, if placed in the dark for 10–15 min before fixing, and fixed in dim red light (beyond 650 nm at the limit of human dark-adapted vision), a zone of clear endoplasmic vacuoles forms a sleeve up to 2 μm wide around the rhabdom (Fig. 2B). These ommatidia are defined as being in the dark-adapted (DA) day state.

The difference between dark and light-adapted eyes in the day state agrees with that described by Horridge & Barnard (1965), although at that time a different species (*Locusta migratoria*) was used, the animals were reared in continuous light from tungsten lamps, the region of the eye and the time of day were not controlled, and the methods of fixation were primitive. Other more recent descriptions will be found that differ from ours: for example, Shaw (1978) in *Valanga*, and Wilson *et al* (1978) in *Locusta*, illustrate ommatidia in which there is a sleeve of vacuoles around the rhabdom in the light-adapted day eye. We interpret this as due to too low an intensity; for example, when the ommatidia are not directed towards the adapting light, and to the sections being too deep in the retina. We arrange the identified ommatidia to point at the lamp and examine the rhabdom tip just below the end of the cone. The experimental intensity given above is about a tenth of sunlight.

Night state

When eyes were fixed during the night from 24.00 to 05.30 h the rhabdom had a diameter of 3–4 μm at its tip (Figs. 1B and 2C). These eyes were also fixed in very

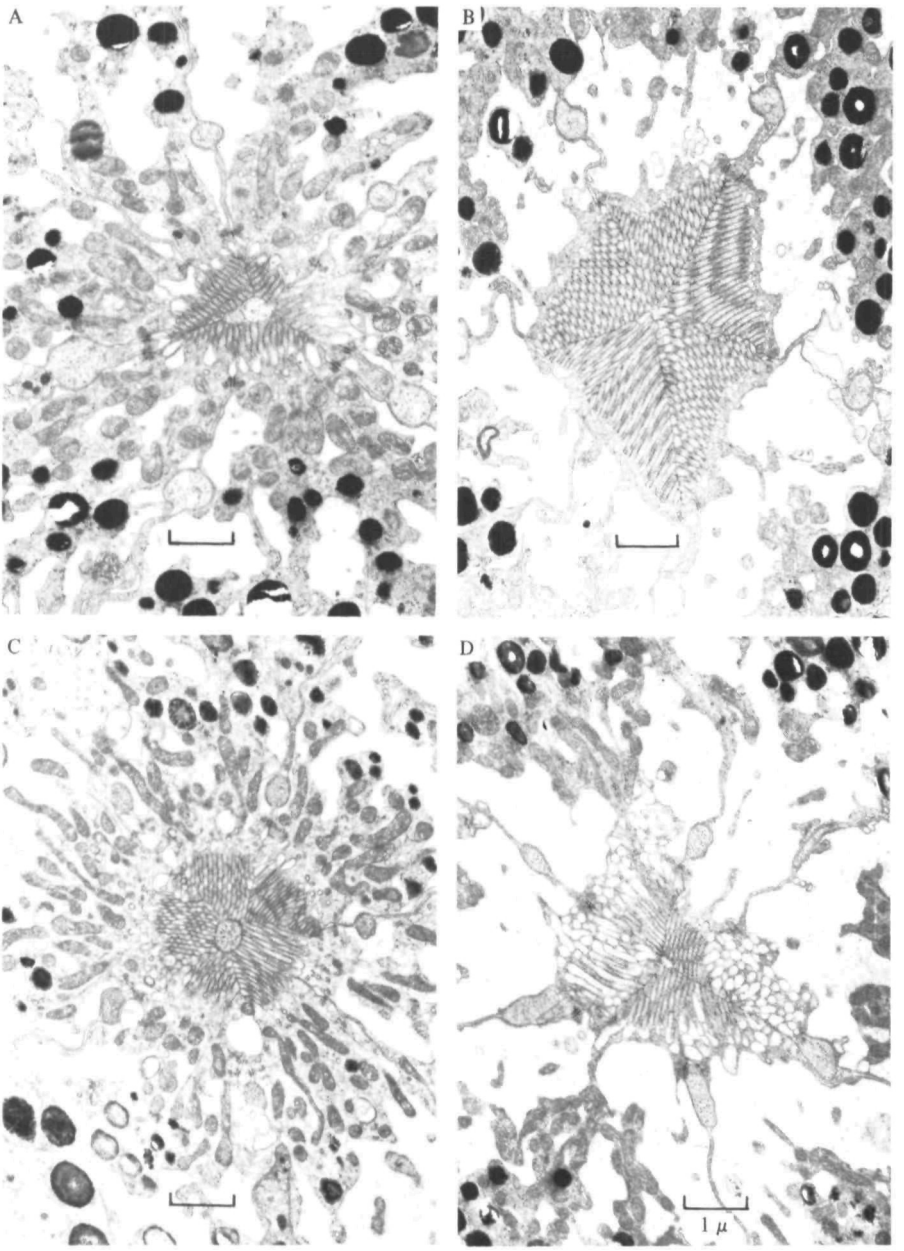

Fig. 1. Transverse sections through the tip of the ommatidium of the locust *Valanga* in the centre of the fovea, (all at the same scale). The level is identified by the tip of the cone in the centre of the rhabdom. Corresponding ommatidia were found by counting facets in the block. Scale 1 μm. (A) Light-adapted day state; cytoplasm rich in mitochondria round a small rhabdom. (B) Dark-adapted night state; vacuoles surround a large rhabdom. (C) An eye that was in the night state but illuminated for 35 min at an intensity of 4×10^{14} photons cm^{-2} s^{-1} (at 544 nm) then fixed at 04·25. The replacement of the vacuoles by cytoplasm and mitochondria is a sign of light adaptation but the rhabdom shows little sign of swelling by this time and is reduced to a diameter intermediate between night and day states. (D) From an animal on a normal cycle (06.00–22.00 h light, 22.00–06.00 h dark), but in this case kept in the dark until 10.00 h and then fixed in the dark. The rhabdom is in the process of spontaneously breaking down from the night stage.

