Biophys. Struct. Mechanism 5, 211–222 (1979)

Biophysics of Structure and Mechanism

Blue Adaptation: An Experimental Tool for the Study of Visual Receptor Mechanisms and Behaviour of *Drosophila**

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Abstract. Physiological and behavioural studies with *Drosophila* to elucidate visual mechanisms have exploited the bi-stability of the visual pigment in the peripheral retinula cells R1–6, and the 'off-on switch' action of blue and orange light. Measurements of flicker fusion and response waveform from both receptor and lamina regions prior and subsequent to blue adaptation, which induces a prolonged depolarising afterpotential and loss of visual function in R1–6, show these retinula cells to have a high fusion frequency and R7/8, the central retinula cells, a lower fusion frequency. Such measurements also allow analysis of the extracellular response in terms of contributing cells, and its potential for studying the fly's ability to respond to various potential visual cues such as a rotating plane of polarised light. Blue adapted flies fail to fixate normally a black stripe, confirming a role for R1–6 in orientation behaviour requiring a competent degree of acuity.

Key words: Blue adaptation – Flicker fusion frequency – Orientation behaviour.

Introduction

Two methods of manipulating the visual system of *Drosophila* have proved useful for the study of its visual receptor mechanisms and behaviour. One, genetic dissection, involves the use of mutants lacking certain parts of the eye (Harris et al., 1976). The other uses the fact that the rhodopsin (R480) of the peripheral retinula cells R1–6 converts with blue light to a *stable* metarhodopsin (M580) (Ostroy et al., 1974), since the consequence of this isomeric change is that these retinula cells are rendered refractory in the blue light – the refractoriness continuing in subsequent darkness, indeed until exposure to orange light. The refractory state has been termed *blue adaptation* (Wright and Cosens, 1977), and is observed electrophysiologically as a prolonged depolarising afterpotential – a late receptor potential that is sus-

* Based on material presented at the European Neurosciences Meeting, Florence, September 1978

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tained beyond the light stimulus. The afterpotential can be cancelled quickly concomitent with return of visual sensitivity by orange adaptation (Cosens and Briscoe, 1972; Cosens and Wright, 1975; Minke et al., 1975), which has been shown also to photoconvert M580 to R480 (Ostroy et al., 1974). The role of the R1–6 subsystem in information processing and behaviour may be assessed by switching it *off* with blue light and *on* with orange light and observing the resultant change in performance. Our studies have been to correlate these two levels of analysis.

The Extracellular Response

The extracellular response, commonly refered to as the electroretinogram ERG), is recorded easily with a Ringer-filled micropipette from the receptor region of the eye and the lamina neuropile – a region largely unexplored. Response waveforms are complex, and despite innumerable studies since that of Holmgren (1865), the ERG has remained ignomatic. For lamina recordings the tip of the micropipette is placed onto a circumscribed area at the mid dorsoventral line of the eye, 0.05 mm above the equator, then inserted along the ommatidial axis to a position where the sustained part of the response to a test-flash disappears in an orange adapted fly. This occurs at a depth of some 100 μm.

Blue adaptation for extended periods of time will create an extracellular prolonged corneal negative afterpotential (the extracellular equivalent of the prolonged depolarising afterpotential), in wildtype flies as well as eye colour mutants. Under such conditions only R7/8 contribute to the ERG – the reponse (no distinction between R7 and R8 can be made), is monophasic (Fig. 1) and can be recorded from

