Ultraviolet and Green Receptors in Principal Eyes of Jumping Spiders

ROBERT D. DEVOE

From the Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT Spectral sensitivities of cells in principal eyes of the jumping spider *Phidippus reqius* were measured using techniques of intracellular recording. Three types of cells were found. UV cells had peak sensitivities at 370 nm and were over 4 log units less sensitive at wavelengths longer than 460 nm. Green-sensitive cells had spectral sensitivities which were well fit by nomogram curves peaking at 532 nm. UV-green cells had dual peaks of sensitivity at about 370 and 525 nm, but the ratios of UV-to-green sensitivities varied over a 40:1 range from cell to cell. Moreover, responses of UV-green cells to flashes of UV light were slower than to flashes of green light. Segregation of receptor types into the known layers of receptors in these eyes could not be shown. It is concluded that jumping spiders have the potential for dichromatic color vision.

The brightly colored, keen-sighted jumping spiders have long been considered the possessers of color vision. Peckham and Peckham (1894) claimed that males of a number of species (*Dendryphantes elegans, Saitis pulex, Phiddipus rufus,* and *Astia vittata*) used color clues to identify females (of the same species) that they would dance before and mate with. When the Peckhams painted the previously attractive females a different color, they were then ignored by the males. With different species, Crane (1949) was unable to duplicate the Peckham's results; she could find no role of color in the attractiveness of the female for the male. However, she did find that a yellow stripe (and not a white stripe) painted on a dead black spider or spider model could elicit male-male threat display in one species, *Corythalia xanthopa,* which possesses such yellow clypeal stripes. She also found that both mating and threat display required uncovered principal (anterior median) eyes, which Homann (1928) had previously found to have superb spatial resolution in *Evarcha blancardi.* Subsequently, Kästner (1950) provided further evidence that the principal eyes might possess color discrimination. In essence, he first showed that *Evarcha falcata* preferred to climb the striped wall in a tunnel as compared to the opposite, plain wall, after viewing both with their principal eyes (Dzimirski

[1959] however, has argued that the lateral eyes may have been used too). Next, he found that although black-gray stripes might be confused with the plain wall (as compared to black-and-white stripes), colored-and-gray stripes (of 26 different shades) never were. From this, Kästner (1950) concluded that the spiders discriminated the hues of his orange or blue colored stripes and not just the brightnesses.

Kastner (1950) also tested the spiders' abilities to see in ultraviolet light: He found that hungry spiders in general did not catch flies in UV light alone and concluded that they were not sensitive to UV wavelengths of light. However, in 1967, Young and Wanlass reported that the jumping spiders *Saltious scenicus* would attack nonfluorescing objects in UV light and "were able to estimate distance and performed courtship antics."

About this same time, but without knowledge of Young and Wanlass' (1967) results, preliminary electrophysiological experiments on spectral sensitivities of principal eyes of jumping spiders (*Phiddipus audax*) were begun in my laboratory (DeVoe and Zvargulis, 1967). Kästner's (1950) results had strongly suggested the presence in principal eyes of two classes of cells maximally sensitive in one or another region of the visible spectrum. Using the ERG to assess retinal sensitivities, we failed however to find the then expected two classes of cells sensitive in the visible (DeVoe and Zvargulis, 1967; DeVoe et al., 1969). Instead, sensitivity was found to peak both in the UV at a wavelength shorter than 380 nm (the shortest wavelength we could then use) and at about 535 nm in the green. Moreover, it was not possible to obtain selective light adaptations with chromatic backgrounds in order to show whether the UV and green sensitivities came from two classes of cells or not (DeVoe et al., 1969).

In 1969, Land (1969 *a*) found a four-layer stratification of rhabdomes in principal eyes of the jumping spiders *Metaphidippus aenolus* and *Phidippus johnsoni*. This has been confirmed by Eakin and Brandenburger (1971). Land (1969 *a*) calculated that red, blue-green, and blue objects at infinity would be focused upon layers 1 (farthest from the lens), 2, and 3, respectively, due to chromatic aberration of the lens. No image would be focused upon layer 4, which was too close to the lens. With the arguments summarized earlier that there might be color vision in principal eyes of jumping spiders, Land (1969 *a*) then predicted that receptors in layer 1 would be sensitive to wavelengths in the red end of the spectrum and that the receptors in layers 2 and 3 would be sensitive to wavelengths in the blue-green and violet-UV regions, respectively.

Taken all together, the above evidence would now seem to point to the existence of at least UV- and green-sensitive receptors in the principal eyes of jumping spiders, and possibly some red-sensitive receptors as well. On the other hand, the failure of chromatic light adaptations to be selective in electrophysiological experiments (DeVoe et al., 1969) seems to indicate that there may be cells sensitive in both the UV and green, as in the principal eyes of

wolf spiders (DeVoe, 1972 *a*). To settle these questions, further electrophysiological experiments were begun, this time using intracellular recording techniques. The results, reported here, show that there are only cells sensitive in the UV, or in the green, or both, but not in the red. Thus they show that the jumping spider has the capability for a dichromatic color vision. A preliminary report of these results has been made elsewhere (DeVoe, 1972 *b*).

