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RECEPTORS IN INSECT

II. Electroretinogram of the Compound Eye in the Oriental Fruit Fly (*Dacus dorsalis* Hendel)

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Chin-Yih Wu (1989) Receptors in insect. II. Electroretinogram of the compound eye in the Oriental fruit fly (*Dacus darsalis* Hendel). *Bull. Inst. Zool., Academia Sinica* **28**(1): 7-13. The changes of the electroretinograms (ERGs) of the compound eye in the Oriental fruit fly (*Dacus dorsalis* Hendel) were studied upon stimulating by different monochromatic lights and intensities.

After a fly allowed to dark adaptation for 30 minutes, the 15 monochromatic flashing photic stimuli were given on the frontal region of the left compound eye at every 15 second intervals, and the ERGs were computed by 16 superimposed. These ERGs have a triphasic waveform designated as gamma, delta and epsilon in the order of their appearance on those graphic responses.

In a spectral response curve, we can suspect that the frontal region of compound eye possessed the U-V (λ max=348 nm), Indigo (λ max=431 nm) and Blue-Green (λ max=494 nm) visual pigments.

Key words: ERG, Oriental fruit fly, Spectral response curve.

The ommatidia of the compound eye in dipterans (including flies) are the real visual structures which possess the colour sensation and the light sensitivity. The fine structure of the ommatidia in the Oriental fruit fly has been studied in detail before (Wu *et al.*, 1985). In this fly, each ommatidium consists of eight elongated retinular cells surrounded by the primary and the longitudinal pigment cells at the periphery. Two of the inner located retinular cells, a superior and a inferior ones, are projected into the central ommatidial cavity (Wu *et al.*, 1985).

It is well established that the receptor layer of the compound eye in the fly responds to the photic stimulation with a slow potential termed the electroretinogram(ERG) (Green and Cosens, 1983; Loew, 1975; Tinbergen and Abeln, 1983).

The responses of the Oriental fruit flies to the various colours have been ecologically studied in Taiwan (Chiu, 1977; Chao *et al.*, 1979, Hsu and Hsu, 1972), but their electrophysiological responses are not yet studied.

The previous studies showed that the artificial light source, especially the ultraviolet light, was attractive some flies and the strength of phototaxis was influenced by the colour of the light (Broda and Willmund, 1981; Goldsmith, 1965; Green *et al.*, 1983; Naka, 1961).

In this study, we want to seek for an indication of the sensitivity and the spectral

range in the Oriential fruit fly, similar to those have been found in other flies; therefore, we intend to change the phototaxis wavelength from the ultraviolet toward the red light, and to measure directly the ERG of the compound eye for the study its responses to the spectral characteristics.

MATERIALS AND METHODS

1. Preparation of animals

The Oriental fruit flies, *Dacus dorsalis*, used in these experiments were derived from our laboratory.

Because the old flies may have the age-related changes in waveform of the ERG (Loew, 1975), they have been only selected for use in 1 to 2 weeks old of age. All experiments were performed at 25°C. room temperature.

After the flies were immobilized by chilling in refrigerator for about 3–5 minutes, they were mounted with bee's wax on the plate. Then the surfaces of the three ocelli and the right compound eye were covered with the black, soft bee's wax.

All experiments were performed at the "frontal region" (Hardie *et al.*, 1979) of the left compound eye.

2. ERG measurement

Under the light stereo-microscope, fifteen to twenty facets of the ommatidia together were pierced with a very fine tungsten pin, and then poured the insect saline into the tiny hole.

The standard extracellular recording techniques and instruments were used. The very fine tungsten electrode, insulated with resin except the tip, was inserted vertically into the retina through the appeared small hole on its cornea.

Another fine tungsten electrode was inserted into the thoracic segment from the lateral side, served as the indifferent electrode. All ERGs were displayed on a minicomputer oscilloscope (ATAC 250, Nihon Kohden) to be computed and then photographed with a camera.