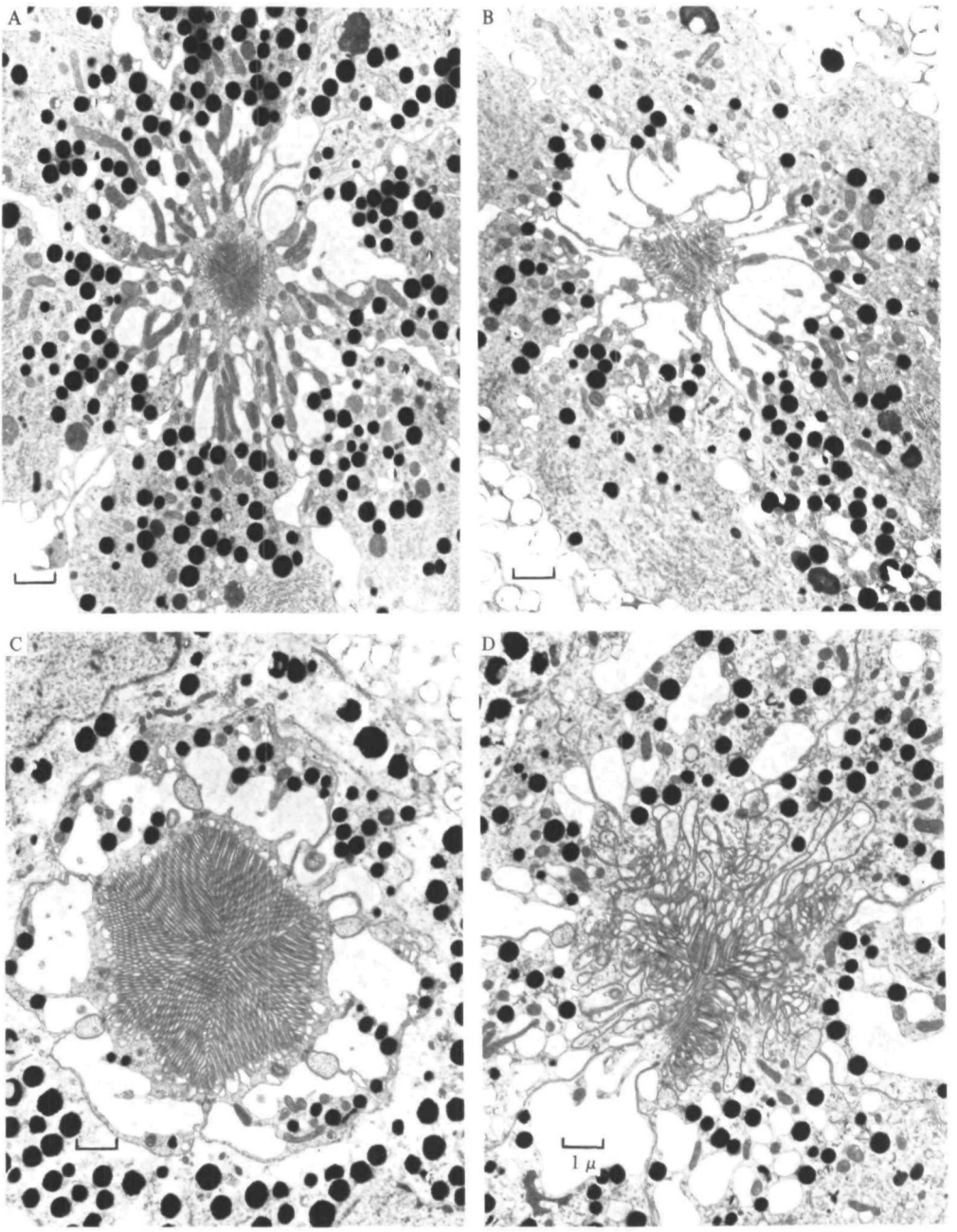

Fig. 2. The effect of light and time of day on the rhabdom tip in the mantis *Orthodera*, all at the same scale. (A) Day state, light-adapted to 4×10^{14} photons cm^{-2} s^{-1} (green photons measured at 544 nm) at 11.15 h. The rhabdom is narrow and cytoplasm around it contains elongated mitochondria. (B) Day state, dark-adapted for 15 min at 11.45 h. There are now clear vacuoles around the rhabdom. (C) Night state. An eye fixed in the dark at 00.30 h. The animal was on a normal daily light cycle and went into the dark at 20.00 h. The rhabdom is large and surrounded by vacuoles. (D) Breakdown of the night state by light. An eye on normal daily cycle as in C but illuminated at 4×10^{14} green photons cm^{-2} s^{-1} for 30 min before being fixed at 00.45 h. The rhabdom microvilli swell, become fewer in number, disorganized and move into the cytoplasm as vacuoles. One rhabdomere, however, isunaffected.

dim red light beyond 650 nm at the limit of human dark-adapted vision. The rhabdom now surrounded by a clear sleeve of vacuoles that can be 3 μm wide. The pigment grains in the retinula cell cytoplasm do not migrate to distant parts of the cell. The rhabdom is remarkably large and regular. This condition, which we call the dark-adapted night state, has not been previously described.

The action of light on the dark-adapted night state is to initiate a complex process that will be described in detail elsewhere, and will be correlated with simultaneous physiological changes in the retinula cells. In brief, light causes the microvilli to become swollen and the palisade rapidly disappears (Fig. 2D). The rhabdom becomes disorganized and eventually shrinks in diameter. Then it becomes ordered once more, and surrounded by cytoplasm rich in mitochondria (Fig. 1 C), as in the light-adapted day state. The diameter, however, is not as small as in the light-adapted day state, showing that the time of day exerts some effect. How these events relate to the normal process at dawn remains to be seen. In view of the many electrophysiological studies carried out by day in dark rooms, it will be of interest to examine day-state eyes that have been put into the dark at various times, and also night-state eyes that have been illuminated then returned to darkness, to see whether they regenerate the wide rhabdom of the night-state eye.