MATERIALS AND METHODS

Animals

All experiments were performed on *Phidippus regius*, a species chosen for its large size rather than bright coloration. This species is black and brown with white markings. However, casual observation suggests that the chelicerae of males may be a bright metallic green, whereas those of females appear mainly a dull metallic red. No pertinent behavioral experiments have been done on the vision of this species, to my knowledge, but there are no reasons at the moment for supposing that behavioral observations on other genera and anatomical findings on other *Phidippus* species would not apply to this species. Animals were collected from holes in unused, concrete, side-rail posts along a road in Suwanee County, Florida. They were kept in separate plastic cages with constant water and periodic feedings of mealworms (larvae of *Tenebrio molitor*). The cages were kept in a room with about 70% relative humidity and a 12:12-h light:dark cycle. In these conditions, the animals survived anywhere from a few months to 15 mon for the last-sacrificed individual. For technical reasons, all successful experiments happened to have been performed on females.

Preparation of the Animals

In early experiments, a number of approaches to the principal eyes were tried. Most failed until it was found that the white tissue which almost completely surrounds these eyes, and which was routinely cut away, was in fact midgut diverticulum (Millot, 1931). The result of gut removal in most cases was destruction of the principal eyes by gut enzymes, although some successful experiments did result after removal of gut. Because of the way the gut encircles the principal eyes, it never did prove possible to avoid damaging it in a ventral approach to the principal eyes, as was used in the wolf spider (DeVoe, 1972 *a*), where there is no gut around the eyes. However, it was found that over the dorsal side of the principal eyes, there were areas between the gut diverticuli through which electrodes could be inserted into the eyes vertically from above.

The final method of preparation was then as follows: Animals were anesthetized with CO_2, the abdomen and legs were cut off, and the cephalothorax was glued dorsal-side up to a cork and immersed in spider Ringer's (Rathmeyer, 1965). A square opening was made in the dorsal carapace over the principal eyes, and the connective tissue sheath binding together the gut diverticuli was gently teased apart. Then, the eye capsules themselves were gently torn with fine tweezers, often allowing the cell bodies of the receptors to be seen within. With this method of preparation, intracellular activity was recorded in six of six tries, whereas in 16 earlier preparations, in which gut was cut, activity was found in only 6 preparations. Occasionaly, for soften-

ing the eye capsules, enzymes such as Pronase (Calbiochem, San Diego, Calif.), collaganase, or elastase were used in earlier experiments, with indifferent success resulting presumably from undesired enzymes contributed by the injured gut. No enzymes at all were used in later, successful preparations.

Finally, principal eyes of jumping spiders each possess six muscles for eye movements (Scheuring, 1914; Land, 1969 *b*), compared to only two in wolf spiders (Melamed and Trujillo-Cenoz, 1971). In both animals, it is necessary to still these muscles for stable intracellular recording. The enzyme Pronase sufficed to do this in wolf spiders (DeVoe, 1972 *a*). In jumping spiders, instead, the eye muscles sometimes had to be cut and torn away with fine scissors and forceps, otherwise they often went into what appeared to be contractures and seriously distorted the shapes of the eyes. Alternatively, it sometimes was possible to find and crush the nerve bundle to these muscles (Land, 1969 *b;* Eakin and Brandenburger, 1971). Similarly, a small band of tissue between the proximal tips of the two principal eyes had to be cut, otherwise it slowly contracted during an experiment and pulled the eye-tips together.

Methods of Recording

Intracellular penetrations were made with micropipette electrodes pulled from Pyrex glass tubing containing a few small glass fibers. The electrodes were filled by injection of 2 M KCl and had resistances of from 40 to 120 MΩ in Ringer's; most were in the 40–55-MΩ range. A few unsuccessful attempts were made to strain cells using electrodes filled with 6% Procion yellow; these electrodes had resistances of 150–200 MΩ. Signals were led to a unity-gain, negative capacitance bridge amplifier. Oscillations caused by overcompensation aided the penetration of cells. Resting potentials were measured relative to a grounded electrode in the Ringer's bath by means of a calibrated bucking potential. Responses and a photocell record of light on and off were displayed on a Tektronix 502 oscilloscope (Tektronix, Inc., Beaverton, Ore.) and were photographed with a Grass C-4 camera (Grass Instruments, Quincy, Mass.). All experiments were carried out at 20–22°C.

Optical Stimulation

The automated optical stimulator used in these experiments, as well as its calibration, have been described in detail elsewhere (DeVoe et al., 1969; DeVoe, 1972 *a*; Chappell and DeVoe, 1975). Basically, stepping motors under paper tape control drove a Bausch and Lomb High Intensity Monochromator (Bausch and Lomb, Rochester, N.Y.) and circular neutral density wedges (Eastman Kodak, Rochester, N.Y., type M) to deliver flashes at each of about 20 wavelengths from 350 to 650 nm which would elicit approximately equal responses. The tapes were prepared from previous measurements of spectral sensitivities of cells in eyes of wolf and jumping spiders. The light beam was focused by a quartz lens upon the end of a ULGM fiber optic bundle (American Optical Company, Southbridge, Mass.), the other end of which lay in the Ringer's bath in front of the stimulated eye.

