J. exp. Biol. (1978), **74**, 47–57
With 9 figures
Printed in Great Britain

SPECTRAL SENSITIVITIES OF THE ANTERIOR MEDIAN EYES OF THE ORB WEB SPIDERS, *ARGIOPE BRUENNICHII* AND *A. AMOENA*

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(*Received* 1 *August* 1977)

SUMMARY

Spectral sensitivities of the anterior median eyes of the orb web spiders, *Argiope bruennichii* and *A. amoena* have been studied by recording extracellular ERGs and intracellular receptor potentials. The anterior median eyes have three types of visual cells, with maximum sensitivities at about 360 nm (u.v. cell), 480–500 nm (blue cell) and 540 nm (green cell). The blue cells are the most sensitive and have a circadian oscillation of sensitivity. The green cells show a hypersensitivity, i.e. the sensitivity is greater for about 90 s after the cessation of illumination than during dark adaptation. Respiration is necessary for the maintenance of hypersensitivity.

INTRODUCTION

Colour vision in web-weaving spiders has not previously been studied. The anterior median eyes of jumping spiders, which are hunters, show some capacity for colour recognition (Peckham & Peckham, 1894; Crane, 1949; Kästner, 1950; Land, 1969), and Yamashita & Tateda (1969*a*) have shown that the anterior median eye of the jumping spider, *Menemerus confusus* Boes. et Str. has four types of visual cells of different spectral sensitivities.

In the present study, the spectral sensitivities of the anterior median eyes of the orb web spiders, *Argiope bruennichii* (Scopoli) and *A. amoena* have been studied electrophysiologically.

MATERIALS AND METHODS

Animals employed in this study were female orb web spiders, *Argiope bruennichii* (Scopoli) and *A. amoena*, collected in the field near Kyushu University.

Recording and stimulating methods were similar to those described previously (Yamashita & Tateda, 1976*a*, *b*). Intracellular potentials were recorded by means of glass pipette microelectrodes (50–100 MΩ) filled with 2·5 M-KCl. The pedicel was transected and the abdomen removed to eliminate possible influence of heart movement. The dorsal part of the cephalothorax was removed with a sharp razor blade, exposing the retinal portion for microelectrode penetration. A small piece of platinum placed in the saline served as an indifferent electrode. ERGs were recorded from the intact eye. A tungsten electrode (about 1 μm in tip diameter) or a microelectrode

filled with physiological saline was inserted through a small hole in the cuticle covering the anterior median eye. An indifferent electrode was inserted in the cephalothorax.

For white light stimulation, the light emitted by a 6–8 V tungsten lamp was passed through a heat-absorbing filter. The duration of illumination was controlled by a mechanical shutter, and the intensity was adjusted by calibrated neutral density filters. The intensity of the light with no filters is referred to as unit intensity ($\log I = 0{\cdot}0$), and had a value of about 2×10^5 lux at the preparation. Two light sources of the same intensity were placed side by side. One of them was used as the control and the test light, and the other was used as the conditioning light. Initially, the control light, serving also as the test light, was presented. This was followed by the conditioning light and after various time intervals by the test light. For monochromatic light stimulation, the light emitted by a 150 W xenon arc lamp was passed through a grated monochromator and focused on the eye. The intensity is referred to as relative number of quanta. The energy of selected monochromatic light was measured by a thermopile. For the experiment on circadian rhythm, a light-emitting diode (560 nm emitting wavelength) placed in front of the preparation was used. The light-emitting diode was automatically driven by an electronic stimulator at regular intervals.

RESULTS

Spectral sensitivities of receptor cells

The spectral sensitivities of the receptor cells were studied by intracellular recordings of receptor potentials. The magnitude of the resting potential was about 30 mV. Light stimulation elicited a depolarizing receptor potential in all cases, and after-hyperpolarization did not occur. Assuming that the intensity–response curves for various monochromatic lights are all parallel to one another, the intensity–response curves were determined only at the wavelength of monochromatic light initiating the maximum response of the cell. The sensitivity is the reciprocal of the relative number of quanta required to elicit a response of 2 mV. Spectral sensitivity curves were measured from 24 cells. Nine of these 24 cells had a single large peak. Three had a maximum sensitivity at about 360 nm (u.v. cell), two at 480–500 nm (blue cell) and four at 540 nm (green cell). The average spectral sensitivity curves of these cells are shown in Fig. 1. Average intensity–response curves are shown in Fig. 2. The intensity–response curves are not taken to saturation because the maximum stimulus intensity was not high enough. The sensitivity of the blue cell is higher than that of the green cell and the u.v. cell.