Signs of the diurnal clock

For *Valanga* we have so far three pieces of evidence for an internal clock acting on the rhabdom. First, the fully developed night eye (Fig. 1 B) only appears at night, in our animals after 22.00 h. Secondly, when the light is switched on during the night, the night-state eye breaks down then reconstitutes at a diameter that is intermediate between the day and night states (Fig. 1 C). Thirdly, if the animals on normal cycle are kept in the dark overnight, continuing in the dark until 09.00 or 10.00 h, the rhabdom eventually breaks down even though it has seen no light (Fig. 1 D). If left until 09.30–10.00 h in the dark and then fixed in the dark the rhabdom is reduced spontaneously to 2·5–2·7 μm in *Valanga*. When left until 10.45 h in the dark then light-adapted for 15 min the diameter was 1·9 μm; when left until 11.30 h in the dark and light-adapted for 25 min the diameter was down to 1·5 μm. The return to the day state is therefore not a sudden shrinkage dependent only on time of day or light.

In the mantids the diurnal clock is obvious from a superficial inspection of the eye colour. At night the eyes turn black or in some species violet. This change does not occur if the lights are left on. In the morning the eyes again become pale green irrespective of whether the animals remain in darkness. The spontaneous change to the day state in the dark can be followed electrophysiologically.

Electrophysiology of single cells

The sampling problem

A way to study long-term changes in nervous systems is to sample single units in batches at different times in a search for differences that are necessarily between the means of samples. We have important indications, however, from the neurobiology of other parts of the arthropod nervous system, that every neurone is morphologically

and physiologically unique, and that the point of interest is lost by taking averages between cells. Even in an eye, it is possible that one type of cell may function during the day and another at night, so that sampling of unidentified cells would confuse the issue.

In the eyes of the locust and the mantid every ommatidium has eight separately identifiable retinula cells (Wilson *et al.* 1978). Also, there is a gradient across the eye for each dimension of the ommatidium, so that there is a corresponding gradient of field size and sensitivity to either a point source or to a diffuse source. The long-term changes in field size and sensitivity are therefore more easily interpreted when they are observed in single cells as compared with samples made at different times. Then there is no question that changes occur in single cells, especially if they can be held for periods comparable with the 24 h cycle and then returned to the initial state.

Changes through the day

The usual procedure was to set up the experiment during the day. When the eye had been resting in the dark for about 2 h during the middle of the day, a retinula cell with healthy responses was found in the eye region which was also used for the morphology. In the locust this region is the fovea near to the lateral ocellus; in *Tenodera* it is 6 facets along the axis from the nipple defined by Rossel (1979, fig. 1). Cells showing bumps during the day are preferred because in our experience these cells also give clear bumps during the night. Many cells were lost because they could not be held for long periods.

Intensity/response curves

When the exact ommatidial axis had been found, the responses to 500 ms flashes of increasing intensity were measured at 3 s intervals. There is little possibility of making meaningful comparisons of the baseline or resting potential in a single cell at different times of day. Therefore the height of the peak of the potential was measured from the point of take-off on the baseline, ignoring the fact that at high intensities there can still be a residual hyperpolarization from the previous stimulus. The point is that the measurements were made with a constant experimental procedure to compare responses to a point source at different times of day, and also to calculate the angular sensitivity curves by interpolation from the heights of the responses to a constant flash at different angles. The eyes were left in absolute dark for 15 min before each set of measurements to allow the physiological state of the membranes and the position of the screening pigment to equilibrate so that the dark adapted day state can be compared to the night stage.

The $V/\log I$ curve measured near midnight is to the left of that measured during the previous afternoon (Fig. 3 for *Valanga*, Fig. 5 for *Tenodera*). Corresponding angular sensitivity curves for these cells are given in Figs. 4 and 6. One way to define sensitivity to a stimulus of fixed angular size subtended at the eye is by the position of the 50% response point on the intensity axis (the PAQ 50 of Laughlin 1976). On this measure the cell in Fig. 3 was four times more sensitive at 23.30 than at 14.30 h with the ambient light turned out at 17.00 h. A similar change is shown for *Tenodera* (Fig. 5). It is important to note that the point source subtended an angle (0·1 °) at the eye that is much smaller than the width of the angular sensitivity curve,

