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Spectral sensitivity of *Calliphora erythrocephala* and other insect species studied with Fourier Interferometric Stimulation (FIS)

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Summary. 1. A fast action spectrometric method with high resolving power and high reproducibility has been developed: Fourier Interferometric Stimulation (FIS). The method, experimental set-up and application of the new technique on investigations of the spectral sensitivity of insects are discussed.

2. FIS intra- and extracellular recordings revealed that *Calliphora erythrocephala*'s R1–6 cells show a fine-structured sensitivity in the UV, with 5 peaks in the range 300–400 nm and probably two at shorter wavelengths (Fig. 6). This can be explained by the absorbance spectrum of retinol being in a special conformation. The peak in the visible spectrum is congruent with the absorbance spectrum of rhodopsin, with a maximum at about 490 nm (Figs. 4, 5, 7).

3. *Drosophila melanogaster* (ERG) also has a fine-structured UV sensitivity due to R1–6 cells. A mutant (*rdgB, w*) which lacks R1–6 receptors shows a non-structured UV sensitivity evoked by R7 receptors (Fig. 8).

4. *Ascalaphus macaronius* (ERG) shows a non-structured UV sensitivity in the frontal eye due, as is known, to a UV rhodopsin (Fig. 9).

5. *Cataglyphis bicolor* (ERG) has a dual-peaked sensitivity with a non-structured UV peak caused by two rhodopsins (Fig. 10).

6. *Periplaneta americana* (ERG) also has a dual-peaked sensitivity with a non-structured UV peak probably caused by two rhodopsins (Fig. 11).

7. The butterflies *Pieris brassicae, Aglais urticae* and *Pararge aegeria* (ERG) show more complex sensitivities from the UV to the red region of the spectrum (Fig. 12). These sensitivities (with the exception of the sensitivity of the ventral part of the eye of *Pieris brassicae*) are caused by 3 rhodopsins. The UV peak is non-structured and, like the other peaks, chromatically adaptable (Fig. 13).

Abbreviations: *AC* alternating current; *ADC* analog-to-digital converter; *DAC* digital-to-analog converter; *DC* direct current; *FFT* Fast Fourier Transform; *FIS* Fourier Interferometric Stimulation; *He–Ne laser* helium neon laser; *rdgB, w* receptor degeneration B, white (*Drosophila* mutant); *sev* sevenless (*Drosophila* mutant); *WLP* white-light position

Introduction

There has been discussion for more than twenty years on why the spectral sensitivity of the visual cells of insects can be dual-peaked: one peak in the visible region, one in the UV (see Goldsmith 1972; Wasserman 1973; Menzel 1979). Many hypotheses have been proposed for the additional UV peak in the R1–6 cells of the blowfly *Calliphora erythrocephala*: (1) an additional UV rhodopsin, (2) increased UV sensitivity due to wave guide effects, (3) increased β-peak of the rhodopsin (Snyder and Pask 1973; Horridge and Mimura 1975; Rosner 1975; Paulsen and Schwemmer 1979 and others). However, many considerations and also experimental data oppose these hypotheses. Kirschfeld et al. (1977) proposed a photostable sensitizing pigment which absorbs the energy in the UV and transfers it to the rhodopsin (and to the metarhodopsin (Minke and Kirschfeld 1979)) with a Förster-type resonance transfer (Förster 1951). These authors and others (Stark et al. 1979; Kuo 1980) presented experimental evidence for this theory. In 1980 Gemperlein et al. were able to show from intra- and extracellular recordings that the UV sensitivity of *Calliphora* has a fine structure

with several peaks. The fine-structured sensitivity curve gave the fingerprint of the UV absorbing pigment. A polyene with 5 conjugated bonds was proposed (Gemperlein et al. 1980). More precisely, retinol in a coplanar arrangement of an ionon ring and a polyene side chain has been suggested (Paul 1981, 1982). Other authors (Kirschfeld et al. 1983) have discussed several candidates, including retinol in coplanar conformation. Meanwhile a retinol derivate (3-OH-retinol) isolated from the blowfly's eye is regarded as the sensitizing pigment (Vogt and Kirschfeld 1984). It is suggested that the 3-OH-retinol (in a coplanar conformation) is bound to the visual pigment less than 2.5 nm from the chromophore of the xanthopsin (Vogt and Kirschfeld 1983). The chromophore of the visual pigment of flies is not retinal but 3-OH-retinal, which can be derived from xanthophylls (Vogt 1983). Vogt therefore suggested it be called xanthopsin rather than rhodopsin.

After apparently explaining the reason for the dual-peaked sensitivity of the R1–6 cells of *Calliphora* (see reviews from Franceschini (1983) and Hardie (1985)), the question arises whether this UV mechanism is a widespread characteristic of insects or whether other mechanisms are more frequent.

Using Fourier Interferometric Stimulation (FIS), a new action spectrometric method with many advantages, the spectral sensitivity of a variety of insects was measured with high spectral resolution and high precision to look for the origins (the absorbing pigments) of their spectral sensitivities.

Materials and methods

The spectral stimulus during FIS is produced by a continuously scanning Michelson interferometer. The individual spectral components of the stimulus light are intensity-modulated in a sinusoidal manner and – most importantly – with a typical frequency. For example, the spectral component 600 nm is modulated at 4 Hz and the component 300 nm at 8 Hz. (The modulation frequency and the spectral wavelength are inversely proportional.) Thus the components are appropriately distinguished. The fixation of the spectral stimulus is not achieved by limiting the width of the spectral band (interference filter, monochromator), but by coding each of the spectral elements. The modulation frequency depends on the moving speed of one of the two mirrors of the interferometer and can be, to a certain extent, freely chosen. The Michelson interferometer transforms the light of a lamp into the Fourier interferometric stimulus. This stimulus (interferogram) presents the sum of sine-shaped intensity modulations each representing a different spectral quality (Fig. 1). The biggest changes in light intensity appear near the white light position (WLP), where all modulations are in phase.

A light-sensitive system can be tested by recording its response to that stimulus. Frequency analysis (Fourier analysis) of stimulus and response leads to spectra, or in other words,

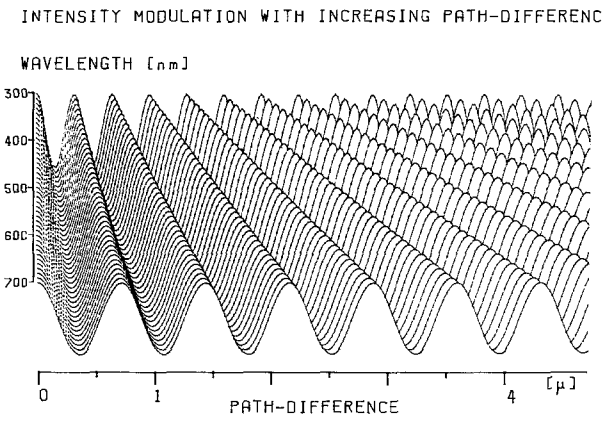

Fig. 1. Fourier interferometric stimulus. The stimulus is the sum of an endless number of sinusoidally modulated spectral elements. Starting at the white light position (path difference: 0), the modulations are shown of the spectral elements of an ideal light source, with a constant amplitude for each wavelength. The sum of the modulations is called 'interferogram'. The duration of the interferogram differs with the moving speed of the mirror in the interferometer. Ordinate: wavelength (in nm), abscissa: optical path difference in the interferometer (in μm)

to amplitude distributions of stimulus and response over the (modulation) frequency interval, reaching for example between 4 Hz and 8 Hz. This is related to the spectral range 600 nm to 300 nm. (The exact relationship between wavelength and modulation frequency is permanently controlled using a He–Ne laser reference.)

