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FLICKER FUSION STUDIES IN THE LAMINA AND RECEPTOR REGION OF THE *DROSOPHILA* EYE

DEREK COSENS and H. CH. SPATZ*

Department of Zoology, University of Edinburgh, Great Britain

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Abstract—Blue adaptation and orange adaptation is used to isolate the contributions of the visual subsystems to the lamina response of *Drosophila melanogaster*. Besides the prominent on-transient and off-response the extracellular lamina response contains also an attenuated component from retinula cells R7, R8. Flicker fusion frequencies measured in the lamina range are indistinguishable from those measured in the receptor region. The response mediated by receptor cells R1 to R6 fuses at a threefold higher frequency than the R7, R8 response.

INTRODUCTION

ERG WAVEFORMS of *Drosophila melanogaster* are quite complex. They consist of an on-transient of lamina origin (ALAWI and PAK, 1971; HEISENBERG, 1971), superimposed on the sustained response of retinula cells R1 to R6, as well as to a lesser extent on the response of retinula cells R7 and R8 (COSENS and WRIGHT, 1975; MINKE *et al.*, 1975; HARRIS *et al.*, 1976). Likewise the off-response is a superposition of a lamina off-response and the drop of sustained responses of R1 to R6 and R7, R8, having together a transient-like appearance. Several techniques have been developed to separate these components. Mutant studies provide an important tool to isolate and identify the different components (HOTTA and BENZER, 1969; PAK *et al.*, 1970; HEISENBERG, 1971; HARRIS *et al.*, 1976). Adaptation with short wavelength light isolates the response of R7, R8 from that of R1 to R6 (COSENS and BRISCOE, 1972; COSENS and WRIGHT, 1975; MINKE *et al.*, 1975; HARRIS *et al.*, 1976; STARK, 1977). Lamina recordings (HEISENBERG, 1971; LAUGLIN, 1975) give access to the on-transient and the negative going lamina off-response. This report is intended to characterize further the extracellularly recorded lamina response of *Drosophila*. In particular it is shown that the response picked up in the lamina region contains contributions from cells R7, R8. Flicker fusion frequency for the response mediated by R1 to R6 is about threefold higher than that for R7, R8. No difference is found whether fusion frequencies are measured in the receptor or in the lamina region.

MATERIALS AND METHODS

Wildtype (strain annamas, kindly supplied by Dr. A. Ewing) and bw^{cn} *Drosophila melanogaster* were raised on standard medium at a 12:12 light dark cycle. Flies 3 to 8 days after eclosion were mounted on their side into a groove in dental wax. Where control of temperature was required a very shallow bed of wax was placed on a metal plate. This was temperature-controlled with a water bath and a cooling unit. Mounting was done in such a way that the abdomen touched the metal plate. Furthermore a piece of parafilm was placed over the wax bed to cover thorax and abdomen. Temperatures were recorded by a telethermometer with the probe placed on the metal plate within the block of embedding wax. ERG recording techniques were as described previously (COSENS and WRIGHT, 1975). A gold wire inserted into the thorax served as the indifferent electrode. The other electrode was a Ringer (0.14 M NaCl, 4 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$) filled glass micropipette, which was inserted through the cornea into the retinula cell region of the eye (Fig. 1). Lamina recordings were obtained using the same techniques, details will be described in the Results section.

Blue and orange adaptation of the white-eyed flies was carried out as described by COSENS and WRIGHT (1975) using a bright adapting light. The wildtype was blue-adapted by placing a piece of parafilm as a diffuser over the eye and adapting with blue light of 10 W/m^2 and 465 nm for 30–60 min (BRODA and WRIGHT, 1978).

Optical arrangements are depicted in Fig. 1. All lamps were powered by stabilized d.c. power supplies. Heat filters, Balzer broad band interference filters (K-series) and a narrow band Balzer UV382 filter, in combination with Balzer neutral density filters were used. Arrangement A gives a testflash as described previously (WRIGHT and COSENS, 1977). For the experiments reported here, 1 sec flashes at 4 sec intervals were used. Arrangement B provides a flickering light by rotating a disc with an 18° slit behind a 3° defining slit, giving a duty cycle of approximately 1:20. The disc is driven by a d.c. motor at variable speeds. Light intensities were measured with a Tektronix J 16 Digital Photometer and a Spectroradiometer (Gamma Scientific Inc.).