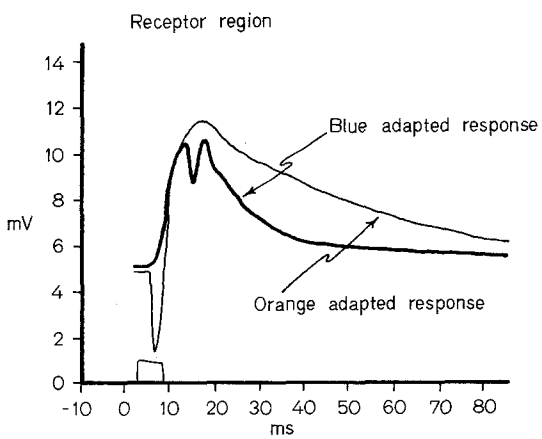


Fig. 1. Extracellular responses from the receptor region recorded with a Ringer-filled micropipette electrode axially inserted to 40 μm, a gold wire indifferent electrode in the thorax, coupled via an impedence probe for display on an oscilloscope and photographed with a polaroid camera. *Note:* The 'notch' in the monophasic (blue adapted) response of R7/8 does not represent the lamina on-transient of the diphasic (orange adapted) summated response at a longer latency. It is a consequence of the high intensity of the test-flash which causes the response to oscillate initially – a longer light exposure is required to show more of the oscillations. The effect is interesting and probably a receptor membrane phenomenon

both the receptor and lamina regions. It is interesting with reference to the idea of receptor and lamina compartments proposed by Heisenberg (1971), that this monophasic response can be recorded from the lamina region where it drops to about 60% of it's almost constant amplitude in the receptor region – but does not reverse in polarity. Proximal to the lamina the response disappears (Cosens and Spatz, 1978). Figure 2 is a direct trace recorded from the lamina of a white-eyed (*bwcn*) fly, which, initially orange adapted, is transferred into the blue adapted state by the intense blue test-flash in the dark period and then is brought back to the orange adapted state by additional orange light. The trace shows the isolation of the monophasic R7/8 response, and that the shift in d.c. potential (the afterpotential), which in the receptor region is characteristic for blue adaptation, is only small in the lamina region. Furthermore, the trace shows that the lamina on-transient and off-response are clearly driven by R1–6 consistent with anatomical observation (Goldsmith and Bernard, 1974). When going from the orange adapted state to the blue adapted state characteristically the lamina off-response disappears first and then the on-transient *before* the R7/8 response develops fully. This is true also for the receptor region. Analysis of these changes when the test-flash is less intense than that used in the experiment figured (i.e., the changes are slower), shows that the waveform is a summation of a constant R7/8 response and a R1–6 mediated response which becomes smaller as the fly is transferred into the blue adapted state (i.e., it's R480 becoming M580) (Cosens and Spatz, 1978). When reverting to the orange adapted state, however, the R7/8 response reduces in amplitude and may disappear temporarily before the summated response with lamina components recovers. From such studies it becomes possible to recognise active cells contributing to the extracellular

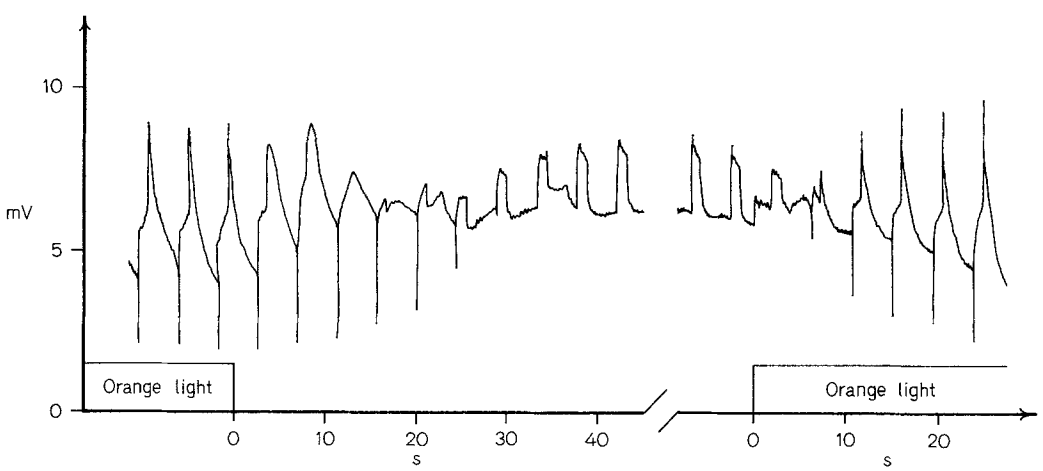


Fig. 2. The lamina response of a *bwcn* fly to a 1 s test-flash of 5 W/m² repeated every 4 s. The fly was orange adapted with an additional ambient orange light of 0.57 W/m², when the orange light was switched off the blue test-flash brought the fly into the blue adapted state, which was reverted again by the addition of the orange light. *Note:* As the *R*1–6 receptors are rendered refractory the lamina cells they drive cease to contribute to the extracellular response, first the off-response disappears and then the on-transient. Ultimately the *R*7/8 response is isolated. It is interesting that the d.c. shift (afterpotential) is small

response, both in the receptor region and the lamina neuropile; we have used this potential to probe some mechanisms of visually mediated behaviour.

