Eur Biophys J (1985) 12: 173–179

European Biophysics Journal

Fly photoreceptors and temperature: Relative UV-sensitivity is increased by cooling

M. Weckström[1]*, M. Järvilehto[2], E. Kouvalainen[1], and P. Järvilehto[1]

[1] University of Oulu, Department of Physiology, and
[2] University of Oulu, Department of Zoology, Oulu, Finland

Received February 4, 1985/Accepted April 3, 1985

Abstract. Intracellular responses from blowfly photoreceptor cells were recorded at various temperatures in order to study the behaviour of the transduction system, with particular reference to spectral sensitivity. With decreased temperature the $V-\log I$ functions showed a reduction in amplitude and the responses showed a slowed time course. For double peaked spectral sensitivity function the UV or 350 nm peak was much less dependent on temperature than the peak in the visible region. The higher UV-sensitivity is interpreted in terms of the sensitizing pigment theory to indicate changes in the effectiveness of energy transfer between the two chromophores.

Key words: Electrophysiology, intracellular, eye, visual, photopigments

Introduction

At least five different kinds of spectral sensitivity functions have been noted from intracellular recordings from photoreceptors of the compound eye of the blowfly. Among them, the retinular cells R1 to R6 have usually been shown to have a double peaked spectral sensitivity function (e.g. Burkhardt 1962) in which the UV-sensitivity is commonly slightly larger than the sensitivity maximum in the visible region. Also single peaked (UV or Green) receptor cells have been found, and they have been identified in some experiments as the central ommatidial cells 7 and 8 (Hardie 1979; Hardie et al. 1979).

Microspectrophotometry of R1 to R6 cells also revealed double peaked spectral absorption, in which peak in the visible (VIS) region is termed *alpha* and the UV-peak *beta* (Langer and Thorell 1966). The discrepancy between the electrophysiologically determined spectral sensitivity and the spectrophotometric absorption measurements is obvious if one compares the *alpha* peak with the VIS peak and *beta* peak with the UV peak. In electrophysiological experiments the sensitivity functions show much greater responses in the UV region than can be derived from the pigment absorption properties.

Two basically different mechanisms for this "UV-enhancement" have been proposed. First, there are known to be factors, e.g. photopigment self-screening properties, special screening pigments and wave-guide effects, which could in principle modify the spectral composition of the light before it is captured by the visual pigment (for review, see Menzel 1973). These factors could cause a shift in the spectrum to shorter wavelength and make the UV peak somewhat more distinguishable. They have not, however, been thought to provide a satisfactory explanation for the discrepancy. The second explanatory mechanism, the theory of a so-called antenna, or sensitizing, pigment has been put forward by Kirschfeld et al. (1977). It explains the UV-enhancement by means of a photostable pigment that, after absorbing a UV quantum, transfers it to the photopigment and the usual transduction process follows. The sensitizing pigment is not easily detectable in conventional microspectrophotometry by virtue of its photostability and it is not thought to be membrane active by itself, but only via the photopigment.

These problems can be approached by electrophysiological determination of the activation properties for the transduction system of the photoreceptor cell at different temperatures. If there really exist two different mechanisms of quantum absorption, then they may be distinguished by their different thermal sensitivities or activation properties, i.e. by changing the thermal conditions under which the visual transduction process takes place.

---

* To whom offprint requests should be addressed

Previously it has been suggested, on the basis of psychophysical measurements made on humans, that visual sensitivity to long wavelength light should decrease when the energy content of the photoreceptor surroundings is lowered by cooling (Stiles 1948). This has also been experimentally confirmed e.g. in Limulus lateral eye (Srebro 1966) and in vertebrates (Lamb 1984, review: Ali 1975). These studies have been performed with a single-pigment system, but in the dipteran eye we are probably experimenting with a double-pigment transduction system, where one pigment is a sensitizer to the other. We shall show that the double peaked spectral sensitivity curves change significantly as a function of temperature, the change being a relative enhancement of the UV-sensitivity. The meaning of this is further discussed in terms of the sensitizing pigment theory.