Terminology

(a) Adaptation with intense light of wavelength below 480 nm for extended periods of time will create

*Permanent address: Institut für Biologie III, Universität Freiburg, West Germany.

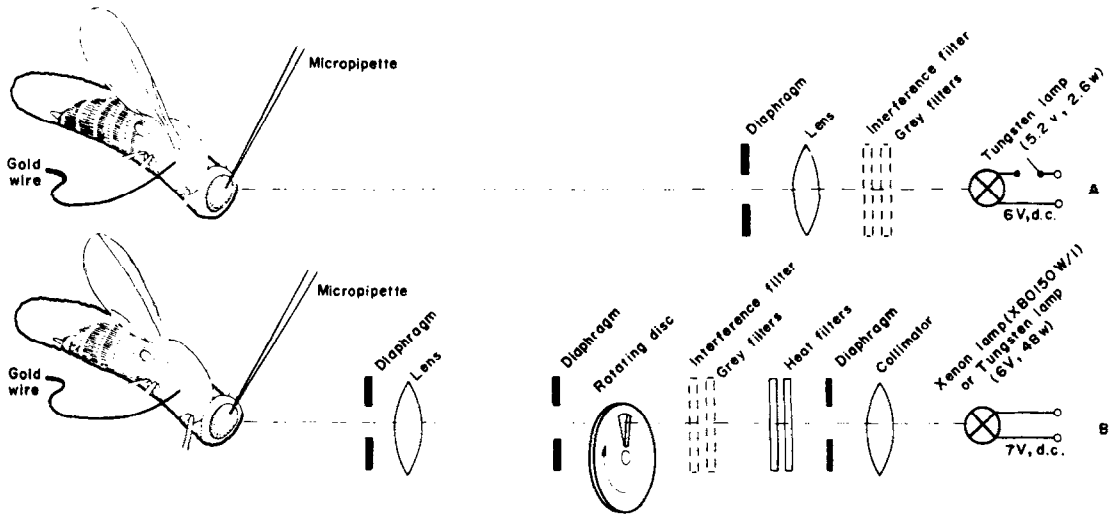

Fig. 1. Schematic drawing of the experimental set up. Filters drawn with dashed lines can be exchanged or taken out of the light beam.

an intracellular PDA (prolonged depolarising afterpotential) (MINKE *et al.*, 1975) and an extracellular PCNA (prolonged corneal negative afterpotential) (WRIGHT and COSENS, 1977). Under these conditions the response of cells R1 to R6 to a testflash disappears (COSENS and BRISCOE, 1972; MINKE *et al.*, 1975; COSENS and WRIGHT, 1975; HARRIS *et al.*, 1976; STARK, 1977). We will call this the blue adapted state throughout. It is inferred that in the blue adapted state only cells R7 and R8 contribute to the ERG.

(b) The blue adapted state can be reverted by exposure to light of wavelength above 580 nm (see above references). We will call this the orange adapted state. It is inferred that under these conditions the ERG is dominated by the response of cells R1 to R6.

(c) As *Drosophila* strains and the methods used here do not provide a certain means to isolate the contributions of R7 cells from those of R8 cells, we will usually refer to the R7, R8 system as a unit.