The Extracellular Flicker Response to Polarised Light

Does *Drosophila* respond to changes of the direction of the E-vector of polarised light, and if so with which receptors? Stark (1977) found no response to the direction of the E-vector of polarised light in white-eyed flies. However, large contributions to the extracellular response of the white eye come from off-axis ommatidia which receive scattered, partially depolarised light. The wildtype red eye where screening pigments provide optical isolation of ommatidia for short wavelength light is a better substrate. And the recent observation of different R7 cells in the eye (Kirschfeld et al., 1978), made further examination hopeful.

Rotating a polarising filter between a light source and a receptor designed to maximise its response to one plane of polarised light will produce for that receptor an intensity modulation: a *flicker*. Cosens and Wright (1975), have established that for white-eyed (*w*) flies the R1–6 and R7/8 subsystems have different flicker fusion frequencies. Thus we have a method – although we need to understand the extracel-

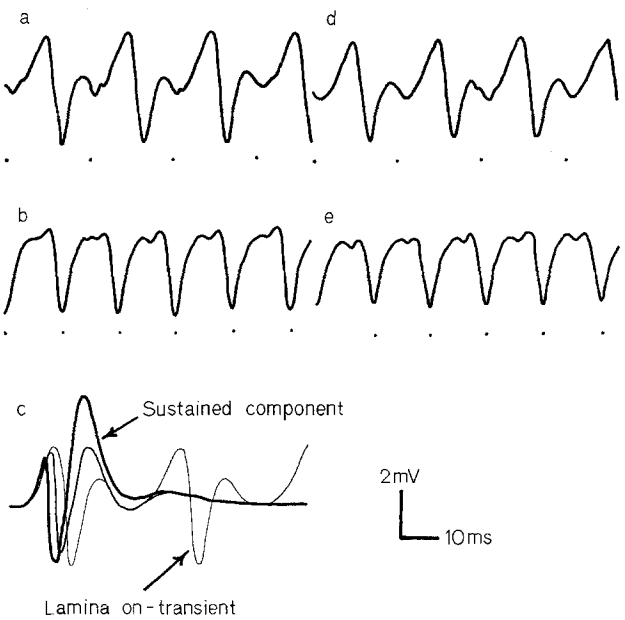


Fig. 3. Extracellular responses of a wildtype fly to a flickering orange light (50 W/m²) recorded from the receptor region: **a**, **b**, and **c**, and the lamina neuropile: **d**, and **e**. The responses show the change in waveform as the component from R7/8 fuses. Note that the responses are the same whether recorded from the receptor or lamina regions. The flickering light was produced by rotating a cardboard disc with an 18° slit (giving a duty cycle of 1 : 20) between the source and a defining slit of 3°, the light being focused onto the eye as a 2 mm spot. The disc was driven at variable speeds by a d.c. motor