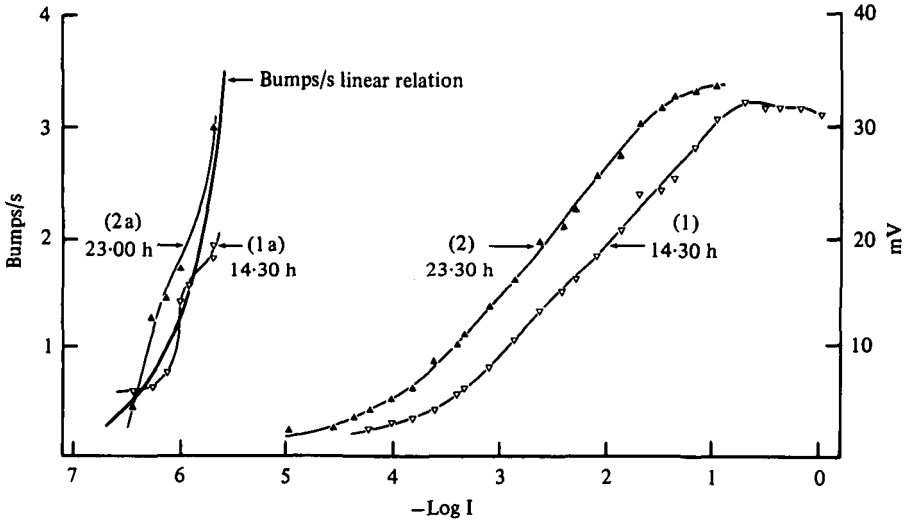

Fig. 3. Locust, *Valanga*. Peak heights of the receptor potential and counts of bumps for a single retinula cell in the day state (curve 1) at 14.30 h and then in the night state (curve 2) at 23.30 h. At the same times the bump rates (curves 1 (*a*) and 2 (*a*), shown at left) are similar, indicating that the optical system is catching the same light flux, although the $V/\log I$ curves are separated by half a log unit. This cell was on the normal cycle. During its expected day (06.00–22.00 h) it experienced a flux of 4×10^{14} green photons $cm^{-2}\ s^{-1}$, and was dark-adapted for 15 min before measurements were made in the day state. Log I = 0 on the intensity scale is $1{\cdot}3 \times 10^{14}$ photons $cm^{-2}s^{-1}$ at 544 nm on this and all plots of $V/\log I$ curves.

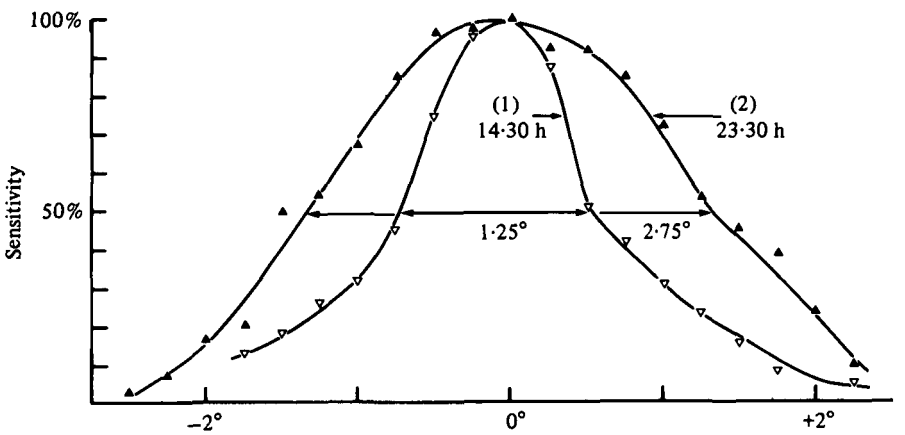

Fig. 4. Locust, *Valanga*. The angular sensitivity curves for the day and night states for the single cell corresponding to the two $V/\log I$ curves in Fig. 2. The cell in the day state was dark-adapted for 15 min before the measurements.

and the point source was centred on axis. The shift in $V/\log I$ curve disagrees with the finding of Rossel (1979) that the position of the $V/\log I$ curve is 'nearly but not exactly identical during the day and at night'. It should also be noted that if there is a standing depolarization during the day, it would be possible that the dark-

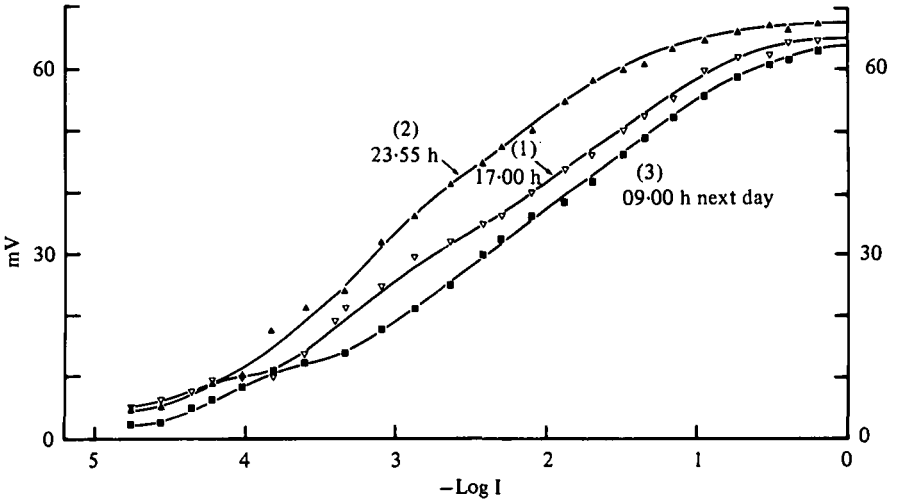

Fig. 5. Mantis, *Tenodera*. Peak heights of the receptor potential (V/log I curves) for a single retinula cell. (1) Dark-adapted for 15 min in the day state at 17.00 h. (2) Night state at 23.55 h (3) Next day at 09.00 h after being in the dark all night and continuing in the dark until the measurements were made. The normal schedule of this animal was 06.00–20.00 h in light, 20.00–06.00 h in the dark, with intensities as for *Valanga*.

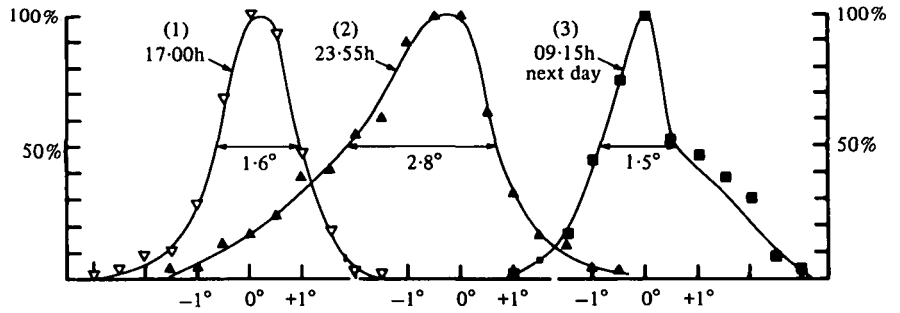

Fig. 6. Mantis, *Tenodera*. Angular sensitivity curves for the single cell shown in Fig. 5, measured at the same times as the V/log I curves. Schedule given with Fig. 5. The acceptance angle becomes narrow in the morning (as shown on the clock) although the animal has not been illuminated since the previous day.

adapted day curve could be lifted on the ordinate and this would reduce the apparent change in sensitivity but would not bring the day and night curves into coincidence.