(d) Following HEISENBERG (1971) we call a region of

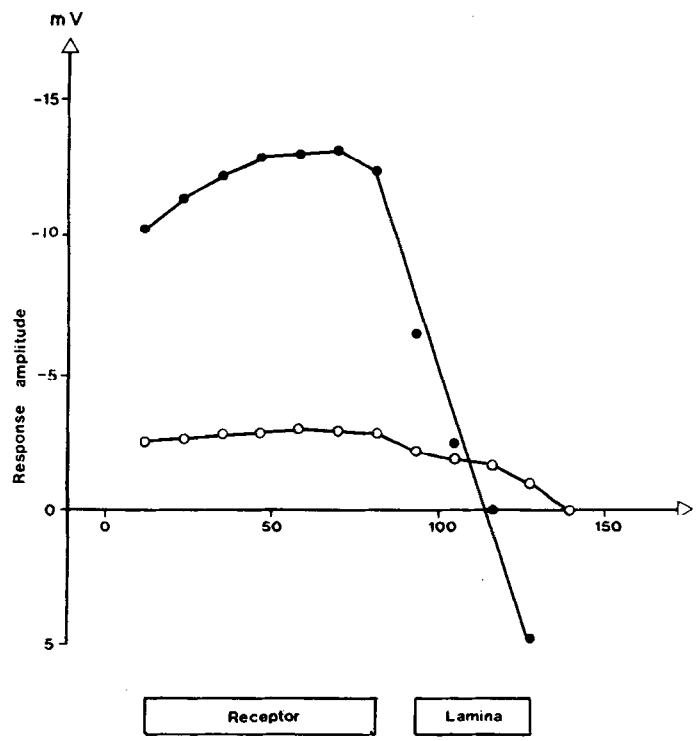

Fig. 2. Response profile. The sustained response of a bwçn fly (● orange adapted, O blue adapted) to a 1 sec blue testflash (465 nm) of 4.4 W/m², arrangement A. The response was recorded along a track through the lamina and receptor region outwards to the cornea, where contact is lost. The abscissa gives the position of the tip of the electrode with respect to the surface of the eye.

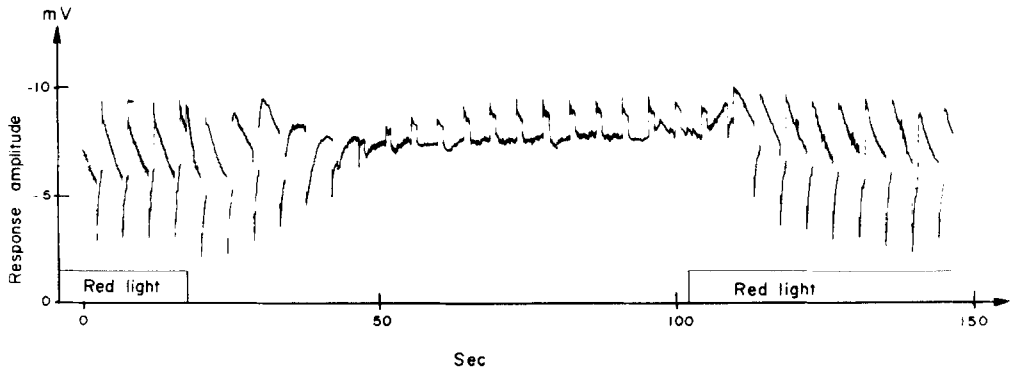

Fig. 3. The lamina response of a bwɛn fly to a 1 sec blue testflash of 5 W/m², arrangement A. The fly was orange adapted with an additional red light of 0.57 W/m². After the red light was switched off, the testflash brought the fly into a blue adapted state, which was reverted again by the additional red light.

the eye, where the sustained part of the response to a testflash in an orange adapted fly disappears, the lamina region (Fig. 2).

(e) Except for the on-transient in Fig. 5 all figures show negative potential upwards (HOLMGREN, 1865).

RESULTS AND DISCUSSION

Individual preparations can vary by about two-fold in the absolute magnitude of the response. Most of the figures therefore show individual records, although all experiments were repeated several times. No differences have been found between male and female flies in the experiments reported here, also no differences between red-eyed wildtype and white-eyed bwɛn flies were found which could not be attributed to the presence or absence of the screening pigments.

(a) *Lamina waveforms*

BURTT and CATTON (1964) have introduced the term potential profile to characterize the changes of the extracellular response from the receptor to the lamina region of the locust. HEISENBERG (1971) used the same procedure for *Drosophila*. Figure 2 shows a response profile of a bwɛn fly, both for the orange adapted state and the blue adapted state. For this and all other lamina recordings the tip of the micropipette was placed onto a circumscribed area approximately at the mid dorso-ventral line, 0.05 mm above the equator.

