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[RAPID COMMUNICATION]

Differences in Flicker Fusion Frequencies of the Five Spectral Photoreceptor Types in the Swallowtail Butterfly's Compound Eye

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ABSTRACT—Intracellular recordings were used to measure flicker fusion frequencies (FFF's) as a function of light intensity (I) in the five types of spectral receptors (UV, violet, blue, green and red) in the compound eye of the butterfly, *Papilio xuthus*. FFF's in all receptor types increase with light intensities when stimulus I's are less than I_{50} (the I that generates 50% of the maximum response amplitude, Vmax, in the V-log I curve). FFF's in all receptor types are maximum at I's between approximately $I_{50}+0.5$ and $I_{50}+1$ log unit. At stronger I's FFF's of blue and green receptors decrease gradually but remain above 80% of the maximum FFF's. But UV, violet and red receptors maintain nearly maximal FFF's at I's above I_{50}. Maximum FFF's of green (107 Hz) and the blue (103 Hz) receptors are significantly higher than those of UV (90 Hz) and violet (82 Hz) receptors.

INTRODUCTION

Flicker fusion frequency (FFF) is a common measure of temporal resolution in vision. It marks the critical frequency at which discrete individual responses to or perception of a flickering light just become fused into a continuous response or perception.

Interspecific differences in temporal resolution of photoreceptors have been reported in Hymenopteran insects [8] and in Dipteran insects [6]. Do photoreceptors of different spectral sensitivities in a single retina have different temporal resolutions? In *Drosophila*, FFF's of peripheral retinula cells (R1-6) are about three times higher than those of central retinula cells (R7, 8). In these experiments, the eyes were selectively adapted with monochromatic light, and the responses were recorded by ERG method which are partially integrated retinal responses [4]. Yet the details of how primary visual processes determine different FFF's in the various types of spectral receptor cells are not known. The butterfly is an insect with compound eyes which cover an unusual wide spectral range [1] and therefore this insect is particularly suitable to investigate this problem. Here we report the first case about comparison of the temporal resolution of the different spectral classes of photoreceptor cells using definitive intracellular techniques.

In the compound eye of the swallowtail butterfly, *Papilio xuthus*, five spectral types of photoreceptors were identified by intracellular recordings. They have respective peak sensitivities around 360 nm (UV), 400 nm (violet), 460 nm (blue), 520 nm (green) and 600 nm (red) [1]. In the present study, electrical responses to flickering light were measured by intracellular recordings from the five photoreceptor types. The FFF's of each type were determined as a function of I.

MATERIALS AND METHODS

Animals, eye preparation, light stimuli and intracellular recording methods were the same as those used previously [2]. Since temperature can affect FFF [4], all experiments were carried out at a controlled room temperature between 22 and 24°C. The insects were dark adapted for 30 min previous to the experiments.

Flicker at various frequencies was produced by rotating a disc with an open sector allowing the beam to pass through. The waveform of each resulting light flash was an asymmetrical trapezoid with no background light. The light and dark periods were equal and fixed. The frequency and phase of the flashes were recorded with a photo-diode connected to the oscilloscope.

We used glass microelectrodes filled with 3M KCl. Since electrode resistance affects the recording condition, noise level, we measured with electrodes which had resistance of 70–80 MΩ. We rejected electrodes which had resistance of lower than 70 MΩ or higher than 80 MΩ for measurements.

When a photoreceptor cell was successfully impaled, its spectral type was determined with isoquantal flashes of monochromatic light of 22 interference filters each with a half band-width of 10 nm and a peak transmission ranging from 290 to 700 nm. The quantum flux of these monochromatic flashes at the corneal surface was adjusted with the optical wedge to 3×10^{10} photons/cm^2.s as measured with a radiometer (Model-470D, Sanso).

Then the V-log I curve for that cell was determined with monochromatic light flashes at each receptor's peak wavelength (λ_{max}). In these experiments I_{50} was defined as the stimulus light intensity evoking a response amplitude 50% of the maximum (V_{max}). This provided a physiological reference point for each cell studied. Stimulus durations for the V-log I measurements were 30 msec.