As can be seen in Fig. 1, each curve has a small secondary spectral sensitivity in the ultraviolet or visible region, suggesting that there may be electrical interactions among different visual cells or a mixture of two or even three different photopigments within a cell.

Circadian change of spectral sensitivity

The amplitude of the ERG changes with time of the day, suggesting that the anterior median eye has a circadian oscillation of sensitivity. Similar phenomena have been reported for beetles (Jahn & Crescitelli, 1940), crayfishes (Arechiga & Wiersma, 1969), scorpions (Fleissner, 1972) and ants (Ventura *et al.* 1976). A typical example of

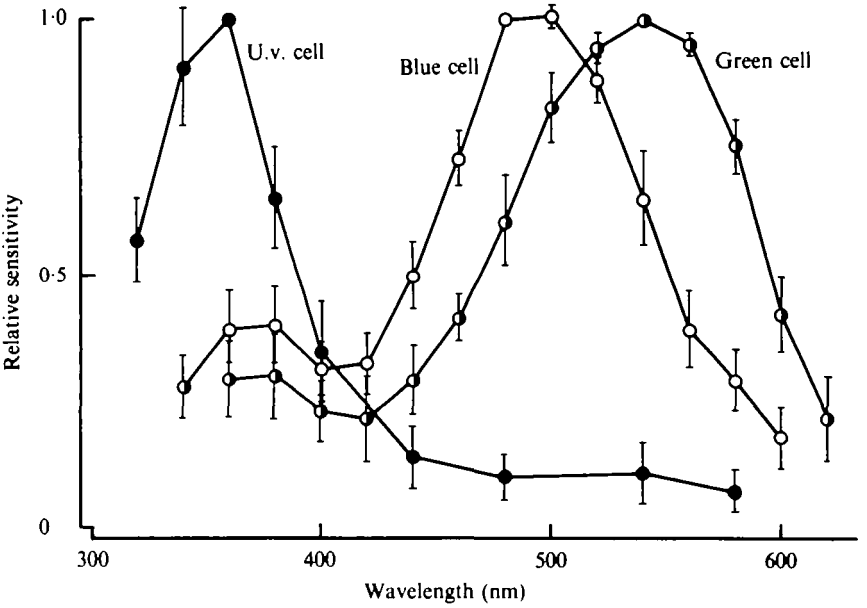

Fig. 1. The average spectral sensitivity curves of three u.v. cells, two blue cells and four green cells. The sensitivities are referred to as 1·0 at 360 nm for the u.v. cell, at 480 nm for the blue cell, and 540 nm for the green cell. The vertical lines indicate the standard deviation.