What corrections and calculations are necessary to find the spectral sensitivity of the system under investigation?

When measuring the spectral sensitivity of a test object with square-pulse-formed stimuli, the relation between stimulus intensity and response amplitude (intensity response function) has to be determined. This is used to transform spectral efficiency into spectral sensitivity (Autrum and von Zwehl 1964). The plot of the logarithm of intensity against response is *S*-shaped.

There is usually a linear relationship between stimulus and response for modulated stimuli with small stimulus amplitudes (e.g. DeVoe 1963; Gemperlein and McCann 1975).

So for FIS the following relationships are valid:

$$R(\lambda)_i = K(\lambda)_i * S(\lambda)_i \quad (1)$$

where
$R(\lambda)_i$: response amplitude, $S(\lambda)_i$: stimulus amplitude, $K(\lambda)_i$: wavelength dependent, proportionality factor.

The spectral sensitivity (or the action spectrum (Rodieck 1973)) of a light-sensitive system is defined as follows: Spectral sensitivity = reciprocal value of the number of quanta of a specific wavelength necessary to obtain a fixed response amplitude from a given test-object.

Different stimulus amplitudes $S(\lambda)_i$ for different wavelengths are necessary to obtain a specific response amplitude R_0:

$$S(\lambda)_i = R_0 / K(\lambda)_i. \quad (2)$$

(Example: $R_0 = 10$ mV, $S(\lambda)_1 = 10$, and $S(\lambda)_2 = 1$ (relative number of quanta) for $\lambda_1 = 565$ nm and $\lambda_2 = 490$ nm in the case of an R1–6 cell of the blowfly.)

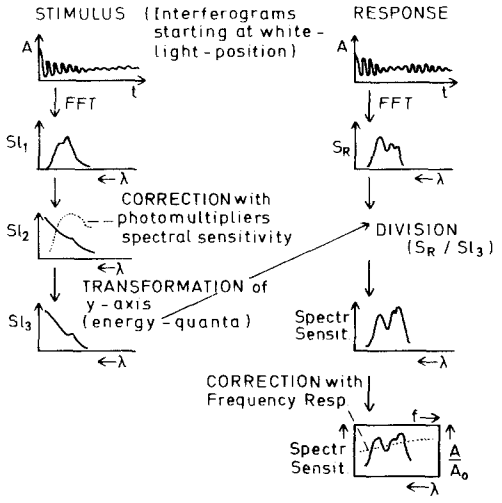

Fig. 2. Correction scheme. Left: calculations on the stimulus, right: calculations on the response. Both time signals are transformed into the frequency domain via FFT (after subtraction of the mean value and sometimes after multiplication with a time window, like a Hamming window). The lamp spectrum is corrected with the spectral characteristics of the stimulus-recording photomultiplier and then the ordinate is transformed from energy units into relative quanta numbers. Division of the response spectrum by the corrected lamp spectrum gives the spectral sensitivity of the system. If necessary, a correction of this spectral sensitivity with the frequency response of the system is carried out

From Eq. (2) we see that the constant $K(\lambda)_i$ is equal to the reciprocal of the number of quanta of a specific wavelength. The distribution of $K(\lambda)_i$ over λ_i is therefore the spectral sensitivity of the system. The constants $K(\lambda)_i$ result from dividing response spectrum by stimulus spectrum.

The division of the response spectrum by the (corrected) stimulus spectrum eliminates the dependency of the response amplitude on the height of the stimulus amplitude and gives the spectral sensitivity. This division would be unnecessary in the ideal case of a stimulus light with an equal number of quanta of all wavelengths. In practice it is necessary to correct for the spectral characteristics of the stimulus recording photomultiplier which modifies the stimulus spectrum. Furthermore, before the division is carried out, the energy measuring scale of the stimulus spectrum ordinate is transformed into relative quanta numbers (Fig. 2). All these calculations and corrections are performed by a computer in a few seconds.

Three points still have to be discussed. First, what happens in the case of a nonlinear relation between stimulus and response? This problem has been discussed in detail by Steiner (1984). He shows that a FIS determination of spectral sensitivity is possible even for quadratic nonlinearities. He also shows that this type of nonlinearity is usually present – if there are any nonlinearities in all – in the investigated insect species during FIS determination of the spectral sensitivity. The nonlinearities can be separated from the linear part of the response. The stimulus only falls into one octave band (e.g. 4 Hz to 8 Hz) so the nonlinearities can be found outsite it. He further shows that nonlinearities can give useful information about the signal processing of the system (Steiner et al. 1986).

The second question is whether different modulation frequencies alone lead to different response amplitudes. The frequency response function must be measured before the influence of the modulation frequency on the determination of the spectral sensitivity can be eliminated. This is done with an electro-mechanical scanner which modulates the intensity of white light in a sinusoidal manner. The influence of the frequency response function on the determination of the spectral sensitivity with FIS could mostly be discounted, because the spectral test was only performed on one octave of the lower frequency range (e.g. 4 Hz to 8 Hz). In most cases the frequency response curve shows in the small frequency bands used (e.g. 4 Hz to 8 Hz) and at the normally used intensity levels only a small continuous increase at the higher frequencies.

The third question is whether adaptational phenomena influence the determination of spectral sensitivities with FIS. Firstly, we have to examine the spectral composition of the Fourier interferometric stimulus at different optical path differences, i.e., at different points of time (see Fig. 1). At a path difference of 0 nm, all spectral elements interfere constructively and, assuming the light source has equal amplitudes for all elements, the stimulus light is white. If the path differences increase, at first the UV elements interfere destructively (at a path-difference of about 150 nm): the stimulus only contains higher wavelengths. If the path difference is further increased, the UV elements interfere constructively again whereas the elements with higher wavelengths (red region) interfere destructively (at a path difference of about 350 nm): the stimulus contains mainly UV light. By further increasing the path difference, the spectral composition of the stimulus becomes more and more complex, but there is a typical spectral composition of the stimulus at each path difference. If the ordinate in Fig. 1 is changed from a wavelength measuring scale (nm) to a frequency measuring scale (THz) by using the relation: $\lambda = c/f$ (c: velocity of light), the spectral composition of the stimulus at different path differences would vary in a sinusoidal pattern (comb-filtered spectra) (see Barlow 1982; Barlow et al. 1983).