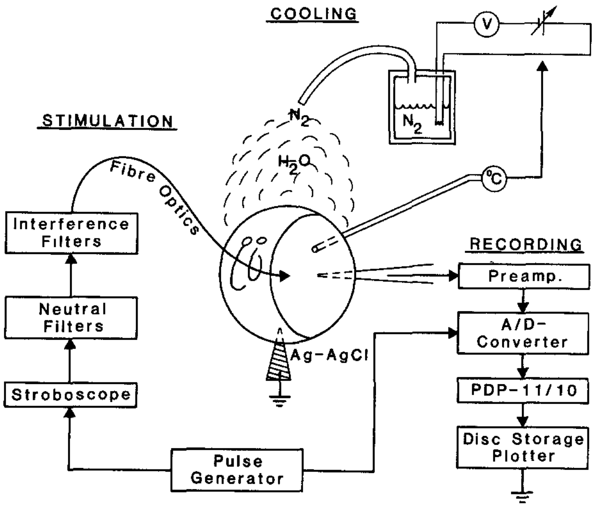

Fig. 1. The experimental arrangement. The head of the fly is fixed into a silver wire serving as an indifferent electrode

Methods

Laboratory raised specimens of blowflies (*Calliphora erythrocephala*), at least 5 day old males and females, were used for the experiments. The larvae were fed on beef with normal vitamin A content, whilst the adult flies were fed on sucrose, beer yeast and water. Capillary microelectrodes, filled with 3 *M* KCl-solution and having resistances of 30 to 70 MΩ, were inserted into the retinae, under the visual control of the dissecting microscope, via a hole made at the edge of the head just outside the eye. The electrodes were dipped in anti-adhesive coating agent before insertion (Järvilehto et al. 1985, in press). Other features of the preparation and recording procedure are described in detail elsewhere (Järvilehto 1978). In the case of cell identification experiments the electrodes were filled with a solution of 4% Lucifer Yellow CH (Sigma Co.).

A schematic view of the whole experimental arrangement is shown in Fig. 1. A PZ-550 Inchworm Controller (Burleigh) was used to control and monitor the movement of the microelectrode. The flies were kept in darkness for at least 5 min before making the recordings in order to complete dark adaptation. Intracellular responses were amplified and subsequently digitized with a 10-bit A/D-converter and analyzed with a PDP-11/10 computer. The stimulating stroboscope, oscilloscope and the A/D-converter were all synchronized using a separate pulse-generator. The light stimulus consisted of a single point-like source subtending an angle of less than 1°. The intensity of the stimulus could be controlled by using neutral density filters. The spectral efficiency was measured with the help of narrow band interference filters (Schott) for which the quantal output was equalized to approximately 2.2×10^8 per flash per cm^2 (calibrated with an 80X Opto-Meter, UDT). The duration of the flash was 30 µs.

The temperature of the preparation was controlled by the smooth flow of vaporized liquid nitrogen, cooling the water vapour in the air, allowed to settle over the head of the fly. The vaporization was produced with a controllable heat element. The temperature of the head of the fly was obtained with a miniature thermocouple inserted into the eye. Temperature measurements were made with a BTU-Multimeter (Passkovsky Messelektronik GmbH, FRG).

Results

It might be possible that the liquid nitrogen, which was used for cooling, could induce artifacts in the functions of the receptor sensitivity. To check this, we studied the effect of nitrogen gas, at room temperature, on the intracellular responses using a gas flow comparable to that used in the cooling experiments. We could measure no effects of this procedure in stable responses during 5 min periods. Only when huge amounts of nitrogen were used did the responses began to diminish, presumably because of the hypoxia produced, and a closed nitrogen atmosphere was found to be lethal for the flies after a few minutes.

When rigorously screened for stability and reversibility of the responses, the main results presented here are based on a sample of 11 cells, from which one or several series of measurements were obtained. Only recordings in which full recovery could be observed after cooling were taken into account. For practical reasons, we rejected cells which did not produce measurable amplitude in all wavelengths at low temperature, even if they showed a considerable response at room temperature. In some cells we were able to measure only the amplitude vs. intensity responses and not the spectral efficiencies at different temperatures before the response deteriorated. Figure 2 presents a sample of original recordings at temperatures of 21 °C and 9 °C with five different stimulation intensities.

Cell identification with Lucifer Yellow was performed in three experiments. The cells were identified as R3, R4 and R6, and they were recorded in the soma. In this series of experiments we have collected cells with double peaked spectral sensitivity paying no special attention to the exact receptor cell type. They are, however, likely to all come from R1 to R6.

The receptor potential is a function of several stimulus parameters (in this paper: intensity, wavelength) and the temperature. We present the results in two parts, in which first the intensity and second the wavelength dependence as a function of temperature is considered.