After isolating a cell, its relative sensitivities at 370 and 520 nm were tested in order to choose the optimum paper tape. Each paper tape programmed a series of flashes of wavelengths increasing from 350 to 650 nm, and then, if the cell survived long

enough, back again. At the end of each one-way scan, a series of flashes of increasing intensity were given at one wavelength, 370 nm in one case, 520 nm in the other. Control flashes at either 370 or 520 nm were interspersed between the other flashes, and corrections were made as needed for progressive or random drifts in sensitivity. From the measurements of amplitudes of response at each wavelength used and from the intensity-response curves obtained at 370 and/or 520 nm, the intensities at each wavelength which should have been used to obtain some criterion amplitude of response (usually 4 mV) could be interpolated.

RESULTS

Figs. 1 and 2 illustrate, respectively, typical waveforms of responses recorded from dark-adapted cells and the spectral sensitivities calculated for these cells. Three different types of cells were found, as determined from spectral sensitivities: UV cells, green cells, and UV-green cells. UV cells were maximally sensitive at 370 nm and had sensitivities at least 4 log units less at all wavelengths 460 nm and longer. Green cells had average peak sensitivities at 532 nm and had shoulders of sensitivity (the *cis* peaks) at 380–390 nm. The UV-green cells had dual peaks of sensitivity at 360–370 nm in the UV and at about 525 nm in the visible. These results will now be considered in more detail.

Green Cells

These were the most commonly encountered cells. They had the highest average recorded resting potentials and longest average survival times. Of 103 such cells, the spectral sensitivities of 10 were determined at 20 wavelengths from 350 to 650 nm. Of these 10 cells, 2 were scanned from 350 to 650 nm and back; the remainder were scanned only from 350 to 650 nm. These cells are the fully analyzed cells listed in Table I. The sensitivities of the remaining 93 cells were tested only at 520 and 370 nm to determine that the UV sensitivity was indeed much lower (by about 0.8 to 0.9 log units) than at 520 nm. Such cells are the partially analyzed cells in Table I. There appear to be no significant differences between resting potentials of fully and

TABLE I

PROPERTIES OF CELLS IN PRINCIPAL EYES OF JUMPING SPIDERS

	Green cells		*UV cells*		*UV-green cells* (two fully analyzed)
	Fully analyzed	Partially analyzed	Fully analyzed	Partially analyzed	
Number of cells	10	93	3	47	17
Average minutes held	15	2	12	3	6
Average resting potentials (± SD) in mV	34±8	31±9	22±9	22±9	17±11
	range 20–53	range 11–59	range 9–28	range 5–46	range 8–43

partially analyzed cells. In the early experiments, all green cells which were stable enough were fully analyzed. Later, a deliberate search was made for UV cells, and many green cells which might have been fully analyzed were not studied further once they had been identified. Thus, the average survival times of partially analyzed cells in Table I are probably biased too low.

Fig. 1 shows some typical waveforms of responses of green cells, all in response to 100-ms flashes. At the upper left is an example of the fastest responses recorded; at the upper right, the slowest. Such waveforms of response were also recorded from UV cells too. Conversely, the waveform of the 370-nm response of the UV cell shown in the lower right was found typically in green