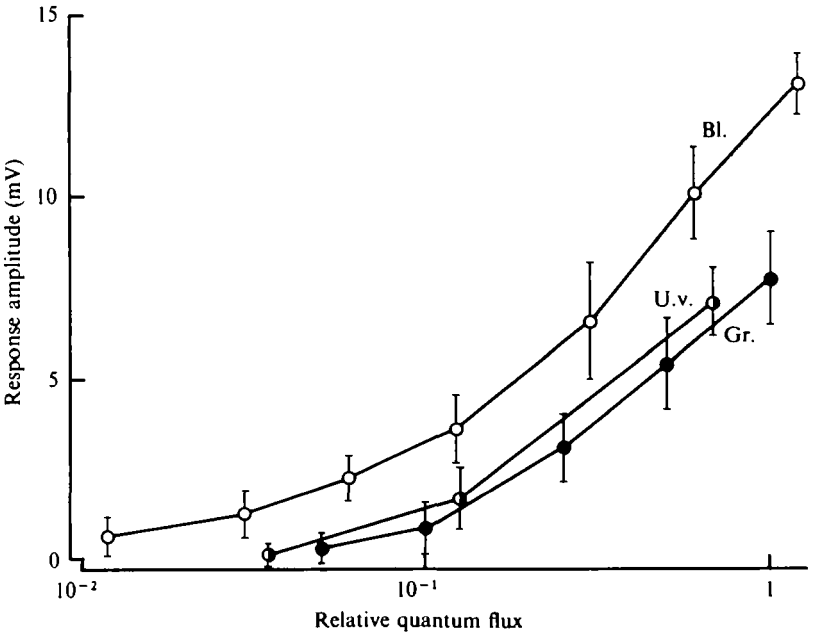

Fig. 2. The average intensity-response curves of three u.v. cells illuminated at 360 nm, two blue cells illuminated at 480 nm and four green cells illuminated at 540 nm. Same cells as in Fig. 1. The amplitude of responses to 67 ms flash are plotted against the log of the relative number of quanta.

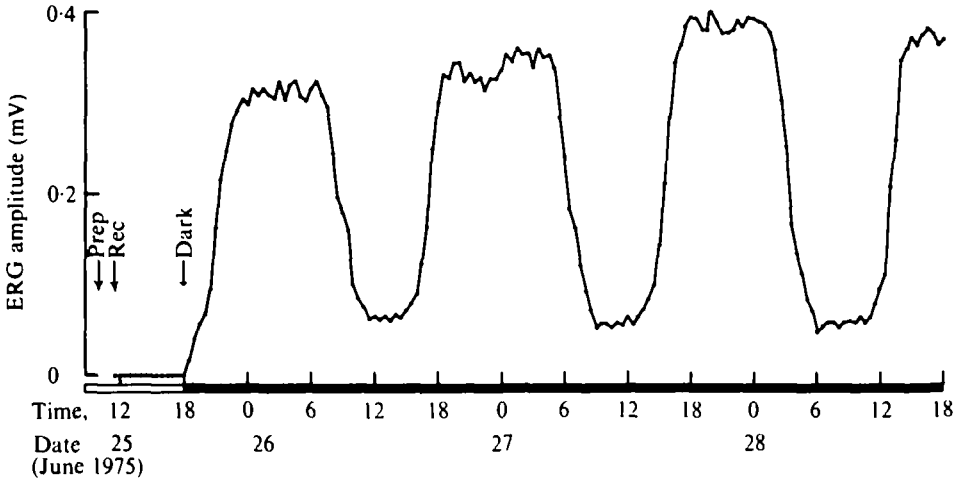

Fig. 3. Circadian changes in ERG amplitude. See text.

a circadian oscillation of the ERG is shown in Fig. 3. The animal was collected from the field in the morning, prepared at 10.00 h, and recording was begun at 11.30 h under background illumination. After the cessation of the background illumination at 18.00 h, ERGs were recorded under constant darkness for 3 days. Throughout these periods, a light flash of 10 ms duration was automatically presented every 2 min. As shown in Fig. 3, the amplitude of the ERG shows a circadian oscillation with a period of approximately 22 h under constant darkness. ERGs of constant low amplitude are recorded for about 3–4 h. We call this period the 'day state'. Then the magnitude of the ERG begins to increase gradually over a period of 4–5 h to a maximum plateau value. This plateau maximum lasts for about 7–10 h, and is hereafter called the 'night state'. The magnitude of the ERG subsequently decreases nearly as fast as it initially increased, until a constant low value is reached again.

The spectral sensitivities of the anterior median eyes during the 'night state' and the 'day state' under constant darkness were studied by recording ERGs from the intact eye. Intensity–ERG response curves were obtained for each 20 nm. In both states, the threshold, which is the relative number of quanta required to elicit an ERG with 150 μV amplitude, was the lowest at 480–500 nm. However, the slope of the intensity–ERG response curves varied with wavelength and was different for the 'day' and 'night' states (cf. Fig. 8). Therefore, spectral responses to equal quanta were obtained from the intensity–response curves for each wavelength during the 'night state' and the 'day state'. Fig. 4 shows the average spectral response curves. The magnitude of the response to the 480 nm flash during the 'night state', is referred to as unity. During the 'night state', peak responses are observed at 360 nm in the ultraviolet and at 480–540 nm in the visible. During the 'day state', the ERG amplitude to each monochromatic light decreases as compared with the ERGs for the 'night state', as shown in Fig. 4. The decrease in the ERG amplitude is larger in the blue, but small in the ultraviolet and the red. Consequently, peak responses for the 'day state' are observed at 360 nm and at 540 nm. These results suggest that the blue cells show a circadian oscillation of sensitivity. If this is true, the same spectral response curve for the 'day state' under conditions of constant darkness should be