The spectral resolving power of the tested light-sensitive system has now to be considered. The spectral composition of the FIS stimulus may become more and more complex because some parts of the spectrum interfere constructively, adjacent parts interfere destructively and the distance between adjacent maxima (or minima) becomes smaller. When this occurs, there are typical threshold values where the FIS stimulus appears to be white to humans (Barlow et al. 1983). The breadth of the sensitivity curve of the investigated system determines the duration of major response variations to the FIS stimulus. Major response variations only occur in the R1–6 visual cells of *Calliphora* (Fig. 4b) at path differences of about ± 2 µm around the white light position.

The stimulus light is always switched on and bigger variations in response are only measurable during 20% of the measuring time. Thus most of the time a constant adaptational level is held regarding the light intensity. Major changes in stimulus intensity (less than one log-unit) and therefore in response only occur near the white light position. There are about 9 major changes in response in a time-span of about 3 s (1 change in 0.3 s). Therefore adaptation to different light intensities can be discounted because the time course of adaptation is slower.

Does the spectral composition of the FIS stimulus influence the rhodopsin/metarhodopsin relation? It has been shown in several invertebrates that light of different wavelengths influences the relation of the concentrations of rhodopsin and metarhodopsin (e.g. Hamdorf 1979). At higher path differences (away from the WLP) the spectral range of the FIS stimulus has such closely adjoining maxima and minima that rhodopsin and metarhodopsin are equally irradiated. Their relationship is determined only by the spectral characteristics of the light source. The concentration of metarhodopsin is only 10% that

of rhodopsin (see Rosner 1975) because a Xenon source with a similar spectral composition to sunlight is used. A change in the relation of rhodopsin to metarhodopsin may occur near the WLP. However, the consistency of the measured spectral sensitivity curves with the absorbance characteristics of rhodopsins (Figs. 4d, 9, 10, 11, 12, 13) shows that changing the rhodopsin/metarhodopsin relation seems to have no important influence on the determination of spectral sensitivities with FIS.

The use of Fourier interferometric stimulation gives several advantages (see also Gemperlein 1982), some of which are those of Fourier spectroscopy, a very valuable tool in spectroscopy.

(1) It is possible to perceive an improvement in the signal-to-noise ratio up to a factor $\sqrt{N}$, as compared with serial methods. Spectral sensitivity is not tested with only one spectral element within a time T, but simultaneously with the N spectral elements of the stimulus light which are absorbed by the light-sensitive system. If an equal signal-to-noise ratio is accepted, the result is a much shorter measuring time. This is called multiplex advantage in Fourier spectroscopy.

(2) Furthermore, (a) the stimulus light is not limited in its spectral content and (b) the interferometer lets light not only from point sources but also from extended sources (up to a certain angle of incidence between the optical axis and the lightbeam) pass through without substantial weakening. This is throughput advantage in Fourier spectroscopy. Both these factors result in a very high potential stimulus intensity.

(3) The spectral resolution depends on the number of recorded modulation periods and can therefore be chosen freely, according to the length of the maximal optical path difference in the interferometer.

Some biological advantages are:

(4) The determination of the intensity/response function for calculating the spectral sensitivity becomes superfluous.

(5) The high spectral resolution together with computer fits allows a detailed analysis of spectral systems using the electroretinogram.

(6) The potentially high stimulation intensity allows sensitivity measurements at many different intensity levels and to separate low and strong light systems (Adamczyk et al. 1983).

(7) The phase information can be used to determine differences in latencies (Steiner 1984).

More information on Fourier spectroscopy can be found in Connes (1968), Loewenstein (1970), Bell (1972) and Parsche and Luchner (1975).

More details on Fourier interferometric stimulation can be found in Gemperlein et al. (1977), Gemperlein (1980), Paul (1981), Gemperlein (1982), Gemperlein et al. (1983) and Steiner (1984).

Experimental set-up (Fig. 3). A Xenon arc lamp with a DC power supply was used as a light source (Osram XBO 150/1 with Suprasil bulb). The optical components of the interferometer consist of triple lensed UV achromats, a Suprasil beamsplitter with an appropriate coating and high planarity ($\lambda/10$) and two flat UV mirrors. The apparatus can work in a range from close infrared to 250 nm in the UV. It is thermostabilized and protected against vibrations. One mirror is moved by a high voltage controlled piezoceramic. A parallelogram-guide guarantees a tilt-free movement. The other mirror is held in a fixed position by two micrometer gauges.

The high voltage source can be controlled either by a 16-bit DAC or a special electronic control. In the first case the mirror is moved in one direction by a continuously rising control voltage produced by the DAC and the computer. The rising speed of this voltage can be selected, giving different modulation frequency bands in the FIS stimulus. Averaging in the time domain is not possible with this technique. The light of a He–Ne laser ($\lambda_L = 632.8$ nm) is lined up closely to the main light beam and brought to interference too. The He–Ne laser reference signal has to be recorded and used in the sampling process which is done by a computer program. Measurements made by the DAC technique are called single runs.

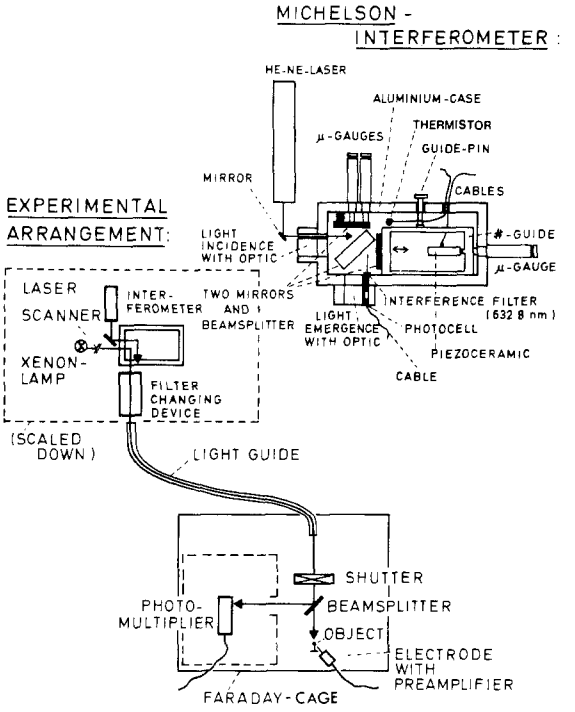

Fig. 3. Experimental set-up. Light of a Xenon arc lamp is transformed into the Fourier interferometric stimulus by a Michelson interferometer. Frequency response curves are measured using an electromechanical scanner to modulate white light. A He–Ne laser is the reference for control electronics. Its fixed wavelength always gives the relation between spectral wavelength and modulation frequency. Light intensity can be changed by neutral density filters; the spectral composition can be changed by colour filters. After passing a light guide (all optical components are quartz) the stimulus light falls onto the eye of an animal, while a part of the light falls onto a photomultiplier which is protected against scattering light. A shutter identifies intracellular responses

A control electronic was developed to allow averaging. A voltage ramp which controls the high voltage source is modified by a signal which results from comparing the sinusoidally modulated signal of the He–Ne laser reference with an electronically generated sinus signal ($f = 4.5$ Hz). Thus the mirror moves at a constant speed. It can also be moved for a selected number of periods by counting the modulation periods of the laser signal. When this number is reached the forward movement of the mirror (caused by the increasing ramp) is stopped. The voltage ramp decreases and the mirror moves backwards, controlled again by the laser reference and the counting system. In this manner the optical path difference between the two mirrors can be reproduced and averaging in the time domain is possible. However, the modulation frequency is fixed to the frequency of the electronically generated sinus signal. Measurements made with this technique are called repeated stimuli.