Counts of bumps

Another measure of sensitivity can be made by counting the frequencies of bumps in response to calibrated dim continuous light over a small range of intensities. Bumps were counted over periods of 50–200 s, to give total numbers of bumps from 100 to 300.

In general we found no obvious difference between bump frequency in the middle of the day (dark-adapted for 10 min) and later in the same cell when it had changed

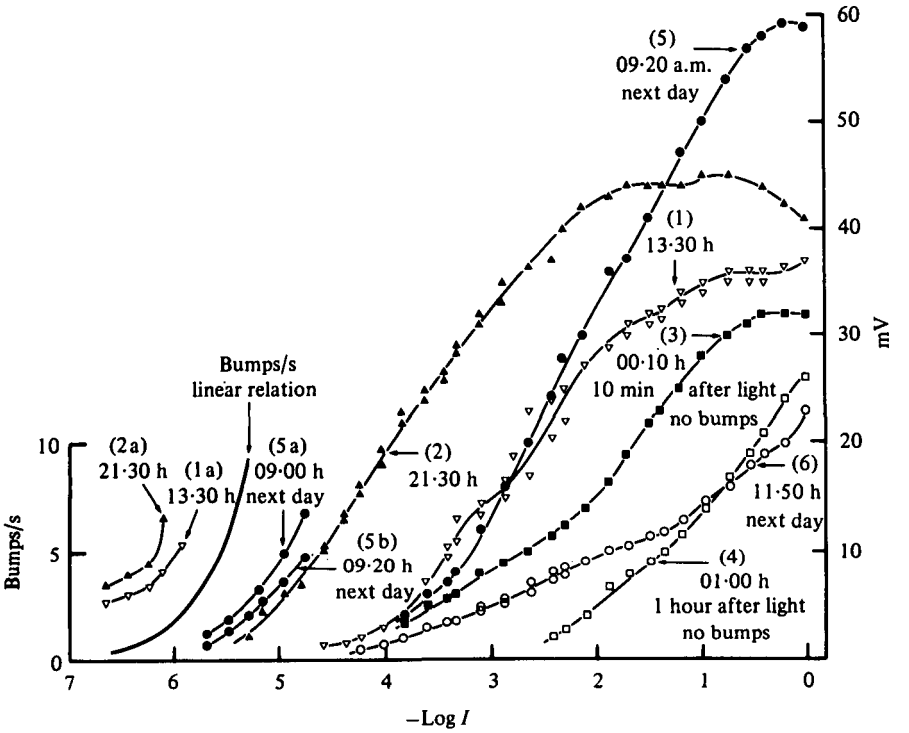

Fig. 7. Locust, *Valanga*. Peak heights of the receptor potential plotted against log of the flash intensity (V/log I curves) in the same cell at different times of day. The normal schedule of this animal was 06.00–22.00 h light, 22.00–06.00 h dark. Ambient day intensity was 4×10^{14} photons $cm^{-2} s^{-1}$ at 544 nm in the laboratory. One example of the linear relation between bump frequency and intensity is shown as a curve on the left. This theoretical curve shows the expected slope, but its position on the graph depends on the ratio of bumps to photons. (1) Day state at 13.30 h after 15 min dark adaptation, with corresponding bump frequencies plotted at (1*a*.) (2) Night state at 21.30 h. The cell is more sensitive as measured by the position of the V/log I curve but not by the corresponding bump frequencies, plotted at (2*a*). (3) Desensitization at 00.10 h immediately after 0.5 min illumination at $1{\cdot}3 \times 10^{14}$ photons $cm^{-2} s^{-1}$ at 544 nm. The V/log I curve moved to the right by two log units and bumps cannot be recorded. (4) Progressive desensitization, at 01.00 h after 1 h in the dark, the V/log I curve has moved further to the right. Bumps can still not be recorded. (5) At 09.20 h the next morning, still in the dark. The position of the bump frequency curve (5*a*) reveals a low ratio of bumps to photons. (6) At 11.50 h after 2 h in the ambient light then 15 min dark adaptation. The slope and position of the curve suggest that the cell has deteriorated, but the small acceptance angle (Fig. 8, curve 6) shows that the optics are normal.

to the night state (Figs. 3, 7). The significance of this finding is discussed below. Lights-out was at 17.00 h: ambient intensity until then was 4×10^{14} photons $cm^{-2} s^{-1}$ measured through the 544 nm filter.

Desensitization of the night eye by light

Illumination of the ommatidium in the night state on axis by a light (at an intensity of 4×10^{14} photons $cm^{-2} s^{-1}$ at 544 nm for several minutes) provokes a strong response that has already been described (Tsukahara & Horridge, 1977, fig. 3). That paper began with a remark (M. Wilson, unpublished) that 'the amplitude of the receptor