lular flicker response of the wildtype red eye in terms of contributing (active) cells. The actual point of fusion of the extracellular response to a flickering light depends on the signal to noise ratio. However, the decrease in response amplitude with increasing frequency is quite sharp near to the fusion point and the signal to noise ratios at the frequencies used (30–100 Hz) are sufficiently high to ignore this dependence. A flickering light was produced by rotating a disc with an 18° slit infront of a source and behind a 3° defining slit, giving a duty cycle of 1 : 20. The disc was driven by a d.c. motor at variable speeds. Three components can be identified as contributing to the flicker response recorded from either the receptor or lamina regions of an orange adapted wildtype fly: a prominent lamina on-transient and the two sustained receptor components of R1–6 and R7/8. The receptor components fuse at different frequencies. By blue adapting the white-eyed fly the component fusing at a lower frequency is identified as the response of R7/8. This measurement (70 Hz at 26° C), enables the R7/8 component contributing to the wildtype response to be recognised because the response waveform changes markedly at this frequency to be dominated by the on-transient. Figure 3 shows this change: a, and b, are receptor responses from an orange adapted wildtype eye; d, and e, are identical responses recorded from the lamina. c, three superimposed responses as frequency is increased towards the fusion point of the R7/8 component – the thin trace recorded at the frequency of a, and d, (i.e., 50/s); fusion occured in this preparation at 66/s measured at b, and e. Further isolation of the components can be effected by lower-

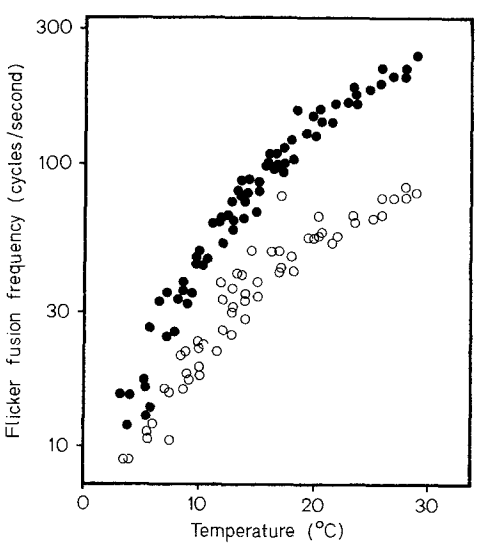


Fig. 4. Flicker fusion frequencies as a function of temperature. The upper curve of solid circles is comprised of data from orange adapted flies: Both wildtype and *bwcn*, and responses recorded from both receptor and lamina regions; the curve defines the behaviour of R1–6. The lower curve of open circles is comprised of data from blue adapted *bwcn* flies, both receptor and lamina responses; and the slow fusing response of the wildtype attributed to R7/8; the curve defines the behaviour of R7/8. *Intensities used were*: Orange light 50 W/m^2, blue light 14 W/m^2; experiments with intensities reduced by 10-fold gave the same flicker fusion frequencies

ing the temperature of the preparation when the lamina on-transient is blocked at a critical temperature of 9 ± 1° C for most preparations. Below this temperature the response is entirely due to receptor activity. Figure 4 shows the flicker fusion frequencies as a function of temperature for wildtype and *bwcn* flies: the data are the same for red-eyed and white-eyed flies for both the components mediated by R1–6 and the component attributed to R7/8. Furthermore, flicker fusion frequencies of the lamina response above 10° C are indistinguishable from those of the receptor region. Interestingly below 10° C the fusion frequencies of R1–6 and R7/8 are quite different. This observation is contrary to the data of Wu and Wong (1977), who found no evidence indicating that the two subsystems have any distinct difference in frequency response. Pertinent here is the fact that the frequencies, particularly above 20° C, can be used as identification criteria; and elsewhere that the curves drawn by the experimental data can be used as standards against which to compare other mutant stocks for normality.