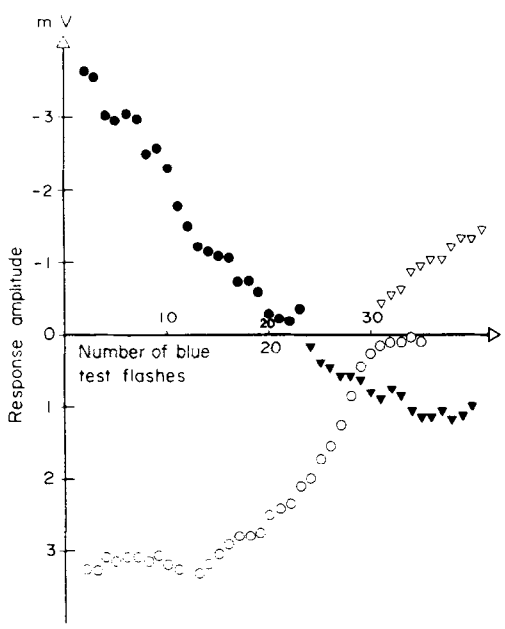

Fig. 4a. On-response (open symbols) and off-response (closed symbols) to a 1 sec blue testflash of 0.5 W/m² in lamina recordings, arrangement A. An orange adapted bwɛn fly is carried into the blue adapted state by repeated testflashes. Due to a small timelag part of the response attributable to R7, R8 can be distinguished from that attributable to the on-transient in the direct traces evaluated for this figure. This is indicated by using triangles for the R7, R8 response and circles otherwise.

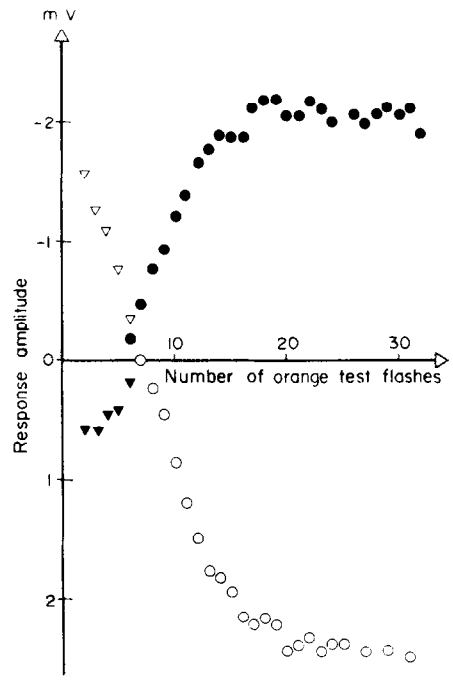

Fig. 4b. On-response (open symbols) and off-response (closed symbols) to a 1 sec orange testflash (603 nm) of 2.7 W/m² in lamina recordings, arrangement A. A blue, 1 min dark adapted bwɛn fly is carried into the orange adapted state by repeated testflashes. Symbols as in Fig. 4a. In contrast to Fig. 4a, the response goes through a point where both on- and off-response apparently disappear.

Inserting the electrode along the ommatidial axis of an orange adapted fly results in the following sequence of events as it is advanced: initial small increase in amplitude of the sustained part of the response, then a marked reduction through a nullpoint and subsequent increase with change of sign. At the nullpoint the on-transient and off-response are the dominant features of the response to a testflash. In the blue adapted state the response is nearly constant in the receptor region but drops to about 60% in the region assigned as lamina.

The stereotactic assignment of Fig. 2 is somewhat different from that given by HEISENBERG (1971). This is due to moving the electrode outwards in the particular experiment shown. Although up to the lamina region the response profiles are in principle reversible, moving the electrode inwards is more likely to lead to distortion of the eye. It is this difficulty which led us to choose a physiological definition of the lamina region, rather than a stereotactic one.

Proceeding further into the eye the response in the blue adapted state disappears. Beyond this point (not shown in Fig. 2) inverted responses are found both for the orange adapted and the blue adapted state. These are of smaller amplitude, but otherwise mirror the responses in the receptor region.