Fig. 1 shows a diagram of the experimental apparatus. After 30-minute dark adaptation, the animals were experimented.

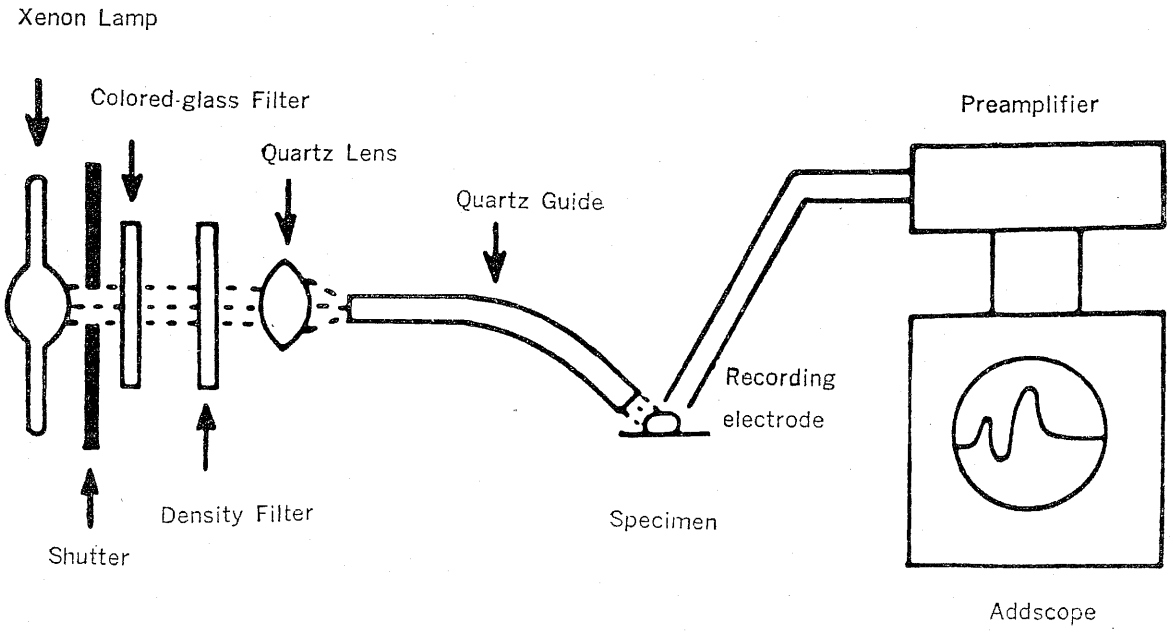

Fig. 1. Diagram of the optical apparatus.

In our previous experiments, the stimulating light was provided at intervals of 120 sec. and the ERGs were computed by sixteen superimposed, but in the late studies, we found that if ERGs were computed by 16 superimposed at intervals of 15 seconds, no visible differences on waveform of the components (ERGs) could be obtained but only their amplitudes were altered, so we flashed the stimulating light every 15 seconds.

The stimulating light of 20 msec. duration, generated by pulse generator, is provided by a 80-watt xenon arc lamp in conjunction with a quartz lens, which converges the light beam and focuses on a quartz light guide (2 mm in diameter, 90 cm in length). Then the left compound eye was illuminated via the light guide close to the eye (ca: 10 mm in distance).

Fifteen wavelengths of the monochromatic stimulating light from 348 to 668 (348, 364, 372, 405, 431, 449, 475, 494, 515, 528, 557, 587, 628, 654, 668) nm were selected by means of the coloured glass filters (Toshiba glass Co.). For measurment of "spectral response curves" (Mayer-Rochow., 1981) under the equal light intensities, the brightness of each wavelength was regulated by neutral density filters.

After a computed ERG was photographed, the responding characteristics of ERGs, represented in such as the waveform and amplitude were evaluated.