The remaining question was empirical: could we record a modulation with the extracellular response? Polarised light with the direction of the E-vector slowly rotating was provided by mounting a polarising filter (Kodak pola screen, 322 6503), in a tube rotated by a belt drive. Changes in light intensity caused by the rotation were minimised by collimating the light from a Xenon arc lamp to a narrow beam to pass through the centre of the rotating filter. Reflections were cut-off by two diaphragms and the light beam focused to a 0.8 mm diameter spot onto the eye of the fly. Notwithstanding, modulations of intensity of 5% could not be avoided. However these were too small to be picked up by our extracellular recordings; and the flies did not noticably respond to the random fluctuations of the Xenon lamp which were of the same order of magnitude. As a reference, a defined intensity modulation was obtained by placing a second, stationary polarising filter into the light beam: modulations of between 2–100% at 600 nm, 1–100% at 450 nm and 4–100% at 360 nm were recorded using a Tektronix J16 Digital Photometer and J6502 probe. Our present data are inconclusive (Cosens and Spatz, unpublished): but indicate some evidence for a response from R7/8 to the changing direction of the E-vector of polarised shortwavelength light. Certainly there is no response from R1–6 nor from units in the lamina region. Figure 5 shows the response of a wildtype fly to polarised light (360–450 nm) with changing direction of the E-vector. Having been either blue or orange adapted, the fly was left in the dark for 1 min (upto min 0), then exposed to the polarised light with slowly rotating E-vector (min 0–1), a second polaroid was inserted into the light beam (min 1–2), then removed (min 2–3), and the light switched off at min 3. The upper trace of the figure is from an orange adapted fly and the lower trace from a blue adapted fly. The modulation of light intensity when the second polaroid is inserted is between 1 and 100% for blue light, the fly responds with a 2 mV response. If this response amplitude is taken proportional to the logarithm of the modulation of the light intensity, the 0.4 mV response of the fly seen between min 0–1 and 2–3 of the lower trace would be equivalent to a modulation in intensity of approximately 60% – certainly more than would be expected in response to the noise of 5%. The amplitude modulation must be a true response to changes of direction of the E-vector of polarised light but it is too small for quantitative measurements. The response has a period of 180° with respect to the rotating polaroid, is of the same order of magnitude in both receptor and lamina regions and

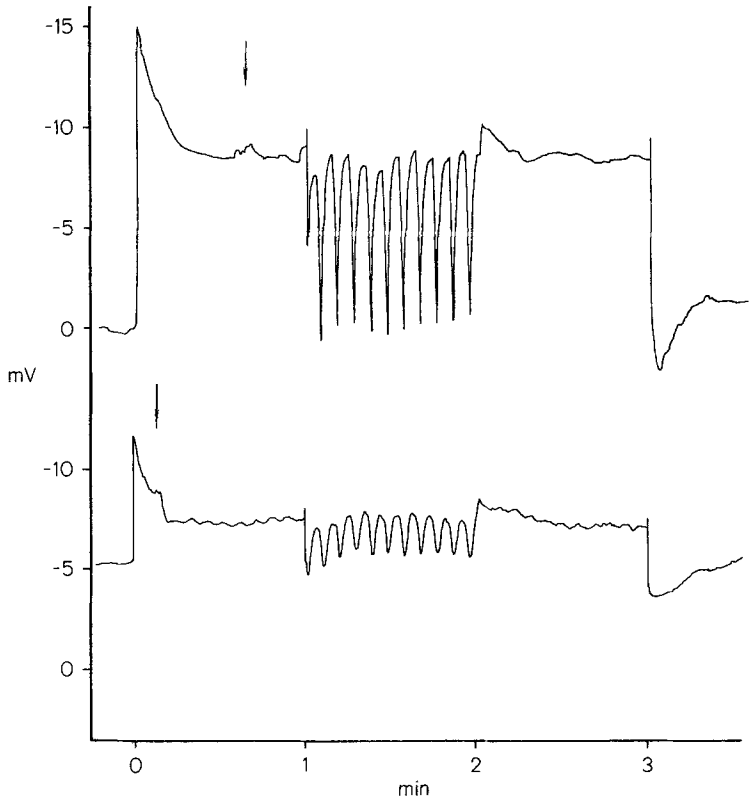


Fig. 5. Direct traces of the response of a wildtype fly to polarised light with changing direction of the E-vector. The experiment was carried out by leaving the fly in the dark for 1 min (up to min 0), exposing it to the polarised light with slowly rotating direction of the E-vector (min 0–1), inserting a second stationary polaroid into the light beam (min 1–2), removing the second polaroid (min 2–3), and then switching off the source (min 3 onwards). The arrows indicate potential changes caused by the movement of the fly's abdomen. *Upper trace*: orange adapted wildtype fly, orange light 70 W/m^2. *Lower trace*: blue adapted wildtype fly (note the afterpotential bracket), blue light 50 W/m^2. The modulation of light intensity when the second polaroid is inserted is between 1–100% for the blue light. The lower trace shows a clear response to the changing direction of the E-vector of the blue light

is only detected at wavelengths between 360–450 nm at intensities above 4 W/m^2. Reducing the aperature from 8°, atwhich most measurements were made, to 2° did not increase the response, neither did blue adapting the wildtype fly and preadaptation with blue light of 40 W/m^2 at a fixed direction of the E-vector for 20 min did not noticably alter the response either.