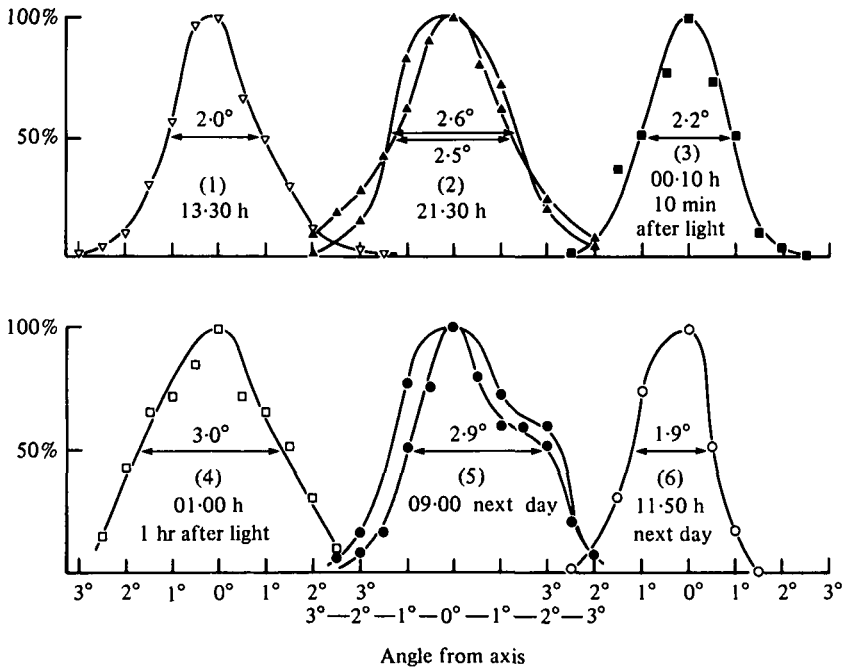

Fig. 8. Locust, *Valanga*. Angular sensitivity curves corresponding to the six $V/\log I$ curves in Fig. 6. (1) Dark-adapted day state. (2) The field broadens at night as expected from the increase in diameter of the rhabdom and of the cone tip. It narrows suddenly after bright light at midnight, but (4) is broad again after an hour in the dark, although the sensitivity has not returned. At 09.20 h the next day (curve 5) the field is still broad, in contrast to the intrinsic rhythm in the mantid (Fig. 6) but after 2 h in the light (6) it is brought back to the dark-adapted day state. In this single cell, the repeated curves in (2) and (5) show the consistency at any one time of day.

potential of a locust retinula cell sometimes does not fully recover for more than an hour after stimulation with an intense flash'. The flash is followed by an after-depolarization that may take many minutes to subside. This after-potential is accompanied by intense noise of several millivolts amplitude. The noise progressively becomes resolved into bumps which continue for at least 30 min further. The bumps, which cannot be directly attributed to photons, we called light-induced dark bumps or after-potential bumps. These bumps are indistinguishable in appearance from those caused directly by light of low intensity on the same cell at the same time. The desensitization following the flash could not be attributed to an increase in membrane conductance.

As illustrated by curve (3) in Fig. 7, the $V/\log I$ curve moved to the right by about 2 log units at 10 min after illumination. The significant point, however, is that 1 h after the illumination the $V/\log I$ curve has moved even further to the right (curve 4) and the record is still noisy (Fig. 9*e*). By 09.20 h the next day, with the animal still in the dark, the $V/\log I$ curve had moved back towards the left and was near that for the dark-adapted day state of the previous day (curves 5 and 1 in Fig. 7). We are unaware of the physical state of the rhabdom at 09.20 h; at that time it may have been breaking down spontaneously as in Fig. 1 D, but even if that was so, the wide

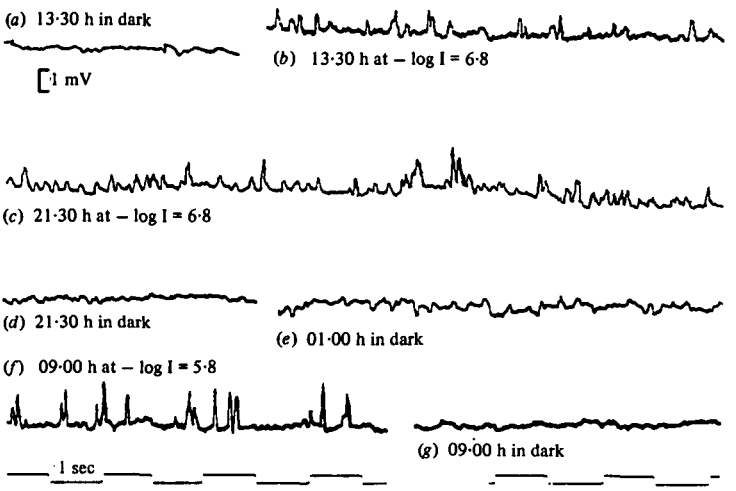

Fig. 9. Waveforms in the same cell at times corresponding to curves 1, 2, 4 and 5 in Figs. 7 and 8. Schedules are given with Fig. 7. (*a*) At 13.30 h in the dark, relatively low noise. (*b*) At 13.30 h at —log I = 6.8, small bumps, making this day-state, dark-adapted eye a suitable one for continuous recording. (*c*) At 21.30 h at —log I = 6·8, with larger bumps. (*d*) At 21.30 h in the dark showing the relatively low noise in the dark-adapted night state. (*e*) At 01.00 h in the dark, 1 h after the bright light. The baseline is still noisy. (*f*) At 09.00 h the next morning at —log I = 5·8, as in curve 5. In this example the bumps are now taller but thinner than in (*c*). (*g*) At 09.00 h in the dark, with baseline as in (*f*) without the bumps.

acceptance angle (Fig. 8, curve 5) indicates that the rhabdom still had the large diameter of the night state. The low counts of bumps (curves 5*a*, 5*b* in Fig. 7), show that for some reason the rhabdom is not catching photons efficiently at 09.20 h, or at least not converting a high proportion of them into bumps.

Changes in the baseline and appearance of bumps are illustrated in Fig. 9. At 13.30 h after 2 h in the dark (and still in the dark) the preparation was quiet (Fig. 9*a*). At this time illumination at a low level yielded bumps on a background of noise (Fig. 9*b*). Some of this noise has the appearance of bumps in coupled neighbouring cells (Lillywhite, 1978). Sensitivity measured by counting bumps at 13.30 h was almost as high as at 21.30 h, as shown by curves 1*a* and 2*a* in Fig. 7. In the night state at 21.30 h the baseline is quiet in the absence of light (Fig. 9*d*), but is violently disturbed for many minutes after a bright flash at $1{\cdot}26 \times 10^{14}$ photons cm^{-2} s^{-1} delivered several times over as part of measurements of the saturated response. Recording was impossible for many minutes; eventually the trace settled down but is still noisy 1 h after the light (Fig. 9*e*). The next morning at 09.00 h the bumps were very clear and of short duration on a relatively quiet background (Fig. 9*f*), but as shown in curve 5*a* of Fig. 7, and by the intensities in Fig. 9, ten times the intensity was required to yield the same frequency of bumps as at 13.30 or at 21.30 h.