Then responses to flickering light were measured. Response

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amplitudes, peak to peak, were averaged. And a response amplitude of 0.5 mV, just strong enough to be discriminated from the background noise, was taken as the threshold for FFF. Flickering light intensity was increased stepwise from weak light intensity, and FFF was determined for each intensity. By these considerations the experimental artifacts can be minimized, and therefore the results obtained here are supposed to reflect almost the actual FFF's.

In the present experiments, responses to light flicker were measured only from photoreceptors which showed resting membrane potentials over 50 mV and a V_{max} response greater than 40 mV to a single flash stimulus. Therefore, our threshold criterion of 0.5 mV for FFF corresponds roughly to 1% of the maximal flash response. Each series of measurements took about 30 min. The reference intensity of each monochromatic light (Log=0) at the corneal surface corresponded to 1×10^{13} photons/cm^2.s.

RESULTS AND DISCUSSION

The data recorded here are based overall on intracellular recordings from 10 UV, 8 violet, 4 blue, 13 green, and 4 red photoreceptor cells. All responded to brief test stimuli of $I_{test}=I_{50}+2$ log units with at least 40 mV depolarization and were active long enough to make three or more series of flicker measurements at different stimulus intensities.

Our preliminary experiments indicated that the logarithm of the flicker response amplitudes (V) declined linearly with flicker frequencies (F) as expressed mathematically as follows:

$$V = a^{-bF}$$

where a and b are constants. The experiments also revealed

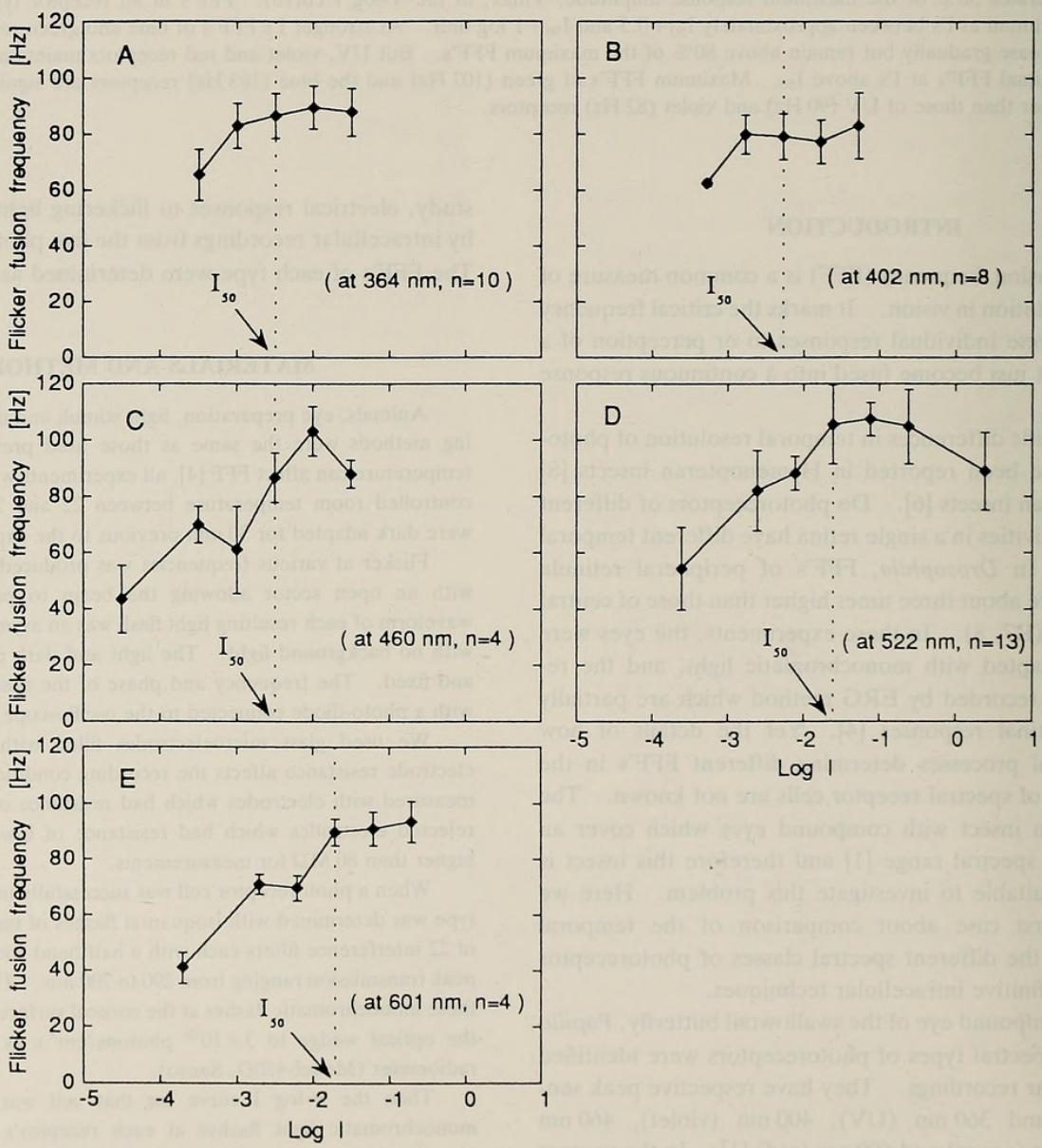

FIG. 1 A-E. FFF as a function of log I for the five types of spectral receptors: UV (**A**), violet (**B**), blue (**C**), green (**D**), and red (**E**). Intracellular recordings were made by 3M KCl-filled glass microelectrodes with resistances of 70–80 MΩ. Resting potentials were −50 to −70 mV. Bars indicate standard deviations. Wavelength of light stimulus is indicated in the parenthesis. Dotted vertical lines indicate I_{50} for each spectral receptors. I_{50} (UV)$=3.23\times10^{10}$ photons/cm^2/s. I_{50} (violet)$=5.31\times10^{10}$ photons/cm^2/s. I_{50} (blue)$=3.18\times10^{10}$ photons/cm^2.s. I_{50} (green)$=2.45\times10^{11}$ photons/cm^2/s. I_{50} (red)$=1.96\times10^{11}$ photons/cm^2.s.

that all five types of spectral receptor cells had almost the same gradients (b in the equation) in the linear relation between F and logarithm of V (Data not shown).

In green receptor (Fig. 1D), FFF's increased with stimulus intensities from 55 Hz at the light intensity of $I_{50}-2$ (log unit) to 105 Hz at I_{50}, then the FFF's stayed nearly constant between 104 and 107 Hz with intensities from I_{50} to $I_{50}+1$. At $I_{50}+2$ which produced nearly saturated responses to flash light stimulus, the FFF finally decreased to 90 Hz. The UV and blue receptors (Fig. 1A, C) followed nearly the same curve as the green receptor. The violet receptor (Fig. 1B), however, did not decrease its FFF's at strong light intensities (I_{50} to $I_{50}+1$), but yielded nearly constant FFF's between 79–83 Hz. In contrast, the FFF's for the red receptor (Fig. 1E) kept on increasing at all light intensities tested up to $I_{50}+1$.

Comparison shows that the green receptor has an FFF_{max} at 107 Hz with an intensity of $I_{50}+0.5$, next the blue receptor at 103 Hz with $I_{50}+0.5$, the red receptor at 95 Hz (or more) with $I_{50}+1$, the UV receptor at 90 Hz with $I_{50}+0.5$. The violet receptor had the lowest FFF at 82 Hz with $I_{50}+1$. A statistical analysis using a student's *t*-test among the highest FFF's, shows that there are significant differences between green-UV, green-violet, blue-UV and blue-violet receptors. The actual highest FFF's of violet and red receptors may be a little higher than those described above, because both receptors still showed increases in FFF's even at the highest intensities so far examined (Fig. 1). The FFF's of violet and red receptors were not recorded at strong light intensities above $I_{50}+2$ in the present experiments because their physiological condition usually deteriorated rather rapidly during intracellular recordings.