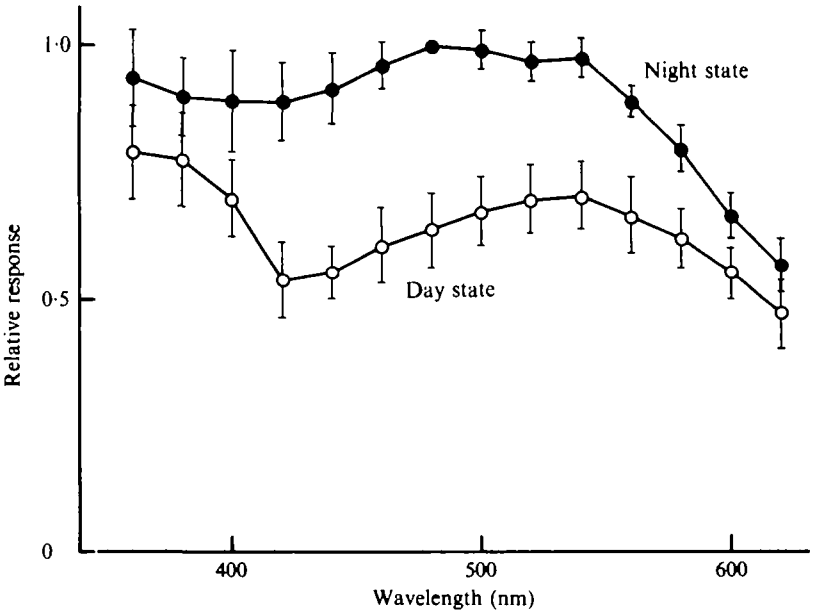

Fig. 4. The average spectral response curves of the dark-adapted eyes of three preparations obtained during the 'night state' (closed circles) and 'day state' (open circles). The relative amplitude of response to a monochromatic light stimulus of equal quanta is plotted against the stimulus wavelength. The magnitude of the response to a 480 nm flash during the 'night stage' is referred to as 1·0. Vertical lines indicate standard deviation.

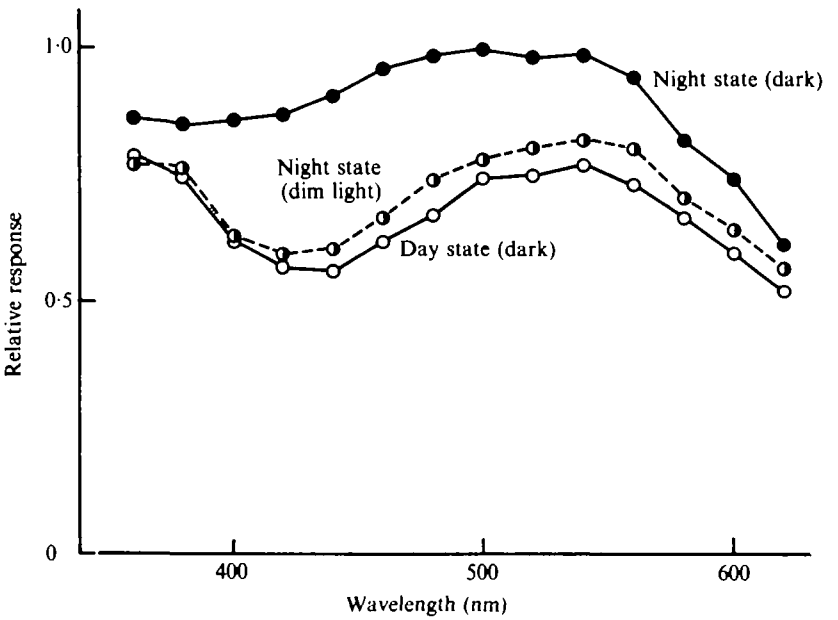

Fig. 5. The effect of dim white light adaptation. The spectral response curve during dim-light adaptation during the 'night state' (half-closed circles) is shown together with those during dark adaptation for the 'night state' (closed circles) and the 'day state' (open circles). The intensity of the background illumination was $\log I = 2{\cdot}5 \times 10^{-5}$.