When we consider these changes in relation to the morphology, several puzzling features emerge. First, we cannot explain why the frequency of bumps at midday is about the same as at midnight with the same light flux but with a wider rhabdom, except by supposing that light from a point source is wholly caught by the rhabdom

tip even in its narrow dark-adapted day state. Calculation from the anatomy (see below) shows that the Airy disc (which is the image of the point source) has about the same diameter as the day-state rhabdom. That would be a satisfactory situation because the calibration of photon captures made by Lillywhite (1977) is then independent of the changes in diameter of the dark-adapted rhabdom with the time of day. Whether this is, in fact, the case awaits further measurements made throughout the diurnal cycle. We then have to explain the lower efficiency of bump production at 09.00 h the next morning. Our tentative explanation, based on the evidence illustrated in Fig. 2D, is that the rhabdom was by that time breaking down under the influence of its own intrinsic rhythm, although kept in the dark, and that either spontaneous or triggered rhabdom breakdown is accompanied by a desensitization that can be measured in terms of bumps or receptor potential.

Measurement of acceptance angle

The field width of a single retinula cell (as measured by the acceptance angle) increases and decreases with, but is not proportional to, the changes in the diameter of the rhabdom tip. The rhabdom is observed histologically in eyes that have been fixed under comparable conditions at the same time of day.

The angular sensitivity curve is the sensitivity of the retinula cell as a function of angle, with sensitivity defined as the reciprocal of the intensity that gives a constant response. The angular sensitivity curve is a section in two dimensions through the peak of a 3-dimensional field; its width at the 50% level is the acceptance angle.

The responses to flashes at 3 s intervals are measured as the source is moved through the field of the retinula cell. The track must pass through the axis, which is defined as the direction of greatest sensitivity. The source subtends at the eye an angle (actually 0·1 °) that is small compared with the angles to be measured. Responses are measured every $\frac{1}{4}$ ° or $\frac{1}{2}$ °.

As shown in Figs. 4 and 8, the acceptance angle near the dark-adapted fovea of *Valanga* in the day state is 1·25–2·0 °. The actual value is a feature of the cell and the recordings can be repeated many times on a single cell without change of acceptance angle. In the same cell in the night state the angle is always larger, sometimes doubled. We find nothing against the view that the increase in angle is a consequence of the increase in rhabdom diameter but, as discussed below, the measured angles are larger than those expected from the geometrical diameter of the rhabdom.

When the ommatidium in the night state is desensitized by a bright light shining directly into it, the acceptance angle is rapidly reduced (Fig. 8, curve 3). This immediate decrease is presumably caused by constriction of screening pigment around the cone tip, because we believe the rhabdom to have the width of that in the night state (see Fig. 2D). After 1 h in the dark the angle had returned to that typical of the night state (Fig. 8, curve 4), at which it remained and was kept in the dark until measured again at 09.00 h the next day (Fig. 8, curve 5). After 2 h light, to 11.50 h, followed by 15 min dark-adaptation, the angle has returned to the dark-adapted day state (Fig. 8, curve 6). With some caution, therefore, the dark-adapted acceptance angle appears to be an indicator of the rhabdom diameter.

In *Tenodera* also, confirming Rossel (1979), we find a large increase in acceptance angle at night (Fig. 6). More obviously than in the locust, the mantis eye in the

[redacted]rning returns spontaneously to the day state if left in the dark after a night in the dark. The eye turns pale and the acceptance angle becomes narrow. In the locust and mantis alike, the return to the narrow field of the dark-adapted day state suggests that the wider angles during the night cannot be explained as deterioration of the optics.

DISCUSSION

Three principal findings have been set out, namely the morphological changes in the rhabdom, the changes in the field sizes and the shifts in the $V/\log I$ curve. Taken together, in a consideration of the whole animal in relation to its environment, these results show that the compound eye changes in such a way that it becomes more sensitive at night. Some species of locust fly at night (Clark, 1969; Farrow, 1974) and mantids are surprisingly active at night, when they are less easily seen by predators (Rossel, 1979).

Taken separately for analysis of mechanisms, the three main findings on rhabdom, fields and sensitivity open out to separate topics which present many new problems.

Changes in the rhabdom

The changes merit a study of membrane turnover and of the control of rhabdom diameter by the balance between the dissolution and the formation of microvilli, as already in progress in mosquitoes (White *et al.* 1980), spiders (Blest, 1980), crabs (Nässel & Waterman, 1979) and flies (Williams, 1980; Williams & Blest, 1980). The pattern of cytological changes is becoming familiar. After the breakdown of the rhabdom when illuminated at night we see abundant multivesicular bodies in the cytoplasm and collections of parallel endoplasmic reticulum membranes in light-adapted ommatidia. At first sight, however, the turnover processes in mantid and locust eyes are difficult to interpret from fixed material at various times.

The sleeve of clear endoplasmic vacuoles around the rhabdom is consistently present in dark-adapted eyes and absent in light-adapted ones, confirming earlier work (Horridge & Barnard, 1965). If we base our argument upon optical theory, the sleeve of vacuoles assists in keeping the light which is caught by the rhabdom tip inside the rhabdom column as it passes down and is absorbed by the visual pigment. In contrast, in the light-adapted eye some rays leak out and are absorbed in pigment grains of the retinula cells. We have no quantitative measure of this attenuation; instead we expect that it extends over a wide range of increasing intensity as the sleeve of vacuoles disappears progressively in the deeper parts of the retina.