An electromechanical scanner with a lamella grid was used to determine the frequency response function. Its steering produces a sinusoidal modulation of the light.

The intensity of the stimulus light can be diminished by a filter changing device containing neutral density filters. The maximal intensity was 20 mW/cm^2 in the range 400 nm to 800 nm, measured using a radiometric device.

After passing through a quartz light guide, the light falls partly onto the test-object and partly onto a photomultiplier. The light guide was fixed about 5 cm from the eye of the test object. The diameter of the light guide is 4 mm and so a visual cell will see its tip at a visual angle of about 5°. The visual cell is well illuminated, as the width of the angular acceptance function at 50% sensitivity (Δp) of a blowfly's visual cell is about 4° (Snyder 1979). The adjustment of the eye was changed till a maximum response of the visual cell was recorded (on-off response). The cell was therefore on-axially illuminated.

The amplified stimulus and response signals are filtered with a low-high pass combination, digitized by a 12-bit ADC and passed on to a 16-bit minicomputer. This minicomputer (Computer Automation Inc.) processes the data, carries out Fourier-analysis (FFT), corrects and graphically displays them (oscilloscope or XY-recording instrument). Extensive program systems have been created to perform these and various other tasks (Paul; unpublished FIS program system).

The working efficiency of the interferometer and the entire experimental arrangement was extensively pre-tested using lamps with characteristic spectra and filters. The apparatus worked satisfactorily in the range near infrared to 250 nm in the UV.

The modulation degree of the different spectral elements of the light source depends on wavelength. It decreases continuously at lower wavelengths due to irregularities in the planarity of the beam-splitter and the mirrors. The transmission of the optic depends on wavelength. It decreases in the UV. Therefore the stimulus was recorded close to the tested biological system to measure the actual stimulus interferogram. Detailed information on the experimental set-up can be found in Paul (1981) and Steiner (1984).

The reaction of the test object is recorded from the eye using glass microelectrodes (filled with 3 mol/l KCl). Intracellular recordings and ERG were used to determine the spectral sensitivity.

The blowflies used were bred at the institute. We used flies raised on bovine liver (high visual pigment content) or on horse skeletal muscle (low visual pigment content). We obtained the *Drosophila* mutants from Dr. Gerresheim in Aachen and Prof. Hengstenberg in Würzburg, the desert ants from Dipl.-Biol. Simmler in Zürich, the butterflies from Prof. Kolb in München and *Ascalaphus* from Prof. Smola in München.

Preliminary notes

The application of FIS to the investigation of insect spectral sensitivity is shown by the ERG response of the blowfly mutant chalky (Fig. 4).

The stimulus (Fig. 4a) and response (Fig. 4b) recordings are shown. Stimulation around the white light position was performed. The movable mirror of the interferometer approaches the point at which the optical distances from the beam-splitter to the two mirrors are equal. It closes in on that point (WLP), passes it and draws away. The intensity modulations of the stimulus interferogram are approximately one log unit of light intensity at the maximum (WLP). Response amplitudes

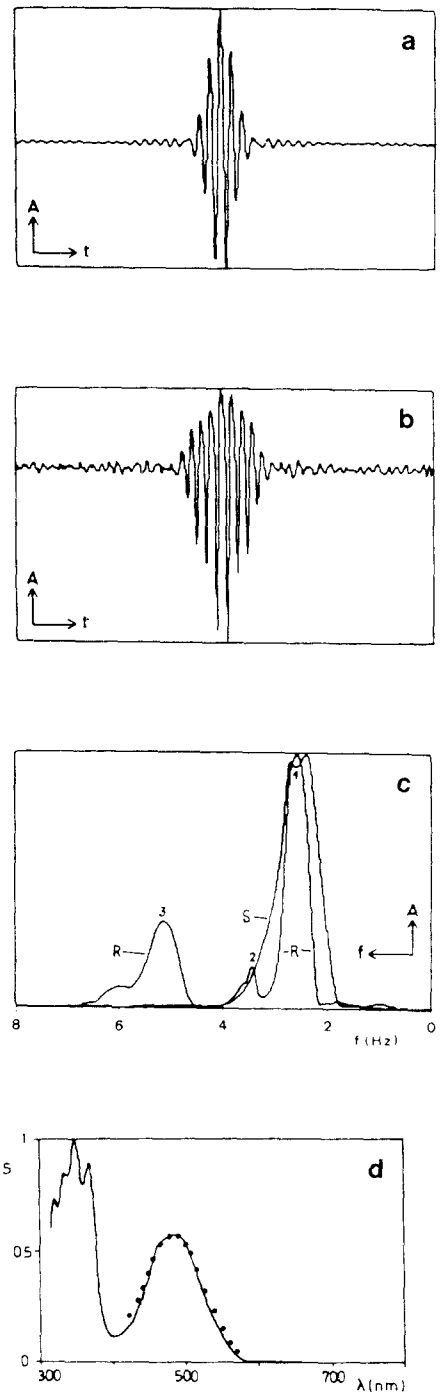

Fig. 4a–d. FIS-stimulus and response of *Calliphora* mutant chalky. **a** Recording of the stimulus (Xenon arc lamp) with the photomultiplier RCA-1P28/VI. *A* amplitude; *t* time (16 s) (Optical path difference: 32 λ_L corresponding to 20.25 µm). **b** Recording of the response, the electroretinogram of the blowfly mutant chalky. Stimulus and response are non-averaged single runs. Major response variations are only seen near WLP (±2 µm). *A* amplitude (peak-peak amplitude at WLP about 10 mV); *t* time (16 s). **c** Uncorrected spectra of stimulus (*S*) and response (*R*). *A* amplitude; *f* frequency (0–8 Hz). **d** Spectral sensitivity of the mutant chalky compared to the absorbance characteristics of a rhodopsin with a maximum at 490 nm (points after Dartnall 1953)

to the FIS are variable. They depend on the investigated species, recording mode (intra-/extracellular) and light intensity. Normally the peak-peak amplitude at the maximum (WLP) varies within 5–15 mV in ERG and intracellular recordings. The response of intracellularly recorded visual cells to a light-on, light-off stimulus was about 50 mV. The DC-plateau at which the interferogram modulation takes place, during intracellular recording is not shown in the following. It is made practically negligible by high-passing the signals. The relevant information for FIS is not the static degree of polarization (de- or hyperpolarization), but the modulation amplitude. The modulation degree of the response interferogram is at most about 80% at WLP.