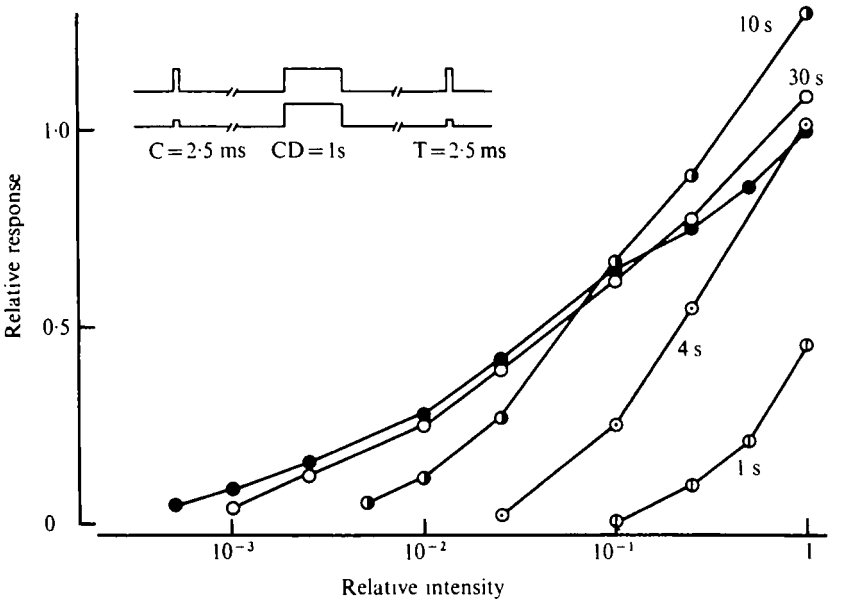

Fig. 6. Intensity–ERG response relations before (●), and after 1 s (◐), 4 s (○), 10 s (◑) and 30 s (○) following a 1 s conditioning stimulus of log I = 0·0. C, control stimulus. CD, conditioning stimulus. T, test stimulus.

obtained for the 'night state' by adapting the blue cells moderately. To test this hypothesis, ERGs were recorded during the 'night state' under various intensities of dim light and compared to those recorded during the 'day state' in the dark. We found that the spectral response curve for the 'day state' was very similar to that obtained for the 'night state' during dim light adaptation. Both blue light and white light have the same adapting effects when their intensities are low. Fig. 5 shows spectral response curves for the 'night state' during dim white light adaptation, together with spectral response curves for the 'night state' and the 'day state' under constant darkness. The intensity of the background illumination was log $I = 2{\cdot}5 \times 10^{-5}$. As can be seen in this figure, the spectral response curve for the 'night state' during dim white light adaptation is almost coincident with that for the 'day state' in the dark.

We conclude, therefore, that the blue cells show a circadian oscillation of sensitivity.

Hypersensitivity

We have reported that photoreceptor cells of the anterior median eye of the jumping spider are hypersensitive following illumination (Yamashita & Tateda, 1976*b*). A similar hypersensitivity has been found for orb web spiders in the present study.

