SPECTRAL SENSITIVITIES OF RETINULAR CELLS MEASURED IN INTACT, LIVING BUMBLEBEES BY AN OPTICAL METHOD

BERNARD, GD; STAVENGA, DG

Published in:
Naturwissenschaften

DOI:
10.1007/BF00405378

Publication date:
1978

Spectral Sensitivities of Retinular Cells Measured in Intact, Living Bumblebees by an Optical Method

G.D. Bernard and D.G. Stavenga

Department of Ophthalmology and Visual Science, Yale University, New Haven, Connecticut 06510 USA, the Department of Biophysics, Rijksuniversiteit Groningen, The Netherlands, and the Marine Biological Laboratory, Woods Hole, Massachusetts, USA

The compound eyes of many diurnal insects exhibit a pupillary response that is a consequence of light-induced migrations of pigment granules within photoreceptor cells. Bright illumination of a retinular cell causes its intracellular granules to congregate at the boundary of its rhabdomere, decreasing the fraction of light within the rhabdomere and increasing the fraction of light scattered from the granules. This mechanism for intracellular control of light was first demonstrated in houseflies [1]. An action spectrum of this kind of pupillary response was first studied in the w^a mutant of the fruitfly *Drosophila* by measuring the antidromic transmission of the rhabdomeres [2]. With a related approach, spectral sensitivities of single spectral types of butterfly retinular cells have been obtained by using decreases in eyeshine as a measure of the pupillary response [3, 4].

Since the methods based on antidromic transmission or on eyeshine are successful in only a small fraction of species that exhibit pupillary responses, it seemed promising to utilize the widely encountered pupillary scattering [5–8] as a tool for measuring the spectral sensitivities of photoreceptor cells in intact animals. Dipteran flies were chosen as first subjects because they have been well studied with other techniques. The results, which compare favorably to published electrophysiological and photochemical work, are summarized elsewhere [8, 9].

The next subject chosen for study was the bumblebee, because: (a) pigment migration in hymenopteran retinular cells is well documented [5, 6, 10–12]; (b) it is easy to observe pupillary scattering in this insect [6]; and, (c) characteristics of its photoreceptor cells are scarcely studied [13, 14].

The technique is to wax an intact bumblebee to the universal stage of an incident-light microspectrophotometer and chronically illuminate a selected, localized region of the eye with an adapting beam of spectral content and intensity chosen so that pupillary granules of the desired cell-type are gathered next to the rhabdom while pupillary granules of other types are dispersed. For example, the adapting beam suitable for measuring sensitivity of the 'green-receptor' is from a 45-W tungsten illuminator covered by a heat filter (3 mm Schott KG-3), a red cutoff filter (Schott RG645), and a neutral filter (density 0.3). Monochromatic flashes (6 s in duration and 5 nm in bandwidth) from a second, superimposed beam are periodically delivered to the eye and thus evoke pupillary responses (increased backscattering of light from the deep pseudopupil, Fig. 15 of [5]), which is detected by a photomultiplier equipped with a barrier filter that is transparent to the adapting beam but opaque to the monochromatic flashes. A criterion increase in pupillary reflectance of 4% is achieved by adjusting the quantum-flux of the flash. If the duration of the flash is short enough the pupillary response is dominated by only one spectral type of retinular cell.

Results from studies of four individuals of three species are presented in Figure 1. Normalized curves of log-sensitivity on a per-quantum basis are shown. The symbols with which the curves are plotted are