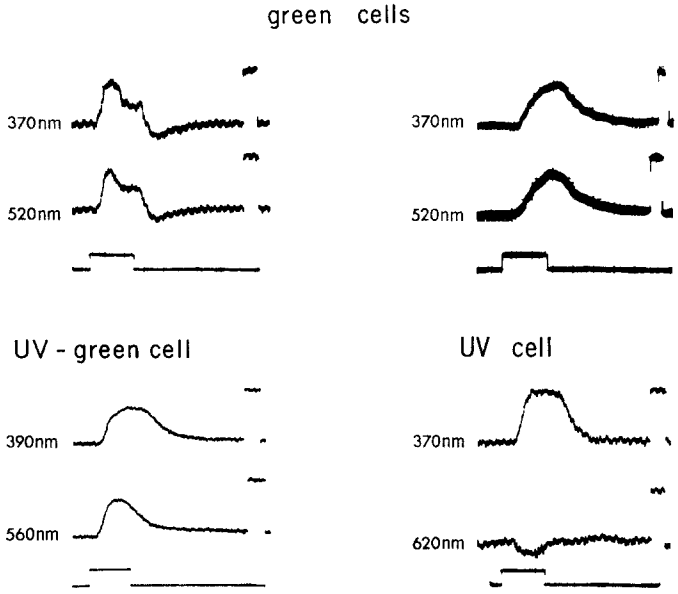

FIGURE 1. Waveforms of responses recorded intracellularly from cells in principal eyes of jumping spiders. Top, left: Examples of the fastest responses recorded to 100-ms flashes; with such flashes, the responses reach plateau amplitudes. Top, right: Examples of the slowest responses; these do not appear to reach maximum amplitudes with flash durations as short as the 100 ms used. There were no variations of waveforms of responses from green cells due to wavelengths. Bottom, right: Square-shaped response of a UV cell at 370 nm is also seen at all wavelengths from some green cells; conversely, fast and slow waveforms at top of figure have also been recorded from UV cells. The responses of UV cells to wavelengths longer than 460 nm are negative going. The response at 620 nm shown here was the largest such negative-going response recorded. Bottom, left: Differerences in waveforms of responses of a UV-green cell with wavelength. The response to the UV flash at 390 nm was slower than the response at 560 nm. Responses illustrated were chosen for equal amplitudes. In all parts of the figure, calibration pulses at end of response traces are 5 mV; upward deflections of bottom-most traces are from a photocell and signify 100-ms flashes.

cells as well. No differences in waveforms of response were found as a function of wavelength in green cells.

Fig. 2 shows the average spectral sensitivity (± SD) of 10 green cells obtained from 12 spectral scans. In the visible, the sensitivity is well fit by a 532-nm nomogram curve (dashed line) (Dartnall, 1953). The subpeak at 380–390 nm in the UV is typical of a *cis* peak of a rhodopsin and occurs at a somewhat longer wavelength than the peak of sensitivity of the UV cells. In

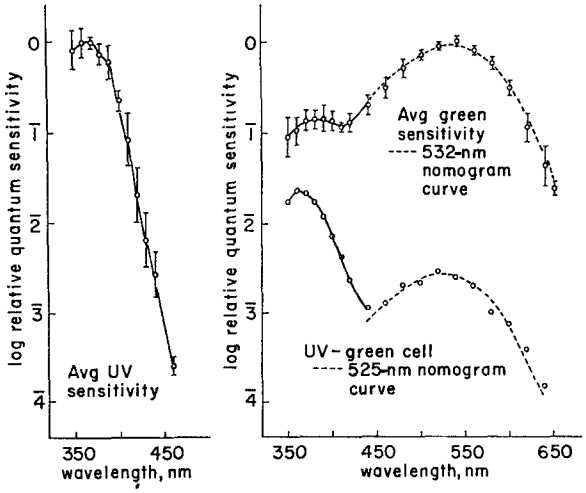

FIGURE 2. Spectral sensitivities of cells in principal eyes of jumping spiders. Left: Normalized average spectral sensitivity (± SD) from four scans on three UV cells. Top, right: Normalized average spectral sensitivity (± SD) from 12 scans on 10 green-sensitive cells. The dashed line represents a 532-nm nomogram curve (Dartnall, 1953). Bottom, right: Spectral sensitivity of one UV-green cell, slid down arbitrarily on the ordinate for clarity. Dashed line represents a 525-nm nomogram curve. Absolute sensitivity of this cell at 360 nm was 4×10^{11} quanta/s upon the eye in a 100-ms flash for a 4-mV response. Zero log relative sensitivity for the other cells was 8.5×10^{10} average quanta/s at 526-540 nm for the green cells and 10^{11} average quanta/s at 370 nm for the UV cells. In all parts of the figure, solid lines were fit by eye. Criterion amplitudes of response for determining spectral sensitivities were 2-4 mV.

two cells tested, intensity-response curves at 370 and 520 nm were parallel. Thus, these green cells most likely contain only a single visual pigment, absorbing maximally at about 532 nm.

UV Cells

Less common were UV cells, and of 50 such found, only 4 were stable enough to be scanned (for technical reasons, the results on one such scanned cell had to be discarded). UV cells were characterized by very low sensitivities at wavelengths longer than 460 nm, such that the intensities available from the

monochromator were insufficient to elicit normal, depolarizing responses. Rather, hyperpolarizing responses, a few tenths of a millivolt in amplitude, were recorded to maximum intensities available at wavelengths longer than 460 nm. The response to a 620-nm flash illustrated at the lower right in Fig 1 was the *largest* such hyperpolarizing response recorded. Hyperpolarizing responses of even a few tenths of a millivolt were recorded only if maximum intensities from the monochromator at visible wavelengths were used, and these were always many log units more intense than those needed to stimulate at UV wavelengths. Thus, the hyperpolarizing responses most likely represent pickup of extracellular currents generated by other, maximally stimulated green cells in the same eye. A similar basis for hyperpolarizing initial responses of cell in the median ocellus of *Limulus* has been proposed by Nolte and Brown (1972).

The average spectral sensitivity of four scans of three UV cells is illustrated ($\pm$ SD) at the left in Fig. 2. The curve is too narrow to be fitted by a nomogram curve. As shown in Table I, the resting potentials of UV cells are considerably lower than those of green cells. The two populations of resting potentials appear to be significantly different ($P < 0.01$). However, longer-lived UV cells had resting potentials closer to those of green cells. If the UV cells are smaller than the green cells, then possibly they are more easily damaged by the electrodes, and this could result in lower resting potentials. No correlation was found, however, between resting potentials and electrode tip resistances, which presumably are indicators of electrode tip sizes.