Changes in intensity dependent responses

In all measurements typical changes were seen in the intensity dependence of the receptor cell response. This is shown as an example in Fig. 3a for recordings from one cell at temperatures of 21°, 15° and 9 °C. Response amplitude was systematically reduced when the temperature was lowered. In the case shown in Fig. 3a the reduction from an initial 51 mV was about 10 mV at 15 °C and about 25 mV at 9 °C with the maximum stimulus intensity. The reduction was more prominent in subsaturation stimulus intensities, and this is probably because of the lack of saturation of the curves at lowêr temperatures. The calculated temperature dependence of the reduction in amplitude has a Q_{10} of about 1.9 at the highest intensities used, and of about 2.4 one log unit below this.

Changes were also found in the latencies of the potential responses; latency was found to increase considerably when the eye was cooled. This is shown in Fig. 3b. The latency is defined and measured here as the time from the flash onset to the point of intersection of the baseline and the tangent to the maximum of the first derivative of the response. The latency obtained with this method is likely to be somewhat longer than that determined by the measure of some arbitrary critical amplitude. In these recordings the increase in the latency as a function of temperature was about 2.5 ms at 15 °C and about 8 ms at 9 °C from the 2 ms value at room temperature and maximal stimulus intensity. The Q_{10} for the increase of latency was about 3.1.

The slowing of the time course of the responses is also reflected by the change in the maximum velocity of rise in the potential response (the maximum of the first derivative), which becomes consistently smaller, when the temperature is lowered. This is shown in Fig. 3c. The intensity-response functions were used for the calculation of the corresponding spectral sensitivity functions of each cell.

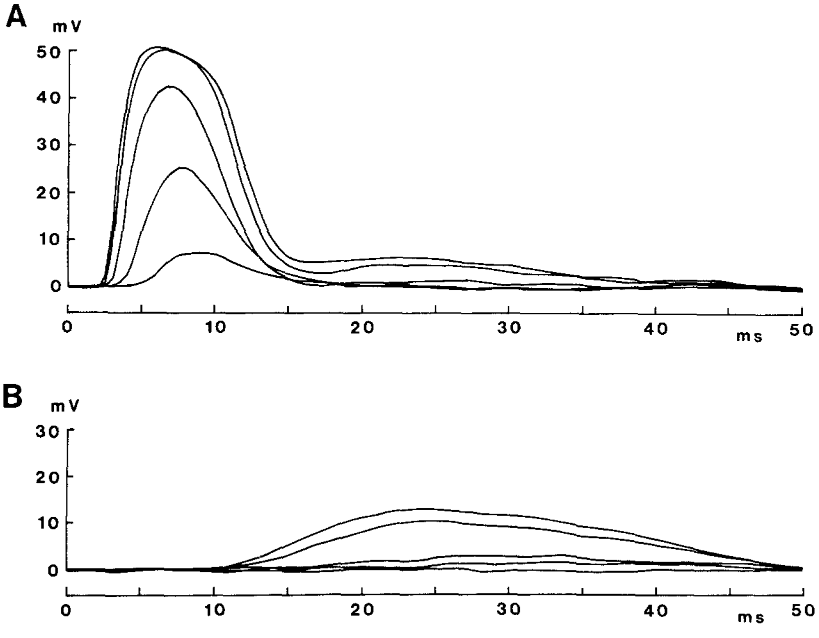

Fig. 2A and B. A set of original intracellular recordings from a photoreceptor cell at two different temperatures, (**A**) 21 °C, (**B**) 9 °C. Each set has been recorded with five different relative intensities of white light pulse (30 µs), 100%, 49%, 14%, 1.5% and 0.09%. The light pulse was applied at the zero time shown in the figures