The duration of the stimulus and the response depends on the mirror-speed of the interferometer. At present stimulus durations of 1 s to several minutes for 32 laser modulation periods are possible. This leads to modulation frequencies of 32 Hz to 64 Hz for the wavelengths 632.8 nm (wavelength of the He–Ne laser) to 316.4 nm or appropriately to frequencies which are much lower than 1 Hz. Stimulation is normally performed in the medium frequency range. This is 2 Hz to 4 Hz for single runs (see Experimental set-up) as shown in Fig. 4a, b; and 4.5 Hz to 9 Hz for repeated stimuli (see Experimental set-up) for the wavelengths from 632.8 nm to 316.4 nm. The duration for one measurement is therefore (for 32 laser modulation periods) either 16 s or 7.1 s.

The sine-cosine Fourier transformation of the interferograms gives the stimulus and response spectra (Fig. 4c). The stimulus spectrum deviates from the proper lamp spectrum because (1) the spectrum is modified by the spectral characteristics of the photomultiplier and (2) the reduced optical transmission and the lower modulation degree at lower wavelengths diminish the amplitudes of these spectral elements (see Experimental set-up). The signal has been filtered and so further deviations at high and low wavelengths ensue. Because the response is filtered in the same way and the stimulus is recorded close to the tested visual system (see Experimental set-up), the division of response spectrum by stimulus spectrum (see Materials and methods) eliminates these influences. The influence of the spectral characteristics of the photomultiplier is eliminated before the division is performed, correcting the stimulus spectrum according to Fig. 2.

The recorded wavelengths or frequencies of the stimulus spectrum (S) range from about 720 nm (1.76 Hz) to 320 nm (3.9 Hz). The response spectrum (R) can be divided into 3 zones. The first maximum (peak 1) is located at a modulation frequency of about 2.53 Hz (500 nm). Another maximum (peak 2) is located at 3.39 Hz (373 nm) and represents the response in the UV. The non-linearity of the response (peak 3), separated from the linear response, is in the 4 Hz to 8 Hz frequency range.

A moving distance of 32 λ_L periods (about 20 µm optical path difference) allows a resolution of about 40 points in the range 300 nm to 700 nm according to Rayleigh's theorem (Bell 1972). (The points are connected by straight lines in the figures.) The spectral resolution is $\Delta\lambda = 6$ nm at 350 nm, $\Delta\lambda = 12.3$ nm at 500 nm, and $\Delta\lambda = 24.2$ nm at 700 nm. The size of the Fourier transformation was adapted to the maximum possible resolution.

The present maximum possible moving distance of 128 λ_L gives a resolution of 1.5 nm, 3 nm, 6 nm for the wavelengths 350 nm, 500 nm, 700 nm, respectively. About 160 points in the range 300 nm to 700 nm are now resolvable.

The division of the response spectrum by the corrected lamp spectrum according to Fig. 2, the change to a wavelength-measuring abscissa and the limitation to wavelengths from 300 nm to 800 nm gives the spectral sensitivity of the blowfly's eye (Fig. 4d).

Results

Spectral sensitivity of Calliphora erythrocephala

The sensitivity of the eye of *Calliphora* mutant chalky (Fig. 4d) shows two maxima: one in the visible region with a maximum at about 490 nm and one in the UV with a maximum at about 350 nm, as reported for the R1–6 cells (e.g. Smola and Meffert 1979). The sensitivity in the visible range fits well with the absorption characteristics of a rhodopsin with a maximum at 490 nm.

The intracellularly recorded sensitivity of an R1–6 cell (Fig. 5) shows no significant difference from the sensitivity curve of the eye (Fig. 4d). The variation of the FIS measurements of spectral sensitivity curves is small (Fig. 5).

The fine structure of the UV sensitivity (Figs. 4d, 5) was first measured in intracellular and extracellular recordings with FIS (Gemperlein et al. 1980; Paul 1981).

In the following experiment (Fig. 6) the stimulus was limited to UV light to improve the signal-to-noise relation in the UV (higher amplification

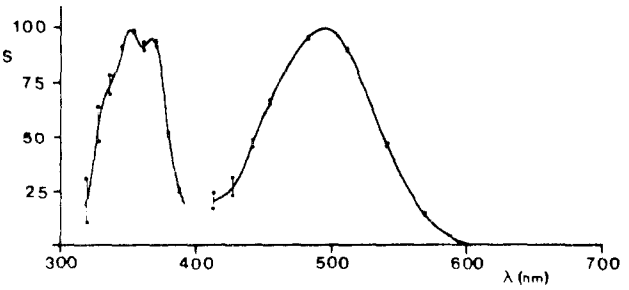

Fig. 5. Variation of FIS measurements. Intracellularly measured spectral sensitivity of an R1–6 visual cell of *Calliphora* mutant chalky. (Optical path difference: 32 λ_L). Visible range: mean sensitivity curve of 12 subsequent single runs. Points: ±standard deviation. UV range (using a BG 24, Schott): mean sensitivity curve of 10 subsequent single runs. Points: ±standard deviation

and better usage of the input range of the ADC). Side lobes appear in the response interferogram (Fig. 6a). This beat structure is a sign that the spectrum is multi-peaked, as can be seen in the spectral sensitivity curve (Fig. 6b). Three large and two smaller peaks in the range 300–400 nm and two below 300 nm are visible. The peaks above 300 nm are located at 370 nm, 350 nm, 333 nm, 319 nm and 302 nm. The peaks below 300 nm are located at 285 nm and 276 nm. If the spectral resolution is lowered by artificially reducing the number of measuring points (Fig. 6c) corresponding to a reduction of the moving distance from 72 λ_L to 18 λ_L, the fine structure disappears (Fig. 6d).

The spectral sensitivity of the mutant chalky was measured in intracellular recordings at 3 and extracellular recordings at 4 different intensity levels. No significant changes in the sensitivity curves were observed (unpublished data).

The spectral sensitivity of the wildtype *Calliphora* (Fig. 7) was compared to that of the chalky (Fig. 4). The former showed an additional small peak (*P*) at 620 nm (Fig. 7a). This leads to a small difference of the sensitivity curve (Fig. 7b) at higher wavelengths in comparison to chalky (Fig. 4d). This is due to the transparency of the screening pigments (ommochrome) in the red (see e.g. Goldsmith 1965). The sensitivity also shows a fine structure in the UV. The relative height of the peak in the visible to that in the UV is variable from animal to animal depending on the vitamin A content of the food (see e.g. Kuo 1981). The UV fine structure was substantiated by reducing the stimulus for the UV (Fig. 7c). Intensity dependent changes in the sensitivity curve have been observed and interpreted as due to migration of screening pigments (Paul 1981).