Orientation to a Black Stripe

Here we wished to study the effect upon behaviour of denying *Drosophila* certain visual cues gathered R1–6. Pattern induced orientation of freely walking flies ('closed loop'), has been studied both for optomotor behaviour (Hecht and Wald,

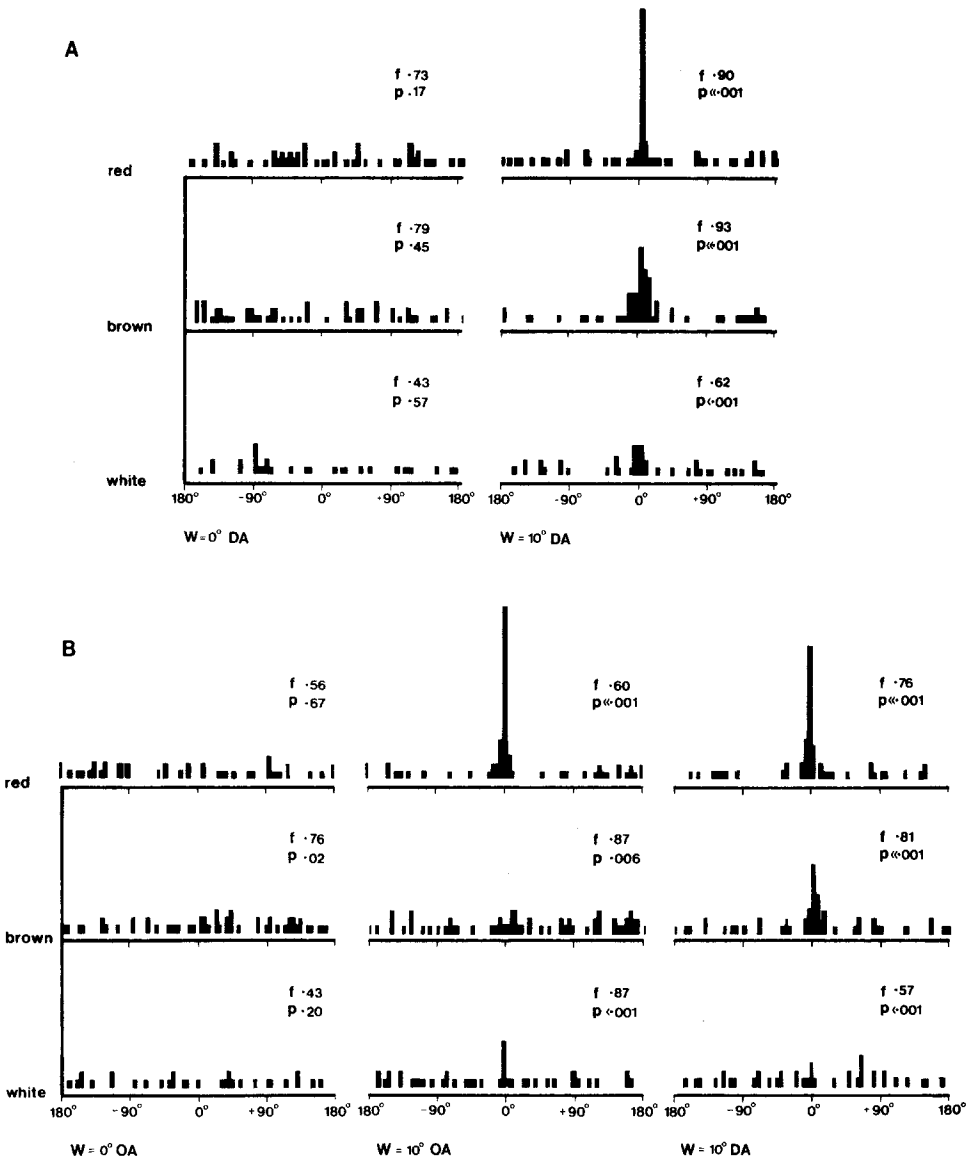
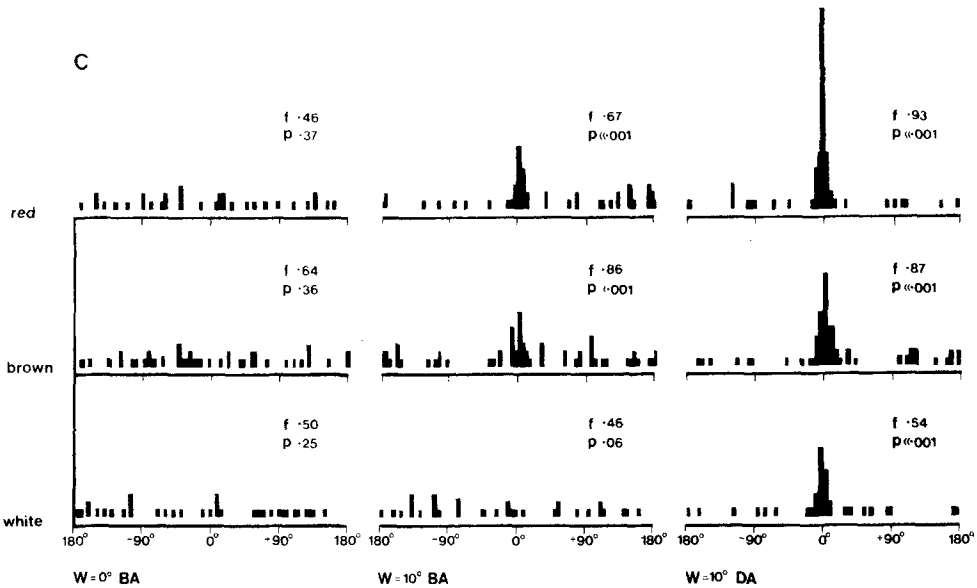