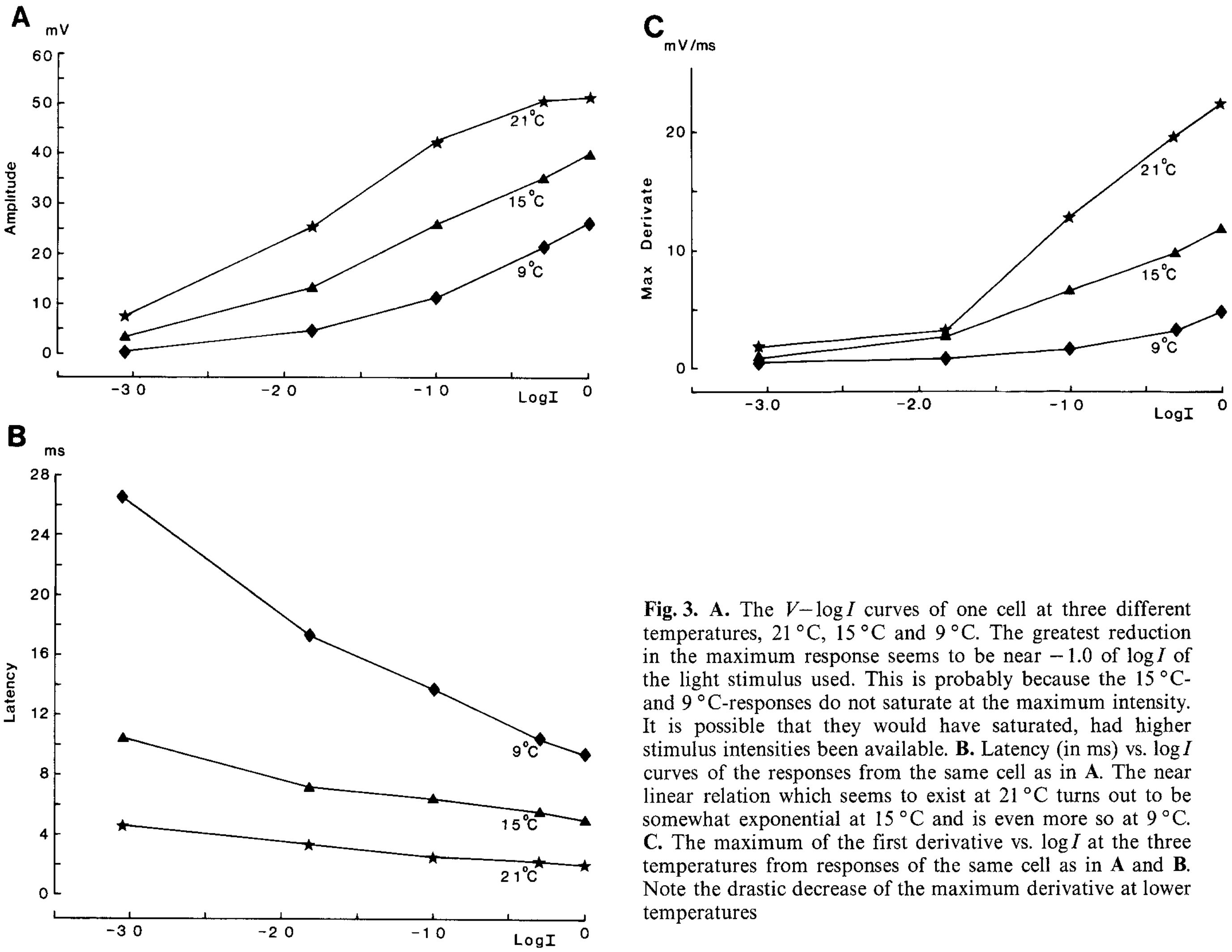

Fig. 3. A. The V–log$I$ curves of one cell at three different temperatures, 21 °C, 15 °C and 9 °C. The greatest reduction in the maximum response seems to be near −1.0 of log$I$ of the light stimulus used. This is probably because the 15 °C- and 9 °C-responses do not saturate at the maximum intensity. It is possible that they would have saturated, had higher stimulus intensities been available. **B.** Latency (in ms) vs. log$I$ curves of the responses from the same cell as in **A**. The near linear relation which seems to exist at 21 °C turns out to be somewhat exponential at 15 °C and is even more so at 9 °C. **C.** The maximum of the first derivative vs. log$I$ at the three temperatures from responses of the same cell as in **A** and **B**. Note the drastic decrease of the maximum derivative at lower temperatures

Changes in spectral sensitivity

The most frequently encountered spectral sensitivity was a double peaked type. In our experiments the peaks lay near 350 nm and 490 nm, and the UV peak was always more prominent. A decrease in temperature caused the responses to monochromatic light to become smaller in maximum amplitude as described above. Similarly the latencies became longer. Surprisingly, however, the sensitivity at the UV peak or the responses to the 353 nm light were consistently lowered less than responses to other wavelengths (Fig. 4). The Q_{10}'s, calculated from the response amplitude maxima, for responses stimulated with 353 nm and 486 nm light differed markedly, by Students t-test $p < 0.05$. The thermal dependence of the amplitude of the UV peak was about 1.7 and that of the visible peak was about 2.1. The latencies of the responses to monochromatic flash stimuli show the same tendency, and the latencies were not increased so much near the UV peak as they were for stimulation with other wavelengths.