Figure 3 shows a direct trace from a lamina recording of a bwçn fly, which was initially orange adapted, then transferred into the blue adapted state by the intense blue testflash and finally brought back to the orange adapted state by an additional red light. The figure demonstrates that the shift in d.c. potential in the receptor region, characteristic for the blue adapted state (WRIGHT and COSENS, 1977) is only small in the lamina region. On-transient and lamina off-response are driven by cells R1 to R6, consistent with the anatomical observation, that these cells synapse in the lamina while the axons of R7 and R8 pass directly through the lamina into the medulla (BOSCHEK, 1971). As Figs. 2 and 3 show, however, the monophasic response of R7, R8 can be picked up in the lamina. In going from the orange adapted to the blue adapted state, characteristically the lamina off-response disappears first and thereafter the on-transient before the R7, R8 response develops fully. This was also observed in the ERG by WRIGHT and COSENS (1977). By going into the orange adapted state, however, the response can disappear temporarily before the on-transient and off-response reappear. Figure 4a and b provide details of this phenomenon. The experiment that led to Figs. 4 a and b also served to answer the question whether the responses picked up in the lamina can be understood as a straight addition of a constant R7, R8 response and an R1 to R6 mediated response, which becomes smaller as the fly is transferred into the blue adapted state. This is not immediately obvious in Fig. 3 as the testflash used is rather intense and the adaptational state quite different at the beginning and end of each testflash. Careful inspection of direct traces using less intense testflashes, however, reveals that under the conditions used for Figs. 3 and 4 response components behave in an additive manner.

(b) *Response characteristics*

As outlined in the Introduction, the different components of the ERG and lamina response can be isolated by using various experimental regimes. Figure 5 shows the response amplitude of the lamina on-transient as a function of the intensity of an orange testflash. At 20°C the on-transient is delayed by about 10 msec with respect to the testflash. The onset of the R7, R8 response is delayed by approximately 18 msec such that it does not seriously interfere with the development of the on-transient even at high intensities. Neither (as in the ERG) does the sustained part of the R1 to R6 response interfere, since

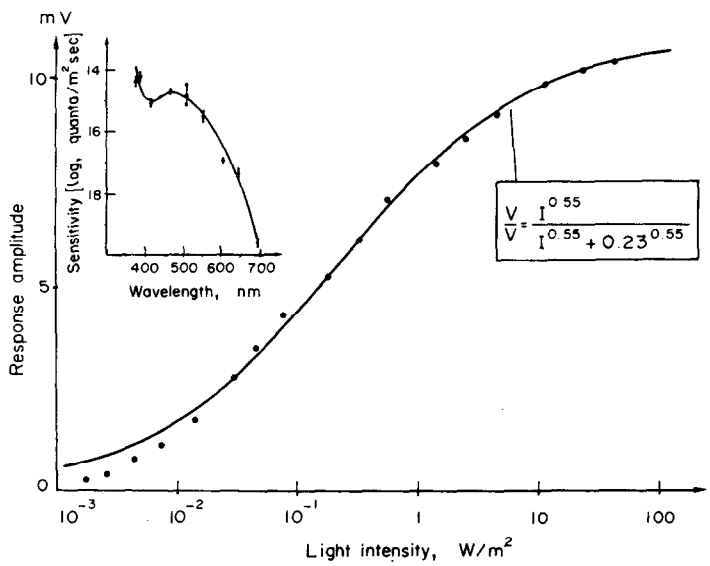

Fig. 5. Lamina on-transient of an orange adapted bwçn fly as a function of the intensity of a repeated 1 sec orange testflash, arrangement A. The solid line represents a theoretical receptor response curve (see text). Insert: Action spectrum for the lamina on-transient obtained as described by HARRIS et al. (1976), 2 mV criterion, $n = 4$. Error bars indicate standard deviations.