The intensity–ERG response curves to white light were obtained at various intervals, before and after a constant conditioning stimulus of log I = 0·0 intensity and 1 s duration. Fig. 6 shows the intensity–ERG response curves obtained before and 1 s, 4 s, 10 s and 30 s after the constant conditioning stimulus. Since the white light used in this study contains few ultraviolet photons, these ERGs reflect only the potentials of blue cells and green cells. If the intensity of the light stimulation is under the threshold of the green cells, the ERG may purely reflect the potentials of the blue cells,

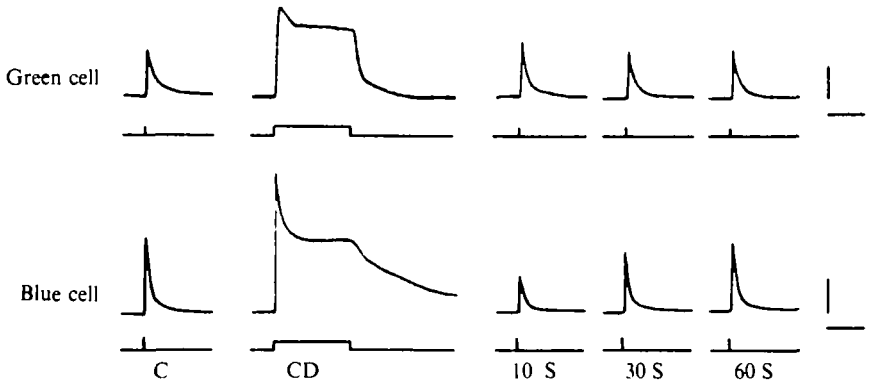

Fig. 7. Increased (green cell) and decreased (blue cell) responses following a light stimulus of 1 s duration and intensity log I = 0·0. The control light (C) of 2·5 ms duration was presented to the dark-adapted eye. The test lights were presented to the eye 10 s, 30 s and 60 s following the conditioning stimulus (CD). Horizontal bar indicates 0·5 s, vertical bar 5 mV for each trace.

and the ERG to bright light reflects the summed potentials from the blue cells and the green cells. As can be seen in Fig. 6, when the intensity of the test light was low e.g. log $I = \bar{3}{\cdot}0$, the response to the test light was very small just after the conditioning stimulus, and then recovered gradually to the dark-adapted level. On the other hand, when the intensity of the test light was high, e.g. log I = 0·0, the response to the bright test light was also very small just after the conditioning stimulus, but increased rapidly beyond the dark adapted level. The increase lasted for about 90 s after illumination and the maximum response occurred 5–10 s after illumination. The recovery process of the eye to a bright test light following illumination was very similar to the dark adaptation process of the anterior median eye of the jumping spider which is known to exhibit hypersensitivity (Yamashita & Tateda, 1976*b*). These results suggest that green cells, the thresholds of which are higher, are hypersensitive, and that blue cells, which have a lower threshold, are not. This hypothesis was examined by recording the receptor potentials of a single photoreceptor cell. In Fig. 7 one potential obtained from a green cell and one from a blue cell are shown. The amplitude of the response of the green cell to the control light was about 7 mV. Ten seconds after the conditioning stimulus, the response to the test light apparently increased (about 8 mV). At that time, the resting potential recovered to the dark-adapted level. The response of the blue cell to the control light was about 10 mV. Ten seconds after the conditioning stimulus, the response to the test light was very small (about 5 mV), and then recovered gradually to the dark-adapted level. We conclude, therefore, that the green cells show hypersensitivity and the blue cells do not.

The hypersensitivity phenomenon was studied for both the 'night state' and the 'day state'. The intensity–ERG response relations before and after the conditioning stimulus were obtained for both the 'night state' and the 'day state'. The test light was presented 10 s after the conditioning stimulus. As can be seen in Fig. 8, the threshold of the ERG to the control light obtained for the 'day state' is higher than that obtained for the 'night state', and the slope of the curves for the 'day state' is steeper than for the 'night state'. However, the intensity–response curves to the test