Discussion

The effect of cooling

The process of visual transduction in photoreceptor cells is thought to be sensitive to changes in ambient temperature in many stages. The normal active temperature of the blowfly is near 20 °C and the visual cells supposedly function optimally in that range. For intensity-dependent responses our results parallel those of previous investigations with different species (Apis: Duruz and Baumann 1968; Limulus: Adolph 1973; Oncopeltus: Dudek 1975). The transfer function analysis of Calliphora photoreceptors (French and Järvilehto 1978) also yielded similar results.

Although the primary conversion of photopigment into metarhodopsin, lasting about 120 µs (Kirschfeld et al. 1978a; Kruizinga et al. 1983), is slowed down by cooling, there are also effects on the membrane processes and perhaps on the mechanism underlying the transfer of information from the photopigment to the membrane. The temperature dependence of the voltage changes across the membrane of some nerve cells has been shown to have a Q_{10} of 1.3 to 1.5 (review: Guttman 1971). In our experiments the Q_{10} of the amplitude of the potential responses was somewhat larger, about 1.9 with the highest available stimulation intensities, which might indicate that other processes are involved. If the Q_{10} is calculated from the responses that are obtained by light one log unit below the maximum intensity, the Q_{10} is larger, about 2.4. The Q_{10} itself is not a clear measure of the temperature dependence and in complex non-linear systems, such as photoreceptors, it may depend, for example, on the stimulus intensities (Järvilehto et al. 1984). For the thermal dependence of the time scale of the responses (as measured by changes in latency) we obtained a Q_{10} of 3.1, whereas Baylor et al. (1982) obtained, for the much slower photoreceptor responses of the frog, a Q_{10} of 2.7 (as measured by the reciprocal of time-to-peak).

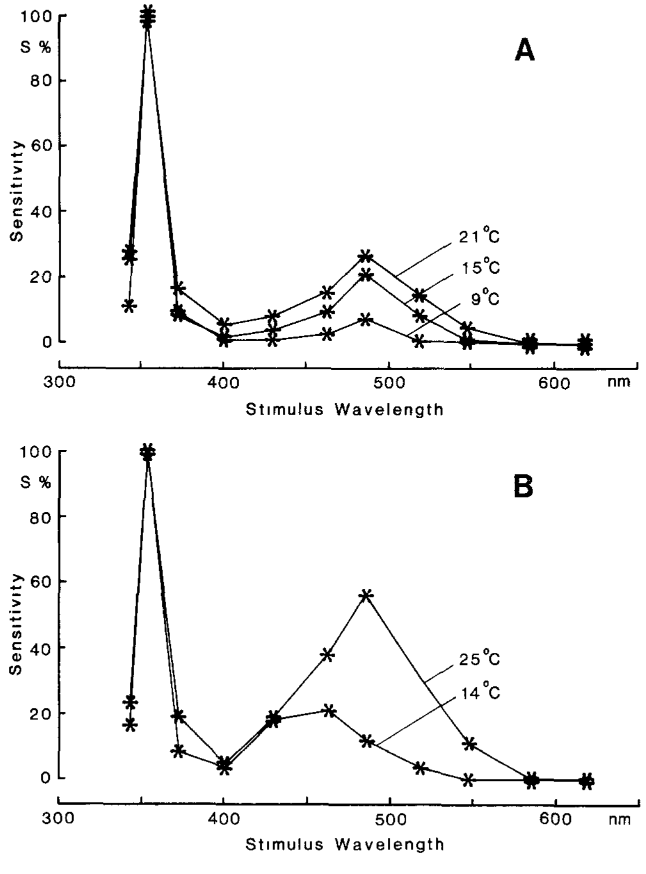

Fig. 4. The spectral sensitivities measured from two representative cells. In the first, spectral sensitivities at three different temperatures are presented, in the second, at two temperatures. Note the clear drop in the sensitivity in the visible or green region of the spectrum with respect to the UV-sensitivity. Sensitivity is calculated from the responses to monochromatic stimuli (the efficiency curve) with the help of the $V-\log I$ function. The maximum intensity received from each function is taken as the maximum sensitivity (100%) value

Spectral sensitivity changes

Two peaked spectral sensitivity functions are the most common in the dipteran retina. As expected, the three identified cell types in our experiments belong to the receptor class with short axons (R1 to R6). In some experiments with a greater number of identified cells this group of receptor cells seems not to be uniform with respect to their spectral sensitivity (Järvilehto and Moring 1976). In our results the two peaks of the sensitivity functions are not of equal height, the UV-sensitivity being consistently higher.