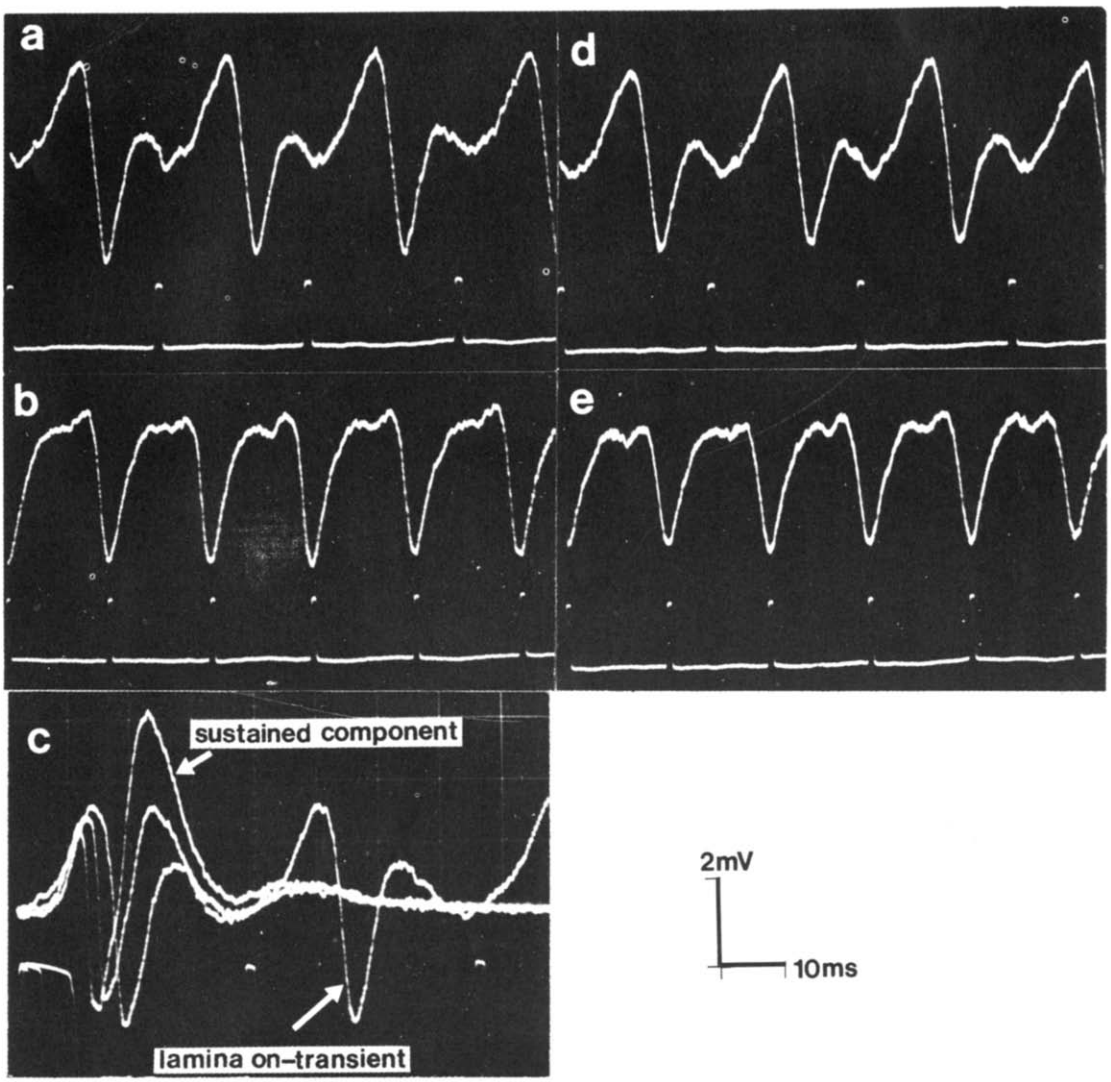

Fig. 7. (a) Response of a wildtype fly to a flickering orange light (50 W/m^2, arrangement B) below fusion frequency of the slow fusing component attributed to R7, R8. (b) Response of the same fly to the flickering orange light near fusion frequency of this component. (c) Three superimposed response traces which show the reducing amplitude of this component with increasing flicker frequency[1] (fusion frequencies of this component are indicated by the open triangle in Fig. 8) (a), (b) and (c), are recorded in the receptor region. (d) and (e) are equivalent lamina responses which indicate that at relatively high flicker frequencies the response is dominated by the lamina components. Lower trace in each picture is the response of a phototransistor. Note: 1. Since a rotating disc was used in this experiment increasing flicker frequency also reduces pulse duration; however COSENS and WRIGHT (1975) obtained data from bwgn flies similar to the present wildtype data. Moreover this was true for a rotating disc as well as when using an LED which provides a constant pulse duration with increasing flicker frequency. 2. Amplitudes in (d) and (e), recorded subsequently to (a) and (b), are reduced due to preparation fatigue: normally the on-transient would be larger.

recordings are made at a point where this is at a minimum. Correspondingly the simple relation

$$I/I_{max} = \frac{I^{\chi}}{I^{\chi}+\sigma^{\chi}}$$ (LIPETZ, 1969; MINKE *et al.*, 1975)

with I being the response amplitude
I, the light intensity
σ, the midpoint of the transition
and χ, characterising the steepness of the curve

approximates the response characteristics rather well, at least for light intensities above 3×10^{-2} W/m^2. The insert shows the spectral sensitivity of the on-transient. It has the characteristic spectrum of the R1 to R6 response (MINKE *et al.*, 1975; HARRIS *et al.*, 1976).

The off-response is slightly more complex (Fig. 6). The deflection in the curve at an intensity of 0.1 W/m^2 coincides with the onset of a contribution from R7, R8 to the response amplitude, which for the off-response is not well separated in time from the lamina off-response going in the opposite direction. At high intensities the initial rise of the off-response decreases slightly while an after-potential not seen at lower intensities is still increasing. This is indicated in the figure. At low light intensities the action spectrum of the off-response has the same characteristics as that of the on-transient. At higher response levels, however, the action spectrum also contains contributions from R7, R8.