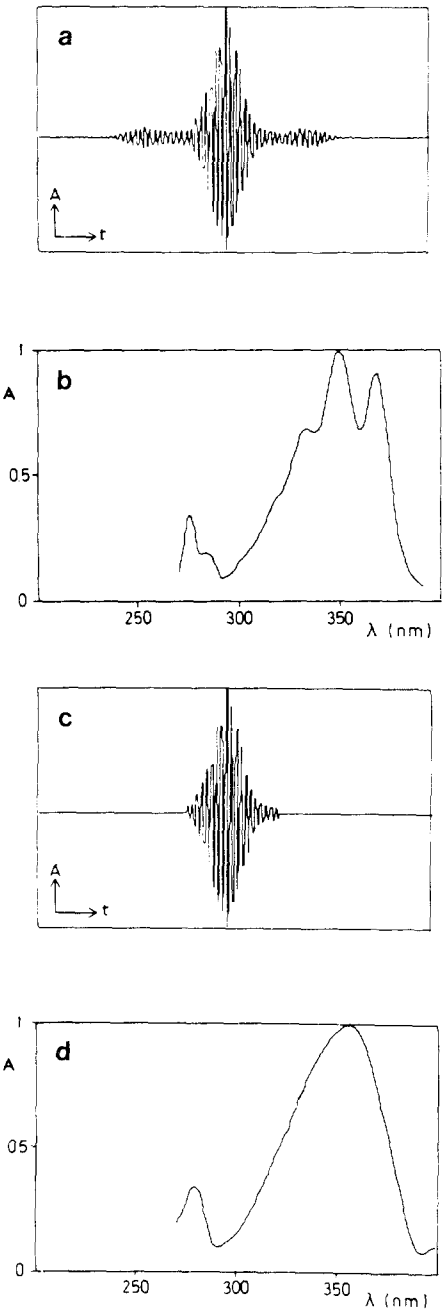

Fig. 6. UV fine structure. **a** Averaged response of chalky. The stimulus is limited to the UV with an optical filter (UV interference filter, Balzers). *A* amplitude (peak-peak amplitude at WLP about 5 mV); *t* time (16 s) (ERG, an average of 100 repeated stimuli, optical path difference: 72 λ_L). **b** Spectral sensitivity of chalky in the UV. **c** Artificial reduction of the path difference which leads to a reduction of the spectral resolution. *A* amplitude; *t* time. **d** Spectral sensitivity of chalky. Lower resolution in (**d**) in comparison to (**b**) destroys the fine structure

Spectral sensitivity of Drosophila melanogaster

The spectral sensitivity of wildtype and different mutants of *Drosophila melanogaster* was measured using the ERG (Nebel 1982). Two mutants are

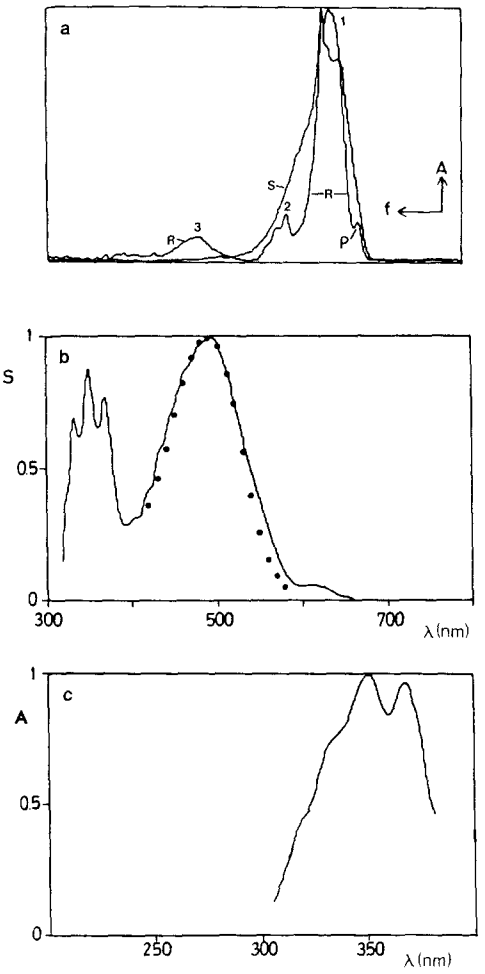

Fig. 7a–c. Spectral sensitivity of *Calliphora* wildtype. **a** Uncorrected spectra of stimulus (*S*) and of intracellularly recorded response (*R*) of R1–6 cells. Notice the peak (*P*) in the red region due to ommochrome absorption. Response in the visible region is indicated by (1), response in the UV by (2), non-linearities by (3). *A* amplitude; *f* frequency (0–18 Hz) (an average of 25 repeated stimuli, optical path difference: 34 λ_L). **b** Spectral sensitivity calculated from (**a**) in comparison to the absorbance characteristics of a rhodopsin with a maximum at 490 nm (points after Dartnall 1953). **c** Intracellularly recorded spectral sensitivity (*S*) of R1–6 in the UV (UG 11, Schott). (An average of 100 repeated stimuli, optical path difference: 34 λ_L)

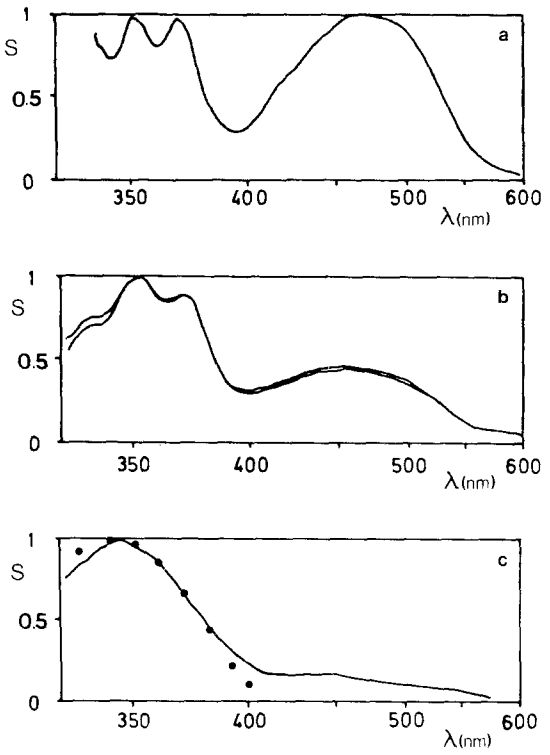

Fig. 8a–c. Spectral sensitivity of *Drosophila melanogaster*. **a** Spectral sensitivity of *D. melanogaster* wildtype (ERG) (an average of 36 repeated stimuli, optical path difference 31 λ_L). **b** Spectral sensitivity of mutant f3/E (ERG). Two subsequent measurements superimposed (an average of 38 repeated stimuli, optical path-difference: 31 λ_L). **c** Spectral sensitivity of mutant *rdgB, w* (ERG) compared to the spectral sensitivity of the frontal eye of *Ascalaphus* (points after Gogala 1967). (An average of 38 repeated stimuli, optical path difference: 31 λ_L. Notice changed abscissa scaling in this and the following graphs)

especially interesting: (1) the mutant f3/E (Gerresheim 1981), similar to the mutant sevenless (sev, Harris et al. 1976), has no visual cell R7 and (2) the mutant *rdgB, w* (Harris and Stark 1977) which has nearly no R1–6 receptors. The mutant f3/E (Fig. 8b) has a sensitivity similar to that of the wildtype (Fig. 8a): a UV fine structure with maxima at about 335, 350 and 370 nm and a maximum in the visible at about 460 nm. Stark et al. (1977) reported a maximum at 470 nm in the visible range. The fine structure was also proven with a pure UV measurement. The differences in the relative height of the two peaks of f3/E and of the wildtype are not significant. The close match of the sensitivity curves of the wildtype and the mutant f3/E shows the great dominance of the R1–6 response in the ERG because the latter has no R7 visual cells. The sensitivity of the mutant *rdgB, w* is different (Fig. 8c), with a dominant maximum in the UV at 345 nm and smaller maxima in the visible (see Stark et al. 1983). The sensitivity in the UV shows no fine-structure. This is also proven by a UV-only measurement. The influence of screening pigments on sensitivity can be excluded as the mutant *rdgB, w* lacks them. The UV-sensitive R7 receptors of *rdgB, w* do not have a fine-structured sensitivity. The comparison of the FIS measurement of the sensitivity of *rdgB, w* with the UV sensitivity of *Ascalaphus macaronius* (Fig. 8c) leads to the assumption that the absorbing pigment is an UV rhodopsin (see below).