The effect of cooling leaves the UV-sensitivity less affected than the visible sensitivity. In other words, the sensitivity peak at longer wavelengths is clearly more dependent on temperature than the peak in the UV. In vertebrates (e.g. goldfish and frog) it has been shown by cooling experiments that the photopigment molecules can use thermal energy in addition to the energy of the light quanta. This happens when the wavelength of the illuminating light is longer than 510 nm, where the energy of a light quantum is not high enough to initiate the photopigment isomerisation reaction (Srebro 1966; Lamb 1984; review: Ali 1975). Quite unexpectedly, our results do not show any clear shift from thermal independence to thermal dependence, but the thermal dependence seems to be small around the UV peak and higher elsewhere (Fig. 4). Clearly we must find some other explanation.

In the light of our results we shall now discuss the three possibilities that might explain the UV-enhancement, namely *1)* the two pigment theory, *2)* the explanation involving the influence of electrical coupling between receptor cells and *3)* the sensitizing pigment theory.

The two-pigment hypothesis

The two-pigment hypothesis has been favoured by Snyder and Pask (1973), Horridge and Mimura (1975) and Rosner (1975). The great difficulty with the theory including two membrane active pigments is that spectrophotometrically only one isosbestic point has been discovered, at about 410 nm. This problem is solved, if we assume that the UV pigment and its photoproducts have about the same absorption properties and cannot, therefore, be easily detected by spectrophotometric measurements. Selective adaptation experiments, though, do not support the two-pigment theory (Burkhardt 1962; Tsukahara and Horridge 1977).

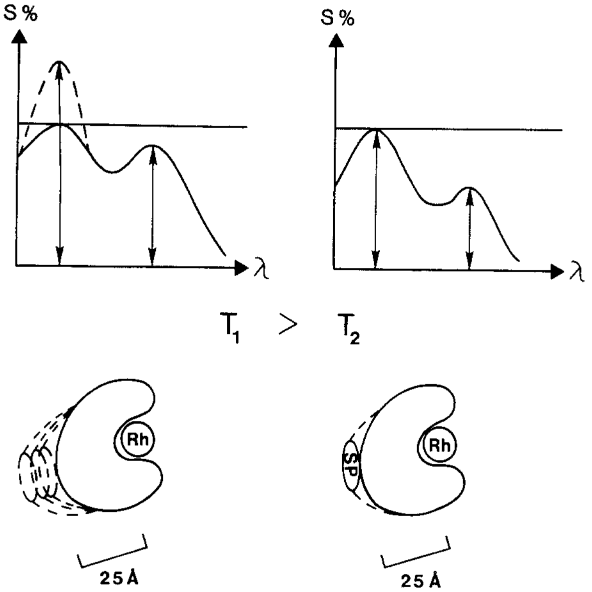

Fig. 5. The explanation of the enhanced UV-sensitivity induced by cooling. The UV-sensitivity is also assumed to be higher at room temperature, but the measured sensitivity is thought to be reduced by some limiting factor, for which the thermal instability of the sensitizing pigment – opsin bond is proposed. Above are shown the measured spectral sensitivities at higher and lower temperature. Below are shown the visual pigments at corresponding temperatures

The effects of electric coupling

The second possible explanation, i.e. that the UV-enhancement at normal room temperature is due to electric coupling, might be compatible with our results. Electric coupling between receptor cells has been proposed in locusts by Shaw (1967, 1969a, b) and in bees by Menzel and Blakers (1976). The effect of cooling on different kinds of photopigments in different cells that are interacting with each other could in principle produce results quite similar to ours. Nevertheless, the dipteran rhabdom is a so-called open rhabdom and the electrical connections – if any – occur in the lamina region between the photoreceptor axon terminals (Shaw and Stowe 1982).

The sensitizing pigment theory

The sensitizing pigment theory proposed by Kirschfeld and his collaborators (Kirschfeld et al. 1977; Kirschfeld et al. 1978b; Vogt and Kirschfeld 1983) explains fairly well the UV-enhancement at room temperature. In essence, it includes a second chromophore that is not permanently attached to the protein part of the photopigment, but is loose in the intracellular space. Then, the inability to detect the second chromophore in conventional subtractive microspectrophotometry becomes understandable: the sensitizing pigment does not change itself, as the rhodopsin photopigment does, but is photostable.