3. Calibration

The transmitting characteristics of each coloured glass filter was measured with the aid of a UV-visible spectrophotometer (Shimazu, Model UV-160). The brightness or the energy of stimulating light through the each coloured glass filter, the quartz lens and the quarts light guide was automatically measured by radiometer (Radiometer 390 U. D. T.), and the equal energy stimulating light was applied to the left compound eye using several neutral density filters and light power regulator.

RESULTS

1. ERG

After a fly allowed to dark adaptation for 30 minutes, the flashing photic stimuli were repeatedly presented to its left compound eye at intervale of 15 sec.

A computed (by 16 superimposed) ERG of the "white light" (control) (Heisenberg., 1971, Mayer-Rochow., 1981) and each variant intensity of the flashing light were shown in Fig. 2. The computed ERG of the compound eye consisted of depolarizing response of about 10 to 12 mv of maximum amplitude. No spike potential was recorded and it was partially very similar to that obtained previously in flesh fly. Its ERG consists of a triphasic waveform designated as gamma(γ), delta (δ) and epsilon (ε) in the order of their responding appearance (Fig. 2a, 4b). The latencies from the light stimulation to their peaks were 30.8 ± 2.1 msec. for gamma, 56.3 ± 5.1 msec. for delta and 114.5 ± 5.6 msec. for epsilon respectively ($n=18$) (Fig. 4b).

2. Sensitivity to white light

When the relative intensity of the white light stimulus was plotted against the resulting depolarization, a response-intensity curve was obtained (Fig. 2b). There was no plateau response appeared even if the maximum intensity ($\log I=0$) was used.

The light stimulus intervals of 15 sec. for a brief duration of 20 msec. throughout the measurement, could guarantee the response very stable and consistent. There was no remarkable change on the action potential.

3. Sensitivity to the flashing light of different wavelengths

The left compound eye of the fly was flashed with the different light wavelengths and the different light intensities, and the resulting waveforms of ERGs were found very much similar to that of the white light stimulation.

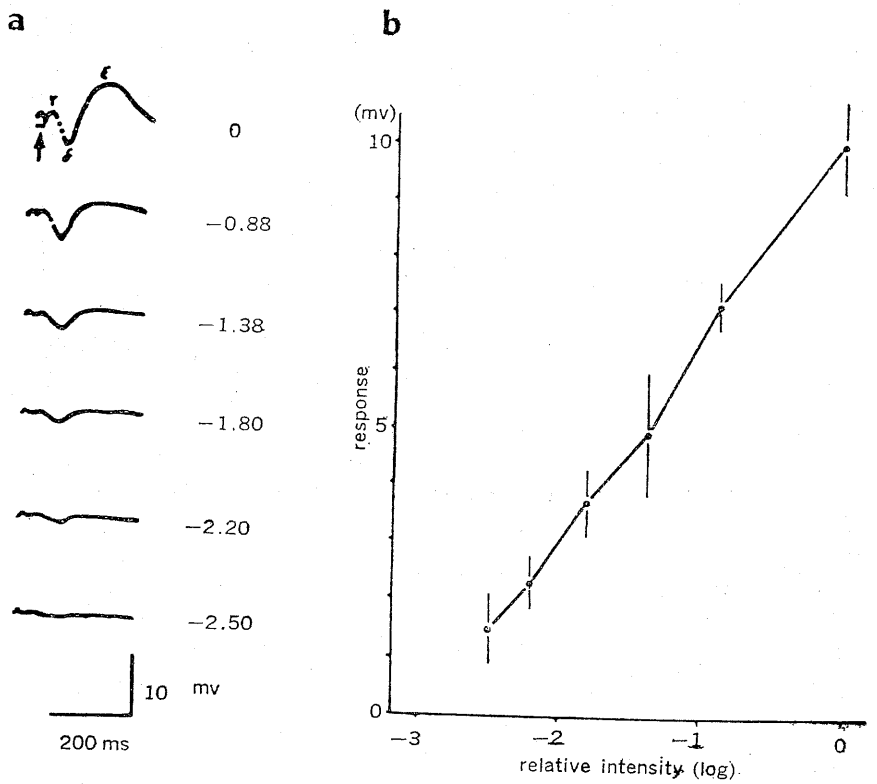

Fig. 2. ERGs and its response-intencity curve. a, computed ERGs of the white light. ERG: 16 superimposed; Light: 20 msec duration, log light intensity; Light intensity (log. I): 0 to −2.5. b, average V/log I curve with standard deviation bars.