UV-Green Cells

A third type of cell, with peaks of sensitivity at both UV and green wavelengths, was found occasionally. The spectral sensitivity of one such cell is shown at the lower right in Fig. 2. This cell was about 0.9 log units more sensitive in the UV than in the green, but the wavelengths of peak sensitivity in the UV and the green were at about the same wavelengths as for UV and green cells, respectively. Different results were obtianed for a second cell whose sensitivity was also tested at 19 wavelength. In this cell, the UV sensitivity was 0.25 log units less than in the green, although still more than the UV sensitivity in green cells. All but one other cell survived only long enough for their spectral sensitivities to be measured at only 370 and 520 nm. The log ratios of these sensitivities ranged from -0.1 (more sensitive in the green) to more than 3 and averaged about 1.3 log units more sensitive in the UV. They thus resemble the extremely variable UV-green cells (which are the only ones found) in principal eyes of wolf spiders (DeVoe, 1972 *a*) and in median ocelli of dragonflies (Chappell and DeVoe, 1975).

Of the 17 UV-green cells found, photographs were taken of the responses of 11 of these cells, and waveforms of responses shown at the lower left in

Fig. 1 are typical of 8 of these 11 cells: The responses to UV light are slower than those to visible lights (in two of the remaining three cells, the responses were equally fast, while in the one cell it was not possible to tell). In Fig. 1, the response to UV light has a longer latency, a longer time to peak, and a broader peak than does the response to 560 nm. In some cells, the photographed responses to UV light were much larger than the responses to visible wavelengths but still had longer times to peak. This indicates that the differences in speeds of response are not simply related to intensities of stimulation. Notably, the only other cells with wavelength-dependent waveform of response are also UV-green cells, those of some cells in the median ocellus of the dragonfly *Libellula* (Chappell and DeVoe, 1975). In *Libellula*, however, it is the response in the visible that is the slower.

UV-green cells had the lowest recorded resting potentials of all groups of cells (see Table I). Possibly, injury by the electrode is an explanation for these low recorded resting potentials, as suggested earlier for the UV cells. Consistent with this hypothesis is that the longer-lived cells that were fully (or partially) scanned had resting potentials of 24, 33, and 43 mV initially and survived 8, 10, and 22 min, respectively. These initial resting potentials slowly declined with time. The resting potentials of all UV-green cells taken together were significantly below those of the green cells ($P < 0.01$) but not those of UV cells ($P > 0.1$).

Localization of Cells

Land (1969 *a*) proposed that the three most proximal rhabdome layers in principal eyes of jumping spiders might each contain a different photopigment, as discussed earlier. The present results, however, do not confirm the existence of cells with peak sensitivities in three different parts of the spectrum. On the other hand, the first UV cells found did appear to lie more distal in the retina (DeVoe, 1972 *b*), suggesting that it was the UV and green cells which were segregated into layers. With a larger population of cells now at hand, this is not substantiated. During each experiment, the points of entry of the vertically oriented electrode into the dorsal or ventral surface of the eye were visually sketched and the depths from the surface where the electrodes penetrated cells were measured. At the end of this work, the entry points of electrodes in all experiments were drawn onto an outline of the eyes, as seen from above, along with the types of cells found in each electrode track. Similarly, the depths from the surface at which each type of cell was penetrated were drawn to scale onto an outline of a transverse section of a principal eye of *Phiddippus regius*. From such drawings, it can be said that no one cell type is preferentially penetrated in any one part of the retina. Histological methods for clearly identifying electrode tracks are lacking, however. Thus, these attempts to localize the points of cell penetration cannot be con-

sidered very precise. It is also possible that penetrations of the different cell types were made at different points along the lengths of the cells. Each receptor cell in the principal eyes has a rhabdome-bearing distal end, a thin intermediate segment, a cell body, and an axon that runs to the optic glomeruli (Land, 1969 *a*; Eakin and Brandenburger, 1971). Possibly the green cells were penetrated predominantly in their large cell bodies, while the UV and UV-green cells were penetrated in their thin intermediate segments, away from their rhabdome endings in one or another layer. Thus, the lack of localization cannot yet be taken to mean that cell types are not stratified, as in Land's (1969 *a*) proposal. Two tries at staining with Procion yellow failed, but assignment of cell types to receptor layers will clearly require just such successful staining methods for cell identification.

In the later stages of this work, a specific search was made for UV and UV-green cells. When found, they alone were scanned to determine spectral sensitivities. While searching, the depth, resting potential, and the sensitivities at 370 and 520 nm were recorded for all cells, giving the large populations needed for the above finding of no localization. When a cell was first penetrated, it was stimulated with 370-nm flashes, then 520-nm flashes, then 370-nm flashes again, with all flashes usually delivered at the same setting of the neutral density wedge. When possible, a number of flash intensities were next used at each wavelength to try to find the intensities needed for a criterion amplitude of response, generally 4 mV. In any event, if the response to the first and second groups of UV flashes were the same but there was no response (or a hyperpolarizing response) to the green flashes, the cell was judged a UV cell (if the responses to the second group of UV flashes were much smaller, the results were discarded). If the responses to the 370- and 520-nm flashes were about the same size, the cell was judged a UV-green cell. This was because the quantum flux from the monochromator was about 0.5 log units less at 370 than 520 nm for any wedge setting, hence the cell must have been at least 0.5 log units more sensitive in the UV. Finally, if the responses to 370-nm flashes were very small compared to the responses at 520 nm, the cell was judged a green cell. When possible, each wavelength was presented at least twice to ensure that small responses were not due to cell deterioration. Possibly, some UV-green cells were missed by this survey procedure. Most UV-green cells were at least 0.5 log units more sensitive in the UV than are the green cells, however, so their identification is relatively certain.