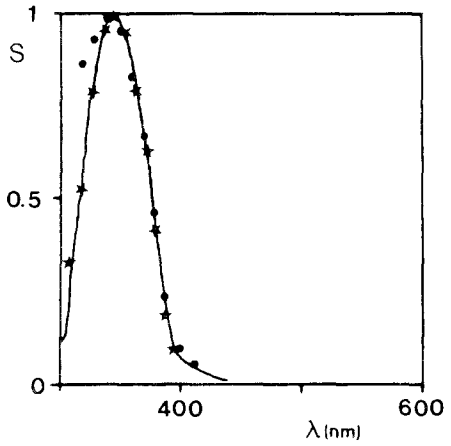

Fig. 9. Spectral sensitivity of *Ascalaphus macaronius*. Sensitivity in the dorsal region of the eye (ERG). Solid points: sensitivity measurement by Gogala (1967); asterisks: absorbance spectrum of *Ascalaphus* UV rhodopsin (after Hamdorf 1979). (An average of 20 repeated stimuli, optical path difference: 30 λ_L)

Spectral sensitivity of Ascalaphus macaronius

The frontal eye of *Ascalaphus* (ERG) has a pure UV sensitivity with a maximum at 345 nm (Fig. 9). No fine structure is present. The FIS measurement of the sensitivity of *Ascalaphus* is in agreement with the results of Gogala 1967 (Fig. 9). The UV sensitivity in *Ascalaphus* is due to a UV rhodopsin (Hamdorf et al. 1971). The FIS measurement is a good match with the absorbance characteristics of this rhodopsin (Fig. 9).

Spectral sensitivity of Cataglyphis bicolor

The ERG of *Cataglyphis bicolor* has a dual-peaked sensitivity with maxima at 350 und 510 nm (Fig. 10) as reported by Mote and Wehner (1980). The sensitivity in the UV shows no fine structure. The spectral sensitivity can be explained by two rhodopsins with maxima at 350 nm and 510 nm, using a computer fit.

Spectral sensitivity of Periplaneta americana

The sensitivity of *Periplaneta* (ERG) displays two peaks in the dorsal region of the eye with maxima at 365 nm and 505 nm (Fig. 11) as reported by Walther and Dodt (1959) and Mote and Goldsmith (1971). The UV peak is not structured, as is also shown by a UV-only measurement. The sensitivity in the visible can be explained by a rhodopsin (Fig. 11). The wavelength of the UV peak is different from the UV peaks of *rdgB*, *w*, *Ascalaphus* and *Cataglyphis* but is similar to the spectral sensitivity of the dorsal eye of the mayfly *Atalophlebia* (Fig. 11).

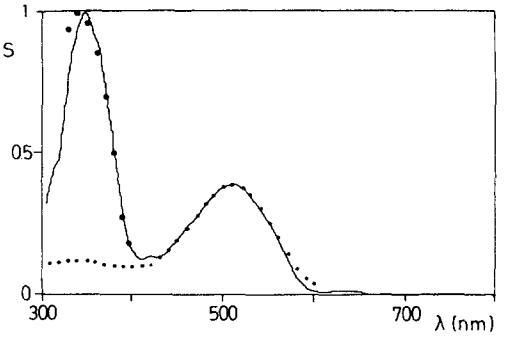

Fig. 10. Spectral sensitivity of *Cataglyphis bicolor* (ERG). Small points: absorbance characteristics of a rhodopsin with a maximum at 510 nm (after Dartnall 1953). Large points: sensitivity of *Ascalaphus* (after Gogala 1967) (an average of 100 repeated stimuli, optical path difference: 40 λ_L)

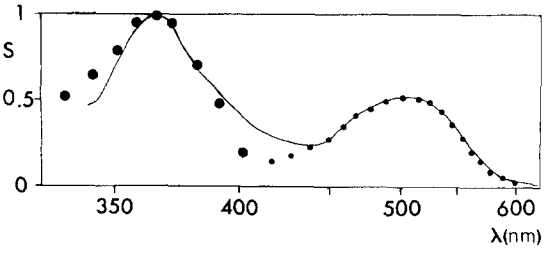

Fig. 11. Spectral sensitivity (ERG) in the dorsal region of the eye of *Periplaneta americana*. Small points: absorbance characteristics of a rhodopsin with a maximum at 505 nm (after Dartnall 1953). Large points: sensitivity of *Atalophlebia* (after Horridge and McLean 1978). (An average of 20 repeated stimuli, optical path difference: 32 λ_L)

Spectral sensitivity of Pieris brassicae, Aglais urticae and Pararge aegeria

The sensitivity of *Pieris* (ERG) shows three maxima in the range 300 to 650 nm (Fig. 12a) in the dorsal part of the eye. The UV peak shows no fine structure. The height of the peaks changes in relation to the height of other peaks by chromatic adaptation (Fig. 13). In *Pieris* the dependence of the height of the maxima on the recording site (distance from the cornea) is characteristic (Steiner 1984). There is also a difference in the sensitivity curve of the dorsal and ventral part of the eye (unpublished data).

The sensitivity of the eyes of *P. brassicae* (dorsal part of the eye), *A. urticae* and *P. aegeria* can be explained by the absorption characteristics of different rhodopsins (Fig. 12a, b, c). The measured sensitivities are compared to the curve which results from adding the absorbance characteristics of three different rhodopsins. All measured sensitivities, with the exception of the ventral part of the eye of *P. brassicae*, can be explained by the