Fig. 6. (Legend see page 219)

1934; Kalmus, 1949), and more recently for tropotactic and fixation behaviour (Wehner and Horn, 1975; Horn and Wehner, 1975). The latter studies have shown that *Drosophila* fixate and walk towards a black stripe of angular width 10° in a circular arena. The efficiency of this behaviour depends on the extent of eye pigmentation, and, it is argued, is mediated by visual cues gathered by R7/8 (Wehrhahn, 1976). We have used this behaviour (as a measure of visual acuity), together with blue and orange adaptation to reassess the role of R1–6 in the visual orientation



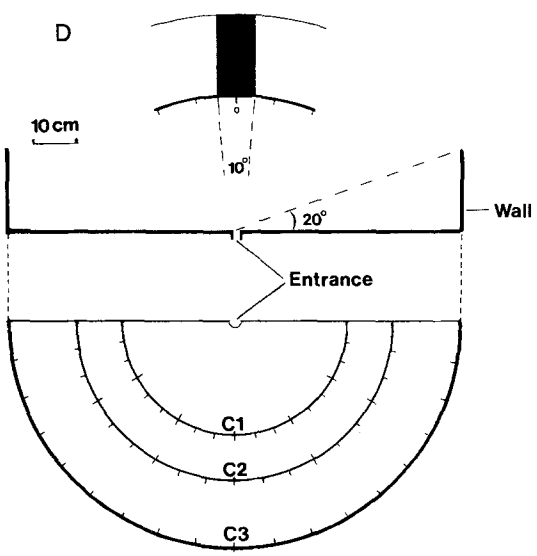


Fig. 6. Angular distributions at C3 for the three phenotypes: eye colour shown on the left. A, run under white light, B, under orange light, C, under blue light. W = 0° : no stripe; W = 10°: with a stripe. *DA* dark adapted, *OA* orange adapted, *BA* blue adapted. *f* = percentage of flies reaching C3 from the population of 70. 1 black square represents 1 fly per 5° sector. Note how the flies do better under blue ambient light *when dark adapted*, but that *blue adaptation* impairs orientation accuracy drastically. The brown eye performance when orange adapted is curious: these flies appeared to move away from the stripe at the last moment. The insert illustrates the arena. Ambient light intensity for each of the colours was 0.7 W/m^2 at the arena centre falling to 0.4 W/m^2 at C3. Flies were adapted in intensities of 1.15 W/m^2 in the case of brown and white-eyed phenotypes, and 7.5 W/m^2 in the case of the red-eyed wildtype