Anterior Lateral Eyes

In five experiments, attempts were made to penetrate cells in secondary eyes, the anterior lateral eyes, as well as in the principal eyes. No cells at all could be held more than 30 s when the electrode was in the eye capsule. The most likely reason is that the cells are quite narrow there (Eakin and Brandenburger, 1971). Likewise, I had no success in attempts to penetrate the cell bodies, which lie in a ring around the eyecup next to the cuticle (Scheuring,

1914; Eakin and Brandenburger, 1971). Possibly, the cell bodies were electrically silent. They were also difficult to approach with a vertical electode, as they lay under a cuticular overhang. An obliquely oriented electrode would have been preferable for penetrating them, but this was not tried.

The spectral sensitivity of one anterior lateral eye was recorded via the ERG, with the electrode in the eyecup. This spectral sensitivity was identical with those of the green cells. Thus there are green cells at least in anterior lateral eyes of jumping spiders. Wolf spiders have *only* green cells in their secondary eyes (DeVoe et al., 1969; DeVoe, 1972 *a*), but it is uncertain if the present results show this for jumping spiders or not. That is, an ERG recorded with a micropipette extracellularly with a principal eye did not always show an UV sensitivity either, although there are UV cells in principal eyes. In the principal eye, this is most likely due to the short space constant of the electrodes used: The electrode tip was too far from UV cells to record their ERG's. However, UV sensitivity of the principal eyes can be recorded in the corneal ERG. Possibly UV sensitivities might be recorded in corneal ERG's of anterior lateral eyes too, but this remains to be tested.

DISCUSSION

The present results show clearly that jumping spiders are at the least dichromatic. They have the physiological capacities for distinguishing colors using the UV and green cells in their principal (anterior median) eyes. All workers do seem to agree that vision which might involve color is handed by the principal eyes (Crane, 1949; Kästner, 1950). The role of the secondary (anterior and posterior lateral) eyes is to turn the animal towards a moving object so that it can be viewed by the principal eyes (Homann, 1928). Once the turning is started, the object does not need to be seen anymore for the correct angle of turn to be made, and it is the darkening of the adjacent visual cells in the secondary eyes which initiate the turning (Land, 1972). Thus color vision is likely to be of minimal or no use to the secondary eyes for such roles. As stated above, the present results show only that green receptors are present in anterior lateral eyes. The receptor complement of the posterior lateral eyes remains unexplored, and the presence of UV cells in any secondary eye has neither been found nor experimentally excluded. However, the failure of hungry jumping spiders in Kästner's (1950) experiments to catch flies when only UV light was present could well be because there are no UV receptors in the secondary eyes, the animals did not see the flies in UV light with the secondary eyes, and therefore they did not turn to view the flies with the principal eyes, which do have UV receptors. Young and Wanlass (1967) did find that jumping spiders in UV light would attack nonfluorescing objects waved in front of them, that is, waved in the narrow fields of view of the principal eyes (Homann, 1928; Land, 1969 *b*). Thus presumably had the

flies in Kästner's (1950) experiments found their way in front of the hungry spiders, they would have been seen and caught.

Color vision in jumping spiders thus most likely is reserved to the principal eyes. Its use in interactions between individuals however is not very large. For mating, the principal eyes must be uncovered, but apparently for shape and not color recognition, and airborn chemoreception takes precedence over color patterns (Crane, 1949). Mating behavior will take place in nearly monochromatic UV light of predominantly 365-nm wavelength (Young and Wanlass, 1967). Such light would stimulate maximally the UV (and UV-green) cells but the green cells much less. This also indicates that color per se has little role in male-female recognition. Color patterns will aid in releasing male-male threat displays in *Corythalia xanthopa*, where, too, the principal eyes must remain uncovered (Crane, 1949). Such threat displays require a black, spider-looking outline with a clypeal patch that does reflect yellow or orange light but does *not* reflect UV light (Crane, 1949). This situation is thus reverse of mating behavior under UV light mentioned above (Young and Wanlass, 1967), for here it would be the green cells which alone responded. In neither case of stimulation of only one set of receptors or the other can color discrimination be said to be involved, any more than it is possible in human scotopic vision when only the rods are operating.