The present experiments demonstrate that there are significant differences in FFF_{max} for the five spectral types of *Papilio* photoreceptors previously reported [1]. The green and blue receptors have significantly higher FFF_{max}'s (107, 103 Hz) than do the UV(90 Hz) and violet receptors (83 Hz) (Fig. 2). If the green and blue receptors of *Papilio* are critical for scanning details of objects such as the green foliage of trees and other plants against the sky, their high FFF_{max}'s would likely increase temporal acuity for perceiving this visual pattern, particularly when flying. If so, the lower temporal acuities of the butterfly's UV and violet receptors may function in other ways to be determined. Presumably the UV receptor aids in discriminating UV light reflection of certain flowers [3] and may be, as it is in honeybees, important for discrimination of polarized light from the blue sky [9].

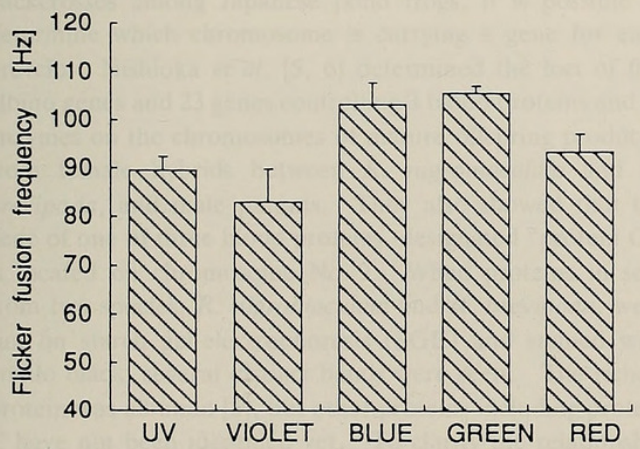

FIG. 2. Comparison of FFF_{max} for 10 UV, 8 violet, 4 blue, 13 green and 4 red receptors. The FFF_{max}'s of green and blue receptors taken together are significantly higher than those of UV and violet receptors with a probability of less than 0.05 that this difference would occur by chance (Student's *t*-test). *Bars* indicate standard errors of the means.

There are several methods to evaluate photoreceptor's temporal resolution. Those are FFF, frequency-response functions by using sinusoidally modulated stimulus, and impulse responses by using very brief flash stimulus. The reason of our choice of flicker fusion method was facility to compare quantitatively temporal resolution in wider stimulus intensity range and to find optimal stimulus intensity of maximal temporal resolution. And limitation of our experimental apparatus was also the reason of measurement by flicker fusion method. Flicker fusion method should be used with care about some aspects, because FFF may differ depending on recording condition, signal to noise ratio. We took care in following aspects to reduce errors of FFF caused by experimental condition, 1) the electrode resistances were kept in 70–80 MΩ, 2) experimental temperature was controlled in 22–24°C, 3) we only measured from photoreceptors which showed the maximal response amplitude of over 40 mV to single flash stimulus, 4) in 1950's and 60's, FFF's were measured by ERG and decided by researcher's own eyes, but in present study 0.5 mV of threshold amplitude was used for definition of FFF, and this definition of FFF by criteria threshold amplitude was used in both intracellular recordings [7] and ERG recordings [5].

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REFERENCES

1 Arikawa K, Inokuma K, Eguchi E (1987) Naturwissenschaften 74: 297–298

2 Bandai K, Arikawa K, Eguchi E (1992) J Comp Physiol A 171: 289–297

3 Barth FG (1985) In "Insects and flowers" Princeton Univ Press, Princeton, New Jersey, pp 116–122

4 Cosens D, Spatz HC (1978) J Insect Physiol 24: 587–593

5 Green DG, Siegel IM (1975) Science 188: 1120–1122

6 Laughlin SB, Weckström M (1993) J Comp Physiol A 172: 593–609

7 Nowak LM, Green DG (1983) Vision Res 23: 845–849

8 Souza JM de, Ventura DF (1989) J Comp Physiol A 165: 237–245

9 Wehner R, Rossel S (1985) In "Experimental behavioral ecology and sociobiology" Ed by B Hölldobler, M Lindauer, Fischer, Stuttgart New York, pp 11–53