The lamina response in the blue adapted state is identical with the corresponding ERG response (MINKE *et al.*, 1975; COSENS and WRIGHT, 1975; HARRIS *et al.*, 1976) in all respects except for the absolute amplitude. We probably observe in the lamina the direct response of cells R7, R8 in a somewhat attenuated form. These may be responses recorded from the axons of these cells, passing through the lamina.

(c) *Flicker fusion frequencies*

The transmission characteristics of visual systems can be characterised by the flicker fusion frequency (AUTRUM, 1958; COSENS and WRIGHT, 1975; WU and WONG, 1977). The actual point of fusion depends of course on the signal to noise ratio. However, the decrease of the signal with increasing frequency is quite precipitous near the high frequency cut off (WU and WONG, 1977). The signal to noise ratios at low frequencies as used in our experiments (30–100 Hz) are sufficiently high that this dependence can be neglected. Figure 7 shows responses of an orange-adapted wildtype fly to the flickering light recorded in the receptor and lamina regions. Three components of the response can be distinguished by flicker fusion, the most prominent being the lamina on-transient, the other two correspond to the sustained parts of the ERG. The two receptor components fuse at different flicker frequencies. An identical response is found in white-eyed flies (COSENS and WRIGHT, 1975). By blue adapting the white-eyed fly the component fusing at a lower frequency is identified as the response of R7, R8. This assignment allows a determination of the flicker fusion frequency of the R7, R8 response even in the orange adapted wildtype.

At temperatures above 15 °C the lamina on-transient of the orange adapted fly is the dominant response, especially at high flicker frequencies. This suggests that the high frequency cutoff is mainly determined by the receptor cells and less by the lamina. When the temperature of the preparation is lowered the lamina on-transient and off-response become smaller. Around 10 °C the off-response disappears, and at a temperature 0.5–1 °C below this point the on-transient also disappears, with the critical temperature of disappearing lamina responses varying somewhat with different preparations. Below this temperature the response to a flickering light is entirely due to the sustained parts of the ERG.