The behavioral responses of Kastner's (1950) experiments were somewhat different. Neither mating nor threat behavior was called for; rather, advantage was taken of the tendency of these plant-dwelling spiders to ascend flower stalks. That is, the animals leapt preferentially on striped walls and proceeded to climb them. With daylight illumination (at a window), they never confused any of 26 different shades of gray with orange or blue stripes on sunny days. They sometimes (but not always) behaved erratically when the sky was, or became overcast. However, similar erratic behavior was then observed with black-and-white striped walls too, which indicates that spectral variations in skylight were not necessarily affecting color discriminations. There was no erratic behavior with blue and gray stripes under incandescent illumination, compared to skylight. This too is consistent with the above conclusion that variations in spectral content of illumination did not alter the animals' abilities to distinguish color (Kästner, 1950).

Before the present study, it appeared from ERG measurements that there might be only UV-green cells in principal eyes of jumping spiders (DeVoe et al., 1969) and that therefore Kästner's (1950) results might be explainable only if his orange and blue stripes reflected UV light as well. This conclusion can now be excluded. Kästner (1950) used colored Ostwald papers lent by Dr. H. Homann, Göttingen, who very kindly has now lent me samples of these papers for measurements of spectral reflectivities. The orange (pa-4)

paper has only minimal UV reflection down to 350 nm, and blue (pa-13) paper reflects maximally at about 430 nm, then drops off in the UV.

Instead, there is now a simpler explanation based on populations of UV and green cells. Each paper, orange or blue, will stimulate the UV and green receptors differently. Therefore no one shade of gray stripes will match the colored stripes for both UV and green cells, and the spider will continue to see the wall as striped with at least one set of receptors as the grays are changed. So too can be explained Kästner's (1950) finding that spiders would confuse interspersed stripes of orange with green (Ostwald pe-22) and yellow (pa-1) papers but not with violet (pc-10), blue (pa-13), or red (pa-7) papers. Low reflectivities of the green, yellow, and orange papers below about 480 nm show that they will stimulate the green receptors alone. The purple and blue papers have about equal reflectivities in the blue and will stimulate both UV and green receptors, while the red paper reflects primarily at wavelengths over 60 nm, to which even the green cells are not very sensitive.

From what has been described above, it remains odd that jumping spiders have evolved so many bright colors, when they seem of little obvious use to the animals. To a normal human observer, they are indeed often brilliant little animals, with irridescent red, green, or blue colors or yellow, brown, or red markings. Possibly the answer is that our color sensibilities are limited. For example, a television camera adapted for the UV (Eisner et al., 1969) could give a somewhat more realistic picture of what the UV cells would be responding to. Combined then with a second, green-sensitive input, such television pictures might provide a far better idea of what jumping spiders see in each ogher.

Layering of the Receptors

As stated earlier, visual observations of electrode tracks and plots of depths of cells indicated no preferential locations of one or another cell within the eye. This is curious, since the principal eyes of other jumping spiders have such a remarkable layering of rhabdome endings (Land, 1969 *a*; Eakin and Brandenburger, 1971). I have seen this layering in horizontal section of *P. regius* also. Land (1969 *a*) has argued that one purpose of layering might be to overcome chromatic aberrations of the lens and aid color vision. As stressed earlier, the present experimental results do not show that the three types of cells found are segregated into any layers at all, but they do not exclude layering either. Only dye stainings of the cell types will be conclusive on this question. For the present, it is instructive to consider in what layers the cells might be if they were indeed segregated. Land (1969 *a*) suggested that blue, green, and red point sources at infinity would be focused on layers 3, 2, and 1, respectively, layer 3 being the most distal and closest to the lens. However, jumping

spiders do not respond to objects much farther away than 25 cm, and then only with their secondary eyes; they run up to within 3–4 cm of prey in order to use their principal eyes (Homann, 1928). Hence the appropriate object distance to use for calculating image distance is not infinity, but 3–4 cm. At such object distances, red light probably would be focused behind the retina, blue-green light (500 nm) would be focused on layer 1 (Land, 1969 *a*), and UV light most likely would be focused on layer 2, although calculations to show this have not been done. It is precisely layers 1 and 2 which overlap nearly completely over the entire cross section of the retina, while layer 3 receptors occupy only a small, dorsal-medial portion (Land, 1969 *a*). Thus if the spider were to counteract chromatic abberrations of the lens by having UV and green cells in two layers, it would be optimal for this dichromatic animal that the layers be completely overlapping and have the same field of view. Alternatively, both layer 1 and 2 might each contain UV and green cell rhabdomes for sharpest vision at two distances. Were this so, then either UV or green wavelengths would be less well focused than the other. There is no reason to believe that this is so. The visual acuity set by the receptors is 12′ (Homann, 1928) or 9′ (Land, 1969 *a*), while the acuity observed behaviorally in white light is 11′, or nearly as good (Dzimirski, 1959).