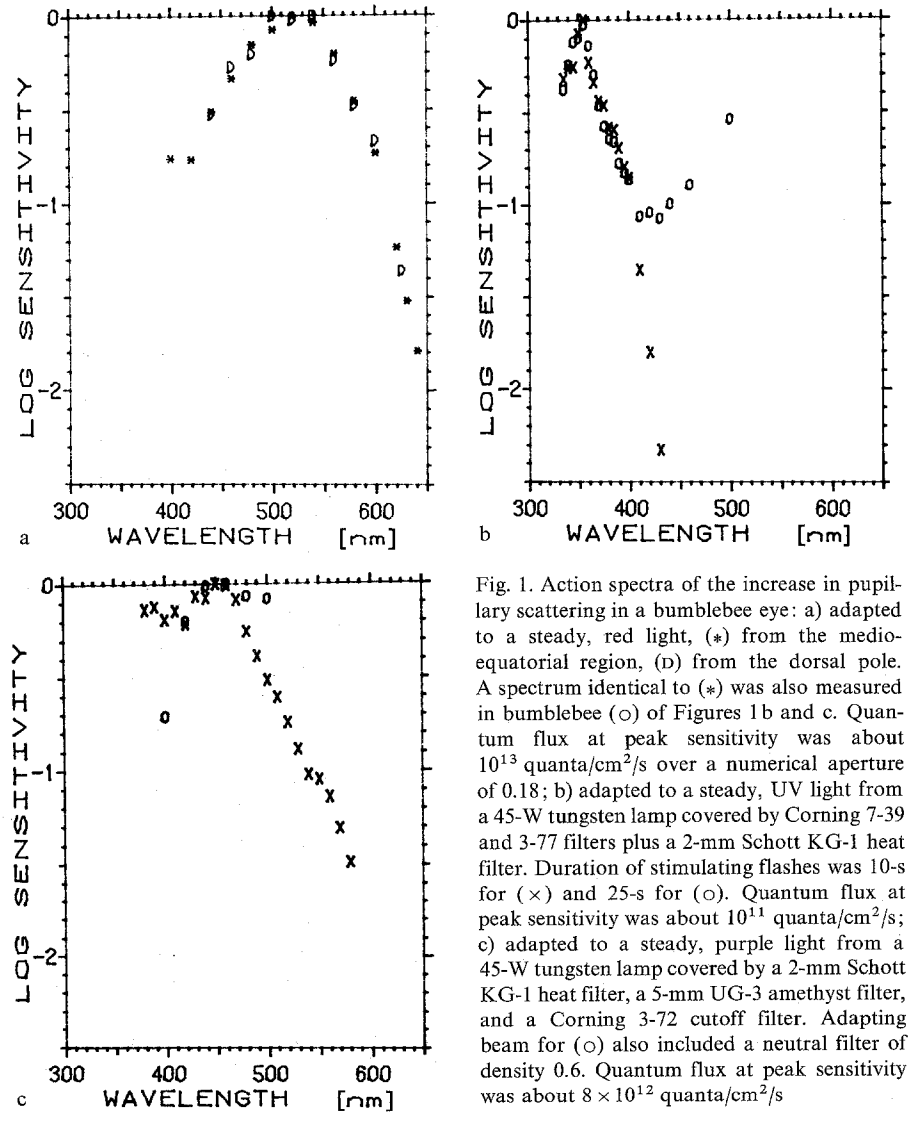

Fig. 1. Action spectra of the increase in pupillary scattering in a bumblebee eye: a) adapted to a steady, red light, (*) from the medio-equatorial region, (D) from the dorsal pole. A spectrum identical to (*) was also measured in bumblebee (○) of Figures 1b and c. Quantum flux at peak sensitivity was about 10^{13} quanta/cm^2/s over a numerical aperture of 0.18; b) adapted to a steady, UV light from a 45-W tungsten lamp covered by Corning 7-39 and 3-77 filters plus a 2-mm Schott KG-1 heat filter. Duration of stimulating flashes was 10-s for (×) and 25-s for (○). Quantum flux at peak sensitivity was about 10^{11} quanta/cm^2/s; c) adapted to a steady, purple light from a 45-W tungsten lamp covered by a 2-mm Schott KG-1 heat filter, a 5-mm UG-3 amethyst filter, and a Corning 3-72 cutoff filter. Adapting beam for (○) also included a neutral filter of density 0.6. Quantum flux at peak sensitivity was about 8×10^{12} quanta/cm^2/s

keyed to individual bumblebees. The symbol * is for a worker and D for a drone of *Bombus affinis* Cresson; × is for a worker *Pyrobombus impatiens* (Cresson), and ○ is for a worker *Megabombus fervidus fervidus* (Fabr.).

When a bumblebee eye is adapted to red light the action spectrum of the pupillary response peaks at about 520 nm and has a shape that is typical of an invertebrate 'green-receptor' [15]. This is true for all four individuals. If the filters covering the adapting beam are changed to create a predominantly UV beam (Corning 7-39 plus 3-77) the action spectrum changes dramatically to that of a 'UV-receptor' if the stimulating flashes are short enough (Fig. 1a, b). However, if the stimulating flashes are too long, more than one spectral type of cell can contribute to the pupillary response (see Fig. 1b). For wavelengths greater than 410 nm the action spectrum of 25-s flashes is strikingly elevated compared to the spectrum for 10-s flashes, because the UV cells are insensitive in that spectral region and because the pupils of the blue and green cells have time to respond to 25-s flashes but not to 10-s flashes.

The action spectra of Figure 1c are not those of a well isolated 'blue-receptor'. However, these curves do demonstrate the presence of a blue-receptor that has peak sensitivity at about 450 nm, and cannot be explained by superposition of pupillary responses from only UV- and green-receptors. Vishnevskaya and Mazokhin-Porshnyakov [14] measured electrophysiological responses of blue-receptors and green-receptors to equi-energy flashes of monochromatic light. Unfortunately, they published neither spectral-sensitivity curves nor an intensity series, so it is not possible to compare their efficiency curves to our sensitivity curves. However, there is agreement that bumblebee eyes contain three spectral types of photoreceptor cells [13, 14].