Changes in field size

The effect of an increase in the solid angle over which the receptor collects light is to increase sensitivity at the expense of resolution. In brief, if the acceptance angle is doubled, the rhabdom tip catches four times the photon flux from any object that is larger than the largest visual field. At the same time, however, the diameter of the smallest black spot that can be detected against a bright background is also doubled.

It has long been known that fields are wider in dark-adapted than in light-adapted locust retinula cells. The new finding is to attribute an additional widening at night to the increase in diameter of the rhabdom tip. The night state contributes towards [redacted]proved night vision.

Predicted and actual field sizes

A theory from which the acceptance angle can be calculated from the anatomy of the ommatidium has been set out in detail (Horridge & Duelli, 1979) and tested against measured values of acceptance angles in various eyes (Horridge, 1980). In this model, the Airy disc moves across the tip of the rhabdom as the point source is moved outside the eye. The theoretical angular sensitivity curve is the mathematical convolution of the Airy disc with the absorption profile of the rhabdom tip. The theoretical minimum acceptance angle $\Delta\rho'$ is given by:

$$(\Delta\rho')^2 = (\lambda/D)^2 + (d/f)^2, \quad (1)$$

where λ is the vacuum wavelength, D is the diameter of the corneal facet, d is the diameter of the rhabdom tip and f is the distance from the rhabdom tip to the posterior nodal point. The angle subtended by the Airy disc at the nodal point (λ/D radians) is the diffraction component: the angle subtended by the rhabdom at the nodal point (d/f radians) is the anatomical component. In several dragonflies and in the fovea of mantids, ommatidia with larger facets have narrower rhabdoms, fitting the relation $(\lambda/D) = (d/f)$, so that the rhabdom diameter is matched to the diffraction component (Horridge, 1980).

We can calculate the acceptance angles from the anatomy and equation (1), once we know that the nodal points of the locust and mantis ommatidia are at one third of the distance from the corneal surface to the rhabdom tip (McIntyre, 1980). For the eye region studied in the locust we have $d = 2$ μm by day and 4 μm by night, $D = 37$ μm, $f = 160$ μm and $\lambda = 0.5$ μm. Then by equation (1), $\Delta\rho = 1.05$ ° in the day state and 1.7 ° in the night state. We see that doubling the rhabdom diameter does not double the theoretical acceptance angle, and that $\lambda/D = d/f$ in the day state, which agrees with the situation in the foveas of mantids and dragonflies (Horridge, 1980).

Calculated values, however, are smaller than values measured in ommatidia that are dark-adapted for 15 min, so the assumed value of (λ/D) or of (d/f), or both, is too small. Because acceptance angles are further reduced by adapting the day state eye to light, and the diffraction component λ/D is not influenced by adaptation, it is the anatomical component (d/f) which must be revised. The focal length f is fixed, therefore d, (the measured value of rhabdom diameter in the model) is too small. From this conclusion it follows that the effective rhabdom diameter that governs the acceptance angle is larger than the geometrical rhabdom diameter, probably because rays are funnelled into the rhabdom by light-guide action in the neck of the cone and the effective transverse section is greater than that at the narrowest point. If this is so, we can infer that the narrowing of the acceptance angle on light adaptation of the locust eye in the day state (Wilson, 1975) is due to the contraction of the pigment sleeve acting as a pupil around the neck of the cone. This inference could be tested directly, disproving previous theory (Horridge, 1966).

Change in the V/log I curves

The $V/\log I$ curve is one arbitrary measure of the response of a retinula cell to light, arbitrary because V is measured as the peak height of a complex response. In this paper the $V/\log I$ curve serves two purposes (*a*) to relate the responses to the poi

rce at different angles back to equivalent intensities so that angular sensitivity curves can be calculated, (*b*) to standardize changes with time of day. There is little chance that the V/log I curves at different times can be related quantitatively to the morphological changes in the rhabdom because too many unknown factors are involved; for example, the shape of bumps or the way they add together may depend on the age of the membrane as controlled by the time of day.

The V/log I curve shows the intensity required for an arbitrary response. The intensity giving a 50% response, called the PAQ 50 by Laughlin (1976), is a measure of sensitivity derived from the receptor potential peak. We observe that as the night state develops the V/log I curve moves to the right: the eye becomes more sensitive by this measure. After bright light at night the eye slowly becomes insensitive and then takes several hours to recover full sensitivity. A full account of these changes would include the standing potentials of the baseline from which the peak responses have been measured, but in insects the second-order neurones respond mainly to the phasic responses of the retinula cells (Laughlin & Hardie, 1978). The positions of the V/log I curves as plotted are therefore some indication of the changes in sensitivity of the whole animal to phasic visual stimuli.

V/log I curves and bumps

We do not wish to elaborate here on the relation between the counts of bumps and the positions of the V/log I curves at various times of day, but even the limited data presented in Figs. 3 and 7 suggests that further study of this point is required. The change in the position of the V/log I curve with time of day is not always matched by the bump frequencies plotted against intensity on the same diagram. Before this problem can be elucidated, two questions need to be settled. First, Lillywhite (1977) showed that one photon causes one bump with an efficiency of 60% in work on dark-adapted locusts in the evening and our tests so far suggest that this value is valid for all ordered rhabdom. When an eye on normal day/night cycle is kept in the dark in the morning, (i.e. given no dawn as in Fig. 1 D), the bump frequency in response to a continuous light is about 0.05 of that expected (curves 5(*a*) and 5(*b*) in Fig. 7) and the V/log I curve has correspondingly moved to the right compared to that for the previous night (curves 5 and 2 in Fig. 7). We suggest that the two separate breakdown processes shown in Figs. 1 D and 2 D are accompanied by a severe drop in efficiency of the transduction process in terms of bumps per photon, a conclusion which can be tested in detail now that the situation has been defined.

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Note added in proof. The morphological difference between day and night states (shown in Figs. 2A, B) also occur in the newly hatched first instar larva of the mantis *Orthodera*, so it is an adaptation not only for the adult. This opens a further question, the establishment of the cycle in the embryonic