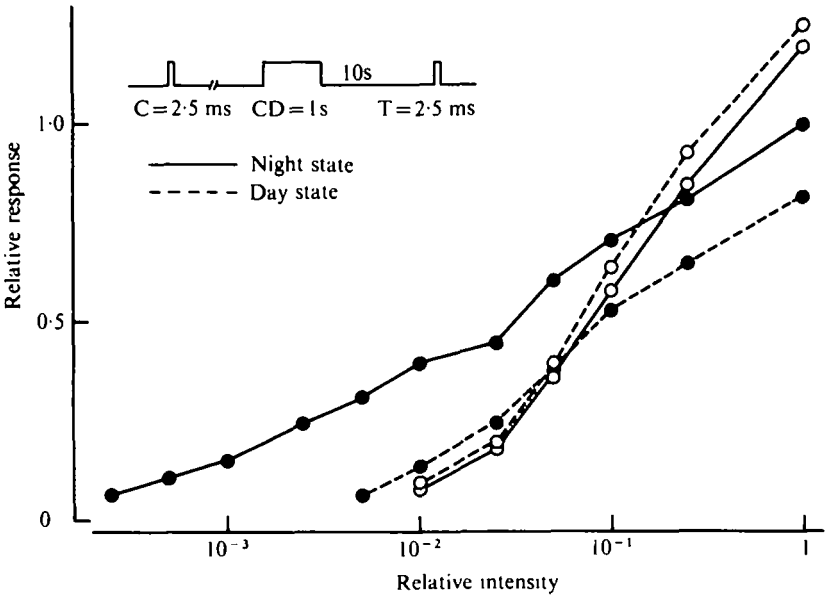

Fig. 8. Intensity–ERG response relation before (closed circle) and after (open circle) a 1 s conditioning stimulus of log I = 0·0, obtained during the 'night' and 'day' state.

light for the 'night state' is almost coincident with that to test lights for the 'day state'. This may be explained by the presence of two types of receptor cells which have different thresholds. As shown previously, 10 s after the conditioning light, the responses of the green cells are greater than those obtained before the conditioning stimulus, while the responses of the blue cells are very small. At that time, one would expect that the greater part of the ERG to the test light is comprised of potentials of the green cells. Therefore, coincidence of the intensity-ERG response curves to the test light for the 'day state' and the 'night state' shows that the green cells have little circadian oscillation of sensitivity. As can be expected, when the eye is adapted to dim light in the night state, both the intensity–response curves before and after the conditioning stimulus are almost coincident to those obtained for the 'day state' in the dark. All data show that only the blue cells have a large circadian oscillation of sensitivity.

Loss of hypersensitivity occurs in jumping spider eyes within a few minutes when the pedicel is transected and the abdomen removed from the intact animal (Yamashita & Tateda, 1976*b*). Since the respiratory organs of the spiders are located in the abdomen, this result suggests that respiration may be necessary for the maintenance of hypersensitivity. In the orb web spiders, we studied the effect of respiration on hypersensitivity in the green cells. The pedicel was constricted repeatedly by means of a V-shaped wire (Fig. 9A). Fig. 9B shows the effect of pedicel constriction on the response to a bright test light (log I = 0·0). The test light was presented to the eye 10 s after the conditioning stimulus. The intensity of the conditioning stimulus was log I = 0·0 and the duration was 1 s. In unconstricted animals, the amplitude of the response to the control light is about 1·3 mV and that to the test light about 1·6 mV. The response apparently increases after illumination. Five minutes after pedicel

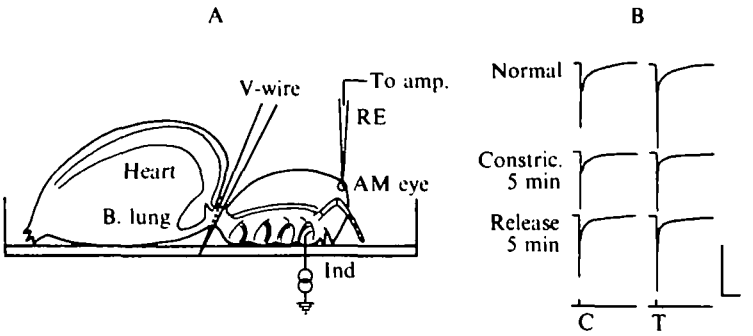

Fig. 9. Effect of pedicel constriction on hypersensitivity. (A) Diagrammatic representation of the method of pedicel constriction and the recording method from the intact animal. B. lung, book lung. V-wire, V-shaped wire for pedicel constriction. RE, recording electrode. Ind, indifferent electrode. (B) ERGs recorded from the anterior median eye before and after a conditioning stimulus. Traces at the left are ERGs of the dark-adapted eye following the control stimulus (C). Traces at the right are ERGs of the same eye following the test stimulus (T) presented 10 s after the conditioning stimulus. The conditioning stimulus was the same throughout (log I = 0·0 and duration = 1 s). Time after pedicel constriction and the time after release of the constriction are shown to the left of the figure. Horizontal bar indicates 0·5 s and vertical bar 1 mV.