behaviour of red-, brown-, and white-eyed flies; since the high flicker fusion frequency of the R1–6 subsystem would suggest that it is better able to discern spatial and temporal characters of a visual cue (form) in relation to the moving fly than the R7/8 subsystem.

Groups of 70 flies of each phenotype were used, males because they are more consistently active (Burnett et al., 1974). Each fly was run across the arena twice, once without the stripe, then immediately with the stripe which subtended an angular width of 10° at the central entrance to the arena. The time from the central entrance, and the angle relative to a fixed point on the arena wall, 0°, designated by the position of the midline of the black stripe for that pair of test-runs, were recorded for the first crossing of each reference circle (C_1 at 25 cm, C2 at 35 cm, C3 the base of the wall at 50 cm). Angles were measured clockwise and anticlockwise from 0–180° and are displayed on a linear scale in the figures. The time for each test-run was 180 s or until the fly reached C3. The binomial distribution was used to test for significant differences from random in the frequency distribution of flies at C3. A 10° sector ($0 \pm 5°$) at C3 was compared with a random expectation of $^1/_{36}$th of the population (0.0278) to be within that sector. The probability of the observed number, x, within that sector was calculated from the probabilities of having 0, 1, 2, 3, . . ., x flies within the 10° sector. The probabilities are additive and hence $P = 1 -$ total number (x) of additive probabilities.

Flies were dark adapted for 1 h, then chromatically adapted either blue (Balzer K2, 416–500 nm) or orange (Balzer K5, 564–644 nm) for 5 min in the case of *bw* and *bwcn* flies; and for 40 min in diffuse light of 7.5 W/m^2 in the case of wildtype flies. Then run under blue, orange or white light in the arena. Figure 6 shows the angular distributions at C3 for the three phenotypes. Orange adaptation is found to have little effect upon accuracy of orientation to the stripe. By contrast blue adaptation significantly reduces the accuracy and demonstrates that orientation in the closed loop situation depends upon some parameter seen by R1–6 and is not predominently mediated by R7/8 (Morton and Cosens, 1978). This finding complements the comparative study by Heisenberg and Buchner (1977) of the optomotor behaviour ('open loop') of receptor deficient mutants and blue adapted white-eyed (*w*) flies to both horizontal and vertical movements of stripe patterns, which has shown that normal responses require cues seen by R1–6. Furthermore, Willmund and Fischbach (1977), reported that following exposure to blue light for several hours wildtype flies show phototactic behaviour in a maze resembling that of mutants with defective R1–6.

Within the natural environment of *Drosophila* there is a preponderence of blue-green to UV wavelengths of light reflected from trees and grass and from the sky. This light could render refractory the peripheral retinula cells in those ommatidia looking directly at the source, for levels of light used experimentally (Broda and Wright, 1978: 2 W/m^2; Morton and Cosens, 1978: 7.5 W/m^2), are considerably less than expected from natural daylight (for example bright sunlight has been recorded as 1600 W/m^2, Young, 1977). Since the R1–6 subsystem is involved in simple phototaxis and more complex behaviour demanding an ability to resolve stationary and moving objects in the visual field, the red eye of *Drosophila* has ecological importance in maintaining the integrity of its visual system and thereby its ability to behave with biological success. What is less clear is why *Drosophila* has selected a

photon driven bi-stable visual pigment system. It is, however, most useful to the experimentalist.

Acknowledgements. The author would like to acknowledge Prof. H. Ch. Spatz who collaborated with this work.

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Received September 6, 1978/Accepted October 20, 1978