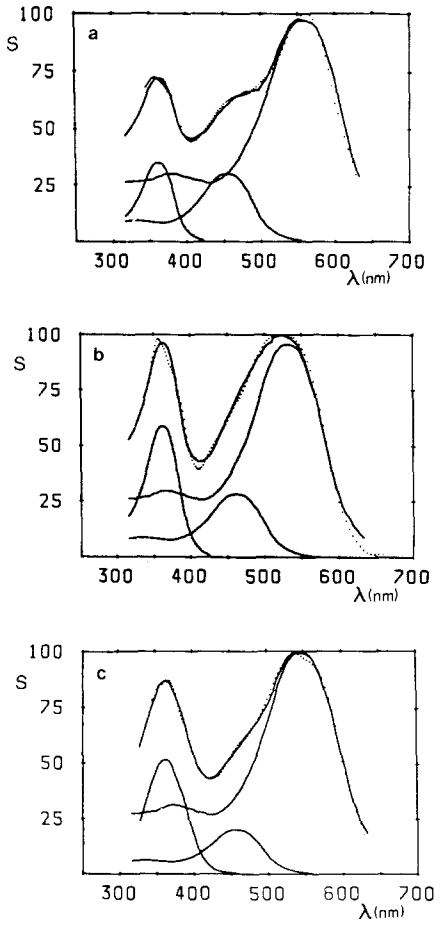

Fig. 12a–c. Sensitivity of Lepidoptera. **a** Spectral sensitivity of *Pieris brassicae* in the outer dorsal region (distance from the cornea: <50 μm) of the eye (ERG). **b** *Aglais urticae* (ERG). **c** *Pararge aegeria* (ERG). Points: sensitivity measurements. Lines: absorbance characteristics of rhodopsins and computer fits of the entire sensitivity curve. (An average of 25 repeated stimuli, optical path difference: 30 λ_L)

absorbance curves of rhodopsins with typical maxima of each species. The sensitivity of the dorsal part of the eye of *Pieris* can be explained by assuming three rhodopsins at 360 nm, 450 nm and 560 nm. The sensitivity of the ventral part of the eye of *P. brassicae* has an additional red peak which cannot be explained by the absorbance characteristics of a rhodopsin (unpublished data).

The measured sensitivities of *Aglais urticae* (Fig. 12b) are independent of the region of the eye and can be explained by the presence of three rhodopsins with maxima at 360 nm, 460 nm and 530 nm. The UV peak is non-structured.

The butterfly *Pararge aegeria* (Fig. 12c) shows a very similar sensitivity curve. This spectral sensitivity can be explained by three rhodopsins with maxima at 360 nm, 460 nm and 530 nm. The UV peak is non-structured.

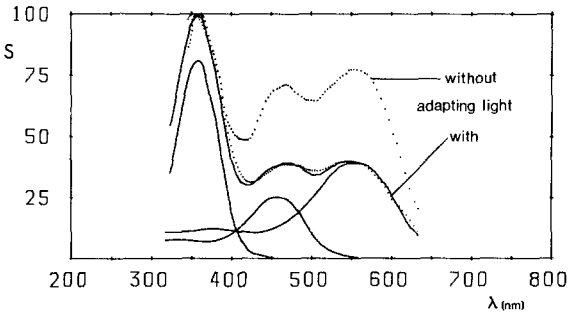

Fig. 13. Chromatic adaptation. Spectral sensitivity of the middle region (distance from the cornea: 200 μm) of the dorsal retina of *Pieris brassicae* (ERG) with and without an adapting light (broad band, non-UV light). Points: spectral sensitivity curves; lines: computer fits and absorbance spectra of rhodopsin

Discussion

The use of Fourier interferometric stimulation leads to the following results.

The spectral sensitivities of the investigated insect species fit well with the absorbance characteristics of rhodopsins in the visible range. The sensitivity curves can be modified by screening pigments (e.g. wildtype *Calliphora erythrocephala*; Fig. 7b), but usually there is a good match between the sensitivity curve and the absorbance spectrum of rhodopsin (Figs. 4d, 10, 11) or the sum of rhodopsins (Fig. 12).

The sensitivities in the UV range can be structured or non-structured. Structured UV sensitivities have only been measured in the Diptera until now. The R1–6 cells in particular show this fine-structured UV sensitivity (Figs. 4d, 5, 6b, 7b, 7c, 8a, 8b). The UV-sensitive R7 cells of a mutant of *Drosophila melanogaster* (*rdgB, w*) do not show a structured UV sensitivity (Fig. 8c), but Hardie and Kirschfeld (1983) found one class of R7 cells (7y) in *Musca domestica* (and one class of R8-cells) which does.

The UV sensitivity of the R1–6 cells of *Calliphora* has 5 peaks in the range 300 nm to 400 nm. This can be explained by the absorbance characteristics of retinol which has a coplanar ionon ring and polyene side-chain (Schreckenbach et al. 1977; 1978a, b; Paul 1981, 1982; Kirschfeld et al. 1983). The stability of the maxima of the fine structure (335, 350 and 370 nm) in two different species (*Calliphora, Drosophila*), in contrast to the position of the maximal absorption in the visible (460 nm vs. 490 nm), indicates that the pigment which absorbs UV is different to that which absorbs visible light.

The fine-structure of *Calliphora* chalky shows two further peaks below 300 nm (Fig. 6b). Goldsmith and Fernandez (1968) reported a UV peak

at 280 nm. It is possible that the absorbed energy of the protein component (opsin) leads to visual excitation. However, the quantum efficiency of this process must be small due to high molar extinction at 280 nm and low response.

The relation of the peak amplitudes of the UV fine structure is variable. The reason for this is unknown. Kirschfeld et al. (1983) have reported that fine structure is reduced or even lost in flies reared on a carotenoid-deficient diet.

Most of the measured UV sensitivities are non-structured and some can be explained by the absorbance characteristics of the *Ascalaphus* UV rhodopsin (Hamdorf 1979) which has a maximum at about 345 nm. This is true for the UV sensitivities of the *Drosophila* mutant *rdgB, w* (Fig. 8c), *Ascalaphus* (Fig. 9) and *Cataglyphis bicolor* (Fig. 10). Other species such as *Periplaneta americana* (Fig. 11), *Pieris brassicae, Aglais urticae* and *Parage aegeria* (Fig. 12) have non-structured UV sensitivities which can be explained by the absorbency characteristics of rhodopsins whose maxima are at 365 nm or 360 nm.

As has been proven in *Cataglyphis* (unpublished) and butterflies (Steiner 1984), non-structured UV sensitivities may change relative to other peaks as a result of chromatic adaptation (Fig. 13). The structured UV sensitivity of the R1–6 cells is not chromatically adaptable (e.g. Burkhardt 1962; Paul 1981). Following Kirschfeld (1979), our experiments show that the rhodopsins which cause non-structured UV sensitivity are separated from the other rhodopsins and located in different visual cells. The UV absorbing pigment which causes structured UV sensitivity is located in the same cell as the rhodopsin (xanthopsin). In the investigated butterflies the rhodopsins in the visible range are chromatically adaptable and very probably located in different cells.

The investigated Lepidoptera have very broad spectral sensitivities which are normally caused by three rhodopsins. Other Lepidoptera also have three or more types of rhodopsin. The sensitivity of the eye of *Deilephila elpenor* can be explained by three rhodopsins absorbing maximally at 350 nm, 440 nm and 525 nm (Höglund et al. 1973). *Spodoptera exempta* has four different visual pigments absorbing maximally at 355 nm, 465 nm, 515 nm and 560 nm (Langer et al. 1979) and *Papilio aegeus* also has four different types of photoreceptors absorbing maximally at 390 nm, 450 nm, 540 nm and 610 nm (Matic 1983).

The high resolving power and reproducibility of FIS gives new insights into the origins of sensitivity curves.

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