constriction, the amplitude of the response to the control light is about 1·1 mV and that to the test light is also about 1·1 mV. The increase in response following illumination apparently disappears. Five minutes after the release of the pedicel both the response to the control and test light recovered to the level observed in the normal preparation. Also, the response to the test light is apparently greater than to the control light. The effect of pedicel constriction was observed repeatedly. We conclude that respiration is necessary for maintenance of hypersensitivity.

DISCUSSION

The anterior median eyes of orb web spiders have three spectrally different types of visual cells with maximum sensitivities at about 360 nm (u.v. cell), 480–500 nm (blue cell) and 540 nm (green cell). Possession of three types of receptor cells suggests the possibility for colour vision, although there is no behavioural evidence for it. We conclude that the blue cells have the lowest threshold and the green cells and the u.v. cells the highest. Orb web spiders build their webs before sunrise. It is reasonable to consider that the orb web spiders have a mechanism which permits adjustment of the visual system over a wide range of light intensities. This can be explained by the duplicity theory of vision. The blue cells may correspond to the rods of the vertebrate retina while the green cells and u.v. cells may correspond to the cones.

We find that both the spectral response curves and the intensity–ERG response curves for the 'night state' are different from those for the 'day state' (Figs. 4, 5, 8), and conclude that the blue cells have a circadian oscillation of sensitivity. The underlying mechanism of the circadian sensitivity change is unknown. Circadian sensitivity changes have been reported for crayfishes by Arechiga & Wiersma (1969) and scorpions by Fleissner (1972). These changes are apparently due to pigment migration in the visual cells in scorpions, and in both the visual cells and pigment

cells in crayfishes. DeVoe, Small & Zvargulis (1969), however, have reported that for the eyes of wolf spiders there are no observable pigment migrations even during light and dark adaptation. We have not observed differences in pigment positions during dark and light adaptation, or at different times of day, so circadian sensitivity changes of the orb web spiders cannot be explained by a pigment migration.

Fleissner & Schliwa (1977) showed that neurosecretory nerve fibres terminate on retinula cell membranes, and according to them, Fleissner & Fleissner (1977) have shown that section of the optic nerve not only leads to degenerative changes of the neurosecretory fibres but also inhibits the circadian pigment movements within the retinula cells. For the eyes of wolf spiders, Melamed & Trujillo-Cenoz (1966) noted the presence of so called 'clear fibres' loaded with numerous dark granules and clusters of small vesicles. These fibres have several morphological similarities to neurosecretory nerve endings. Melamed & Trujillo-Cenoz (1966) suggest that these fibres may be centrifugal fibres. There is the possibility that efferent signals may control the sensitivity of the receptor cells. If this is true for orb web spiders, only the blue cells should be affected by the efferent signals.

We reported for jumping spiders that the sensitivity of the photoreceptor cells is greater for about 60 s following illumination than it is during complete dark adaptation (Yamashita & Tateda, 1976*b*). In the present study, we have shown that the response following illumination of the green cells increases while that of the blue cells decreases (Fig. 7). If we record receptor potentials from an animal whose abdomen has been removed, the increase in response after illumination is soon lost. Therefore, we did not obtain stable intensity–response relations before and after the illumination of single receptor cells which demonstrated the hypersensitivity. However, the recovery process of the eye to a bright test light following illuminations was very similar to the dark adaptation process of the anterior median eye of the jumping spider which is known to exhibit hypersensitivity (Fig. 6). The hypersensitivity mechanism facilitates the recovery of the response after illumination.

The authors are indebted to Dr D. R. Stokes, Emory University, Atlanta, Georgia, U.S.A. for revision of the original manuscript. This research was supported in part by grants from the Ministry of Education of Japan.

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