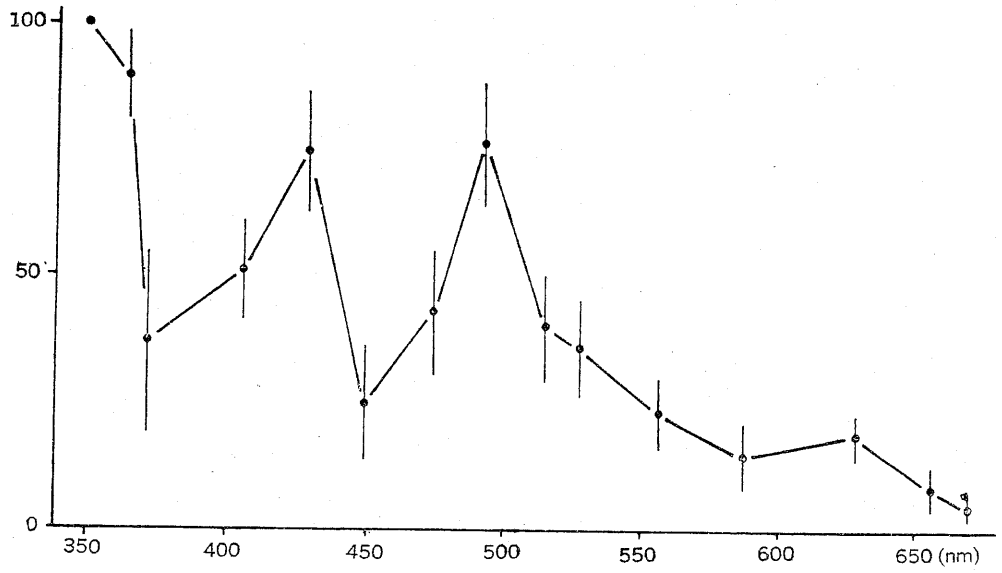

Fig. 3. Spectral response curve of the frontal region of the ommatidia as determined by ERG recordings. log. I=0

4. Spectral response curve

The spectral response curves were obtained from 14 flies' ERGs. Several distinct classes of the colour receptors were assumed in this spectrum (Fig. 3).

All of the ommatidia possessed the ultraviolet sensitivity. They had a large peak in range from 348 to 364 nm following a rapid fall at 372 nm wavelength.

The second and the third peaks appeared at 431 nm and 494 nm in order of their responding appearance respectively. Beyond this peak, the responding curve was greatly sloped down to a relatively low level under the light stimulation of the wavelength longer than 515 nm.