By means of the sensitizing pigment theory the relative UV-enhancement at lower temperatures, as detected in our experiments, could be explained. We must make some additional propositions, though. First, quite recent experiments reveal that the sensitizing pigment probably is an alcohol derivative of a xanthine-molecule, which attaches itself to the photopigment proper with two rather weak H-bonds (Vogt and Kirschfeld 1984; Kirschfeld 1984). The critical distance of 25 Å favouring the energy transfer between the two opsin-bound chromophores could be more often attained and, therefore, the energy transfer would become more effective, when the cells are cooled. This would produce the relative enhancement of UV-sensitivity in electrophysiological measurements.

The relative UV-sensitivity would, according to this model, be gradually increased during the cooling procedure to the level where it actually should be according to the absorption properties of the sensitizing pigment. These ideas are pictured in Fig. 5. Further experiments with higher temperatures than the near optimal 25 °C should then show a gradual decrease in relative UV-sensitivity. The calculations of Vogt and Kirschfeld (1983) yielding a quantum efficiency for the sensitizing pigment at room temperature greater than 75% can be accounted for, because we have discovered a increase in the *relative* UV-sensitivity. The overall sensitivity of the eye is clearly lowered on cooling, as can be seen in Fig. 3A.

Another inference from this model is that the relative UV-enhancement induced by cooling should also be distinguishable in the microspectrophotometric experiments. This is because the sensitizing pigment theory assumes that phototransduction always takes place via the rhodopsin isomerization process. Another possible explanation is to assume two completely or partially separate reaction chains from the pigments to the electrical phenomena in the membrane (Weckström et al. 1984). We have no further evidence for this at the present time.

On the other hand, lower temperatures also reduce the mobility of the solute molecules, including those of the sensitizing pigment and the photopigment. In addition, the fluidity of the lipid membranes, where the photopigment molecules are attached, is reduced. The effect of these changes on the probability of energy transfer between the two chromophores is at present unknown and can only be speculated upon. One possible explanation for the raised relative UV-sensitivity could be the movement of the pigment molecules, which is known to happen in light adaptation (Vogt et al. 1982). The spectral sensitivity function is changed in a somewhat similar manner as in our experiments: the relative sensitivity in the visible region is reduced. It is possible that cooling affects the same mechanisms that are responsible for sensitivity changes during adaptation. The pupil mechanism would, however, shift the whole spectral sensitivity curve in the blue direction, which is not consistent with our results.

Acknowledgements. We would like to thank Mrs. Anneli Rautio for excellent technical help during this work. We are also grateful to Prof. K. Kirschfeld and Prof. S. R. Shaw for constructive and critical discussion.

References

Adolph AR (1973) Thermal sensitivity of lateral inhibition in Limulus eye. J Gen Physiol 62:392–406

Ali MA (1975) Temperature and vision. Rev Can Biol 34: 131–186

Baylor DA, Matthews G, Yau K-W (1982) Temperature effects on the membrane current of retinal rods of the toad. J Physiol (Lond) 337:723–734

Burkhardt D (1962) Spectral sensitivity and other response characteristics of single visual cells in the arthropod eye. Symp Soc Exp Biol 16:86–109

Dudek FE (1975) The visual response from the compound eye of *Oncopeltus fasciatus:* Effects of temperature and sensory adaptation. J Insect Physiol 21:517–528

Duruz C, Baumann F (1968) Influence de la temperature sur le potentiel de repos et le potentiel recepteur d'une cellule photoreceptrice. Helv Physiol Acta 26:CR341–CR342

French AS, Järvilehto M (1978) The dynamic behaviour of photoreceptor cells in the fly in response to random (white noise) stimulation at a range of temperatures. J Physiol (Lond) 274:311–322

Guttman R (1971) The effect of temperature on the function of excitable membranes. In: Adelman WJ Jr (ed) Biophysics and physiology of excitable membranes. Van Nostrand, New York

Hardie RC (1979) Electrophysiological analysis of fly retina I: Comparative properties of R1–6 and R7 and 8. J Comp Physiol 129:19–33

Hardie RC, Franceschini N, McIntyre PD (1979) Electrophysiological analysis of fly retina II: Spectral and polarisation sensitivity in R7 and R8. J Comp Physiol 133: 23–39