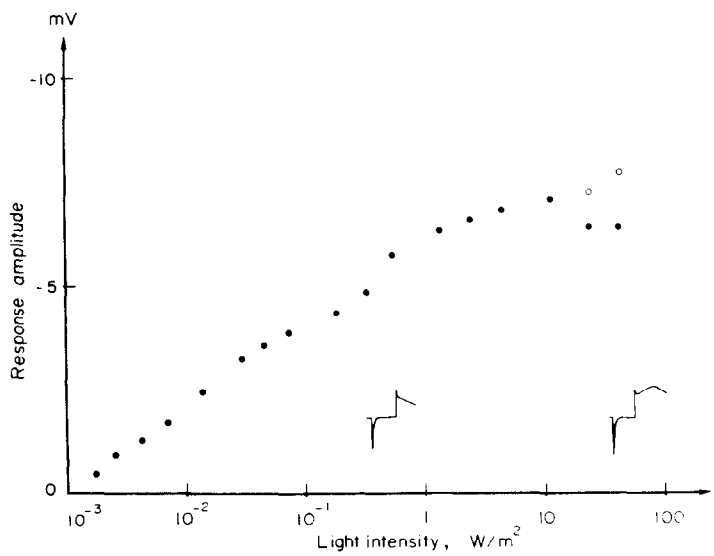

Fig. 6. Lamina off-response of an orange adapted bwcn fly as a function of the intensity of a repeated 1 sec orange testflash, arrangement A. In order to allow a direct comparison, data from the same fly and the same experiment as that for Fig. 5 are shown. At the highest intensities used, the immediate off-response drops slightly and an afterpotential develops; this reaches a peak (open symbols) up to 2 sec after the initial rise. Typical responses for two light intensities are sketched below the response curve.

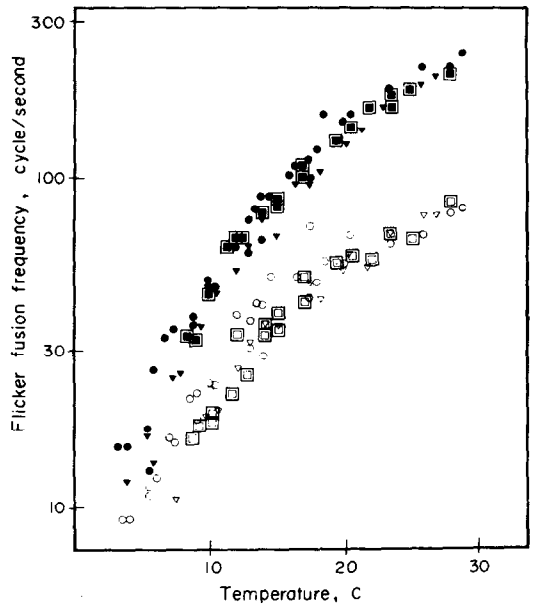

Fig. 8. Flicker fusion frequencies as a function of temperature. Closed symbols: orange-adapted flies, ▼ wildtype receptor, ● bw$\underline{c}$n-receptor, ■ bw$\underline{c}$n lamina region. Open symbols: blue-adapted bw$\underline{c}$n flies, ○ receptor, □ lamina region, ▽ slow fusing response of wildtype (note not blue-adapted, see Fig. 7). Arrangement B, orange light 50 W/m^2, blue light 14 W/m^2. Experiments with the light intensities reduced by 10 fold gave the same flicker fusion frequencies.

Figure 8 shows the flicker fusion frequency as a function of the temperature for wildtype and bw$\underline{c}$n flies. The flicker fusion frequencies of the receptor responses are the same for the wildtype and the bw$\underline{c}$n flies, both for the components mediated by R1 to R6 and the component attributable to R7, R8. The figure also shows that the flicker fusion frequency of the lamina response above 10°C is indistinguishable from that of the receptors.

WU and WONG (1977) "found no strong evidence indicating that R1 to R6, R7 and R8 have any distinct difference in the frequency response". In contrast Fig. 8 shows that even below 10°C, where the lamina response has disappeared, the flicker fusion frequencies of the R1 to R6 and R7, R8 are different. Particularly above 20°C they can be used as an identification criterion and as a further tool to isolate the contributions of the visual subsystems of *Drosophila melanogaster*.

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