This leaves open the identities and locations of the UV-green cells. Possibly they are couplings of the known UV and green cells. Alternatively, they may be individual cells with sensitivities in both the UV and green. Distinguishing between these two possiblilities is a vexatious problem even in far better studied UV-green cells (cf. DeVoe, 1972 *a*; Chappell and DeVoe, 1975), much less here. Whatever the UV-green cells may be, one hypothesis is that they are located in layer 4 (nearest the lens). Layer 4 receives no images even for objects as infinity (Land, 1969 *a*) and still less so for objects nearby. Possibly it serves for detection of polarized light (Land, 1969 *a*; Eakin and Brandenburger, 1971). Such a polarized light detector would benefit by cells sensitive in both the UV and visible. The UV-green cells would not necessarily fill this role well, however, since they are about 1.3 log units on the average more sensitive in the UV. Again, as for the UV and green cells, the question of localization of UV-green cells must await intracellular stainings.

I thank Dr. H. Homann for the loan of the Ostwald colored papers, Dr. R. Cone for the measurements of their spectral reflectivities, Dr. C. Harper for collecting animals, and Dr. A. Lall for suggestions on the manuscript.

This research was supported by Research Grant Ey 00008 from the National Eye Institute.

Received for publication 13 January 1975.

REFERENCES

CHAPPELL, R. L., and R. D. DEVOE. 1975. Action spectra and chromatic mechanisms of cells in the median ocelli of dragonflies. *J. Gen. Physiol.* **65**:399.

CRANE, J. 1949. Comparative biology of salticid spiders of Rancho Grande, Venezuela. Part IV. An analysis of display. *Zoologica* (*N. Y.*). **34**:159.

DARTNALL, H. J. A. 1953. The interpretation of spectral sensitivity curves. *Br. Med. Bull.* **9**:24.

DEVOE, R. D. 1972 *a*. Dual sensitivities of cells in wolf spider eyes at ultraviolet and visible wavelengths of light. *J. Gen. Physiol.* **59**:247.

DEVOE, R. D. 1972 *b*. Spectrally-different cells in principal eyes of jumping spiders. IV International Biophysics Congress, Moscow, 1972. EXVI-a2/10.

DEVOE, R. D., R. J. W. SMALL, and J. E. ZVARGULIS. 1969. Spectral sensitivities of wolf spider eyes. *J. Gen. Physiol.* **54**:1.

DEVOE, R. D., and J. E. ZVARGULIS. 1967. Spectral sensitivities of vision in wolf spiders and jumping spiders. *Fed. Proc.* **26**:655.

DZIMIRSKI, I. 1959. Untersuchungen uber Bewegungssehen und Optomotorik bei Springspinnen (Salticidae). *Z. Tierpsychol.* **16**:385.

EAKIN, R. M., and J. L. BRANDENBURGER. 1971. Fine structure of the eyes of jumping spiders. *J. Ultrastruct. Res.* **37**:618.

EISNER, T., R. E. SILBERGLIED, D. ANESHANSLEY, J. E. CARREL, and H. C. HOWLAND. 1969. Ultraviolet video-viewing: the television camera as an insect eye. *Science* (*Wash. D.C.*). **166**: 1172.

HOMANN, H. 1928. Beiträge zur Physiologie der Spinnenaugen, I. Untersuchungsmethoden. II. Das Sehvermogen der Salticiden. *Z. Vgl. Physiol.* **7**:201.

KÄSTNER, A. 1950. Reaktion der Hupfspinnen (Salticiden) auf unbewegte und farbige Gesichtsreize. *Zool. Beitr.* NS. **1**:12.

LAND, M. F. 1969 *a*. Structure of the retinae of the principal eyes of jumping spiders (Salticidae:Dendryphantinae) in relation to visual optics. *J. Exp. Biol.* **51**:443.

LAND, M. F. 1969 *b*. Movements of the retinae of jumping spiders (Salticidae: Dendryphantinae) in response to visual stimuli. *J. Exp. Biol.* **51**:471.

LAND, M. F. 1972. Mechanisms of orientation and patter recognition. *In* Information Processing in the Visual System of Arthropods. R. Wehner, editor. Springer-Verlag, Berlin. 231.

MELAMED, J., and O. TRUJILLO-CENOZ. 1971. Innervation of the retinal muscles in wolf spiders (Araneae-Lycosidae). *J. Ultrastruct. Res.* **35**:359.

MILLOT, J. 1931. Les diverticules intestinaux du cephalothorax chez les Araignees vraies. *Z. Morphol. Oekol. Tiere.* **21**:740.

NOLTE, J., and J. E. BROWN. 1972. Electrophysiological properties of cells in the median ocellus *of Limulus*. *J. Gen. Physiol.* **59**:167.

PECKHAM, G. W., and E. G. PECKHAM. 1894. The sense of sight in spiders with some observations on the color sense. *Trans. Wis. Acad. Sci. Arts Lett.* **10**:231.

RATHMEYER, W. 1965. Neuromuscular transmission in a spider and the effect of calcium. *Comp. Biochem. Physiol.* **14**:673.

SCHEURING, L. 1914. Die Augen der Arachnoideen. II. *Zool. Jahrb.* **37**:369.

YOUNG, M. R., and F. WANLASS. 1967. Observations on fluorescence and function of spiders' eyes. *J. Zool. Proc. Zool. Soc. Lond.* **151**:1.