Horridge GA, Mimura K (1975) Fly photoreceptors: I. Physical separation of two visual pigments in Calliphora retinula cells 1–6. Proc R Soc Lond [Biol] 190:211–224

Järvilehto M (1978) Light information transmission and processing in the visual system of the fly. Acta Univ Oul D4, Pharmacol & Physiol 10, pp 1–29+123

Järvilehto M, Moring J (1976) Spectral and polarization sensitivity of identified retinal cells of the fly. In: Zettler F, Weiler R (eds) Neural principles in vision. Springer, Berlin Heidelberg New York

Järvilehto M, Weckström M, Kouvalainen E (1984) Spectral sensitivity in insect photoreceptors at a range of temperatures. Acta Physiol Scand Suppl 537:81–86

Järvilehto M, Meinerzhagen I, Shaw SR (1985) Anti-adhesive coating for glass microelectrodes. J Neurosci Methods (in press)

Kirschfeld K (1984) Chemical identity of sensitizing pigments. Lecture presented in XVII Int Congress of Entomology, Hamburg, 20–26 Aug 1984

Kirschfeld K, Franceschini N, Minke B (1977) Evidence for a sensitising pigment in fly photoreceptors. Nature 269: 386–390

Kirschfeld K, Feiler R, Minke B (1978a) The kinetics of formation of metarhodopsin in intact photoreceptors of the fly. Z Naturforsch [C] 33:1009–1010

Kirschfeld K, Feiler R, Franceschini N (1978b) A photostable pigment within the rhabdomere of fly photoreceptors No 7. J Comp Physiol 125:275–284

Kruizinga B, Kamman RL, Stavenga DC (1983) Laser induced visual pigment conversions in fly photoreceptors measured in vivo. Biophys Struct Mech 9:299–307

Lamb TD (1984) Effects of temperature on toad rod photocurrents. J Physiol (Lond) 346:557–578

Langer H, Thorell B (1966) Microspectrophotometry of single rhabdomeres in the insect eye. Exp Cell Res 41:673–677

Menzel R (1973) Colour receptors in insects. In: Horridge GA (ed) The compound eye and vision in insects. Oxford University Press, Oxford

Menzel R, Blakers M (1976) Colour receptors in the bee eye – morphology and spectral sensitivity. J Comp Physiol 108: 11–33

Rosner G (1975) Adaptation and photoregeneration in the eye of the blowfly. J Comp Physiol 102:269–295

Shaw SR (1967) Simultaneous recording from two cells in the locust retina. Z Vergl Physiol 55:183–194

Shaw SR (1969a) Interreceptor coupling in ommatidia of drone honeybee and locust compound eyes. Vision Res 9: 999–1029

Shaw SR (1969b) Sense-cell structure and interspecies comparisons of polarized-light absorption in arthropod compound eyes. Vison Res 9:1031–1040

Shaw SR, Stowe S (1982) Freeze-fracture evidence for gap junctions connecting the axon terminals of dipteran photoreceptors. J Cell Sci 53:115–141

Snyder AW, Pask C (1973) Spectral sensitivity of dipteran retinula cells. J Comp Physiol 84:59–76

Srebro R (1966) A thermal component of excitation in the lateral eye of Limulus. J Physiol (Lond) 187:417–425

Stiles WS (1948) The physical interpretation of the spectral sensitivity curve of the eye. In: Transactions of the optical convention of the worshipful company of spectacle makers. Spectacle Makers Company, London, pp 97–107

Tsukahara Y, Horridge GA (1977) Visual pigment spectra from sensitivity measurements after chromatic adaptation of single dronefly retinula cells. J Comp Physiol 114: 233–251

Vogt K, Kirschfeld K (1983) Sensitizing pigment in the fly. Biophys Struct Mech 9:319–328

Vogt K, Kirschfeld K (1984) Chemical identity of the chromophores of fly visual pigment. Naturwissenschaften 71:211

Vogt K, Kirschfeld K, Stavenga DG (1982) Spectral effects of the pupil in fly photoreceptors. J Comp Physiol 146: 145–152

Weckström M, Järvilehto M, Kouvalainen E, Järvilehto P (1984) Visual responses of the photoreceptors in the fly (Calliphora) measured in various temperatures. Paper presented in the XVII Int Congr of Entomol, August 20–26, 1984, Hamburg, FRG