This study demonstrates the utility of exploiting pupillary scattering to investigate invertebrate retinular cells. Non-invasive, optical techniques have the following advantages over classical electrophysiological techniques: (1) the experimental animal is completely intact: (2) responses are stable and reproducible over very long periods of time; (3) single cell-types can be isolated; and (4) both physiological and photochemical measurements can be performed on identical photoreceptor cells [7, 8, 16].

We thank Prof. R. Wehner for suggestions and comments. This work was supported by research grant EYO1140 and Vision Center Grant EY00785 from the National Eye Institute, US Public Health Service, by the Connecticut Lions Eye Research Foundation, and by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

Received April 25, 1978

1. Kirschfeld, K., Franceschini, N.: Kybernetik *6*, 13 (1969)
2. Franceschini, N.: Thesis, Grenoble 1972
3. Bernard, G.D.: J. Opt. Soc. Am. *66*, 1120 (1976)
4. Bernard, G.D.: ibid. *67*, 1362 (1977)
5. Franceschini, N., in: Photoreceptor Optics, p. 98 (eds. A.W. Snyder, R. Menzel). Berlin: Springer 1975
6. Stavenga, D.G., Kuiper, J.W.: J. comp. Physiol. *113*, 55 (1977)
7. Stavenga, D.G., et al.: ibid. *113*, 73 (1977)
8. Stavenga, D.G., in: Handbook of Sensory Physiology, Vol. VII/6A (ed. H. Autrum). Berlin-Heidelberg-New York: Springer 1978
9. Bernard, G.D., Stavenga, D.G.: Biol. Bull. *153*, 415 (1977)
10. Menzel, R., in: Information Processing in the Visual Systems of Arthropods, p. 37 (ed. R. Wehner). Berlin: Springer 1972
11. Brunnert, A., Wehner, R.: J. Morphol. *140*, 15 (1973)
12. Kolb, G., Autrum, H.: J. comp. Physiol. *77*, 113 (1972)
13. Mazokhin-Porshnyakov, G.A.: Insect Vision, p. 182. New York: Plenum 1969
14. Vishnevskaya, T.M., Mazokhin-Porshnyakov, G.A.: Biofizika *17*, 635 (1972)
15. Menzel, R., in: The Compound Eye and Vision of Insects, p. 121 (ed. G.A. Horridge). Oxford: Clarendon 1975
16. Bernard, G.D.: in preparation

Suppression of Leukemogenesis in Hairless Mice

by Anti-Type C Viral Immune Gamma Globulins

R. Pottathil and H. Meier

The Jackson Laboratory, Bar Harbor, Maine 04609, USA

R.J. Huebner

National Cancer Institute, Bethesda, Maryland 20014, USA

Suppression of exogenous and endogenous murine leukemia virus(MuLV)-mediated tumors in inbred strains has been successful through immunotherapy with type-specific viral vaccines and immune gamma globulins (IgGs) [1–3]. Although a direct etiological link between virus expression and certain types of tumor must still be proven, available evidence indicates that high titers of endogenous ecotropic MuLV correlate well with the occurrence of leukemia in a number of inbred and hybrid strains of mice [4]. A pertinent example is the hairless mouse, HRS/J $-hr/hr$ which is characterized by a high incidence of leukemia compared with its haired littermates, HRS/J $-hr/+$ and HRS/J $-+/+$, respectively. The incidence of leukemia in hairless mice is 45% at 8–10 months of age compared to 1% in haired mice (HRS/J $-hr/+$) [5]. Hairless mice have vastly elevated levels of ecotropic [6] and, by eight months of age, also xenotropic [7] MuLV compared with haired littermates; in addition, polytropic or mink cell focus (MCF)-forming recombinants between these two virus classes occur in most, if not all, leukemic and preleukemic hairless mice [7].

We have found in HRS/J mice that suppression of ecotropic MuLV by passive neonatal immunization significantly decreases the development of leukemia. Offspring from crosses between HRS/J $hr/+ \times hr/hr$ were treated neonatally with a total of 0.45 ml anti-RadLV goat IgG (Pool #3 NIH C5682). The IgG preparation had a neutralizing antibody titer of 1:800 to 1:1600 based on 70–100% inhibition of 60–70 AKR-XC plaques on SC-1 cells. Each mouse received 0.05 ml on days 0, 4, and 7, 0.1 ml on day 10 and 0.2 ml on day 14, as a subcutaneous injection. Control mice received no treatment. We separated the offspring, at weaning, into haired ($hr/+$) and hairless (hr/hr) phenotypes.

At specified ages (Table 1) 1 cm terminal segments of tails were cut and assayed for MuLV as previously described [2]. All mice were set aside for long-term observation of tumor occurrence, and killed only when moribund. At necropsy, thymus, spleen, and lymph nodes as well as other visceral