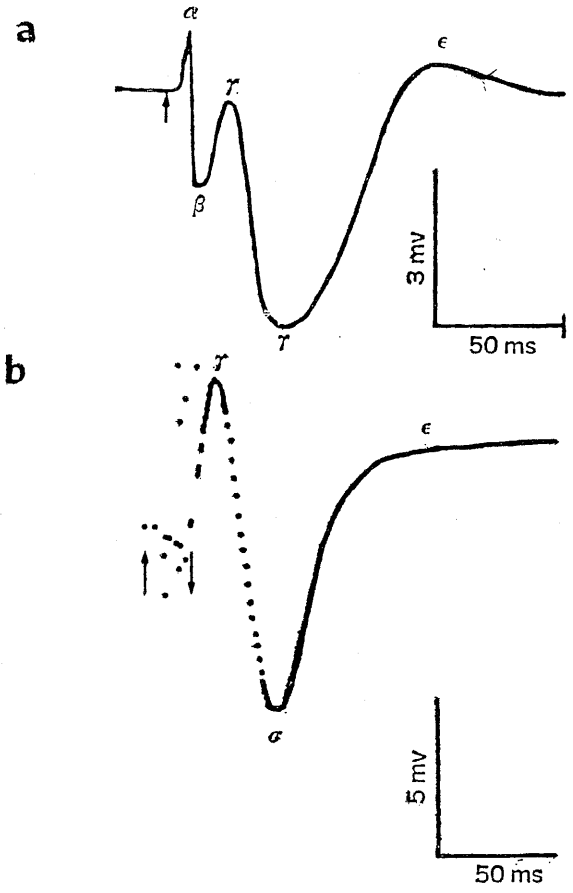

Fig. 4. ERGs from flesh fly and Oriental fruit fly. a, flesh fly ERG from Loew (Loew 1975). ↑: stimulating light. b, fruit fly ERG. Stimulation: white light, 20 msec. duration. 16 superimposed. ↑: stimulating light on; ↓: stimulating light off.

DISCUSSION

As in the eye of another fly species, the increasing intensity of the stimulating white light flash, the amplitude of the depolarizing response rises.

In many studies showed that the amplitude of the action potential was recovered within 10 sec. after a flashing stimulation (Autrum and Burkhardt, 1961; Horridge and Mimura, 1975; Mayer-Rochow, 1981; Moring, 1978), therefore, we suggest that 15 sec. protocol was adopted enough as a precautionary measure.

Using the short sparkling light, the ERG records, obtained from the dark adapted compound eye and then under control conditions in the flesh fly, showed at least five components, which were designated as alpha, beta, gamma, delta and epsilon in order of their responding appearances (Loew, 1975). Since there are insufficient data about their latencies, we can not discuss about it in detail. But upon our handling measurement on the ERGs of the Loew's report, we found that their latencies (n=3) from the point of the offering light stimulation to each of peaks were measured corresponding to 9.7 to 11.7 msec. for alpha, 13.5 to 22.2 msec. for beta, 16.1 to 30.8 msec. for gamma, 43.9 to 54.6 msec for delta, and 103.4 to 116.1 msec. for epsilon, respectively (Fig. 4a).

In our present experiments, only the last three components (i. e., gamma, delta and epsilon) are appeared in the flesh fly. It is assumed that the duration of the stimulating light used in this experiment is fixed in a 20 msec. period of time. From this point of view, apparently we may postulate that this light duration probably just interferes the appearance of alpha and beta waveforms described by Loew (Loew, 1975).

In this experiment, the attenuation of

stimulus ($\log I = -0.88$ to -1.38) caused a steady decrease in epsilon wave and delta wave, but the amplitude and value of the remaining gamma wave were not affected by the decreasing intensity. However, at a relatively low intensity ($\log I = -1.8$ to -2.5), all the waves were appeared with their low amplitudes; nevertheless, the three components were still distinguished (Fig. 2a).

In order to discuss the origin of the waveform of each component of the ERG, it would be isolated each one from others to observe it in an uncomplicated form by means of CO_2 application, selective recording, physical isolation of the receptor cell layer, and drug application, etc. (Loew, 1975). To this problem, it will be left for further studies in the future.

There are U-V sensitive cells located among the retinular cells in *Dipterans*, like that of the *Calliphora* (λ max = 345 nm, Burkhardt 1962., λ max=344 nm, Smola and Meffert, 1975, 1976), and the blue sensitive cells in that of *Drosophila* (λ max=350, 370 nm, Stark, 1975) were also observed in that of *Dipterans* (λ max=490 nm in *Calliphola*, Burkhardt, 1962; λ max=470 in *Drosophila*, Stark, 1975).

In our experiment, λ max of Ultraviolet (348 nm), Indigo (431 nm) and Blue-Green (494 nm) were found by extracellular experiment. Although we can not respect this experiment to determine the visual pigment in the fruit fly eye, we may have some clues for the future study.

When the various relative intensities of white light plotted against the responding amplitude of ERG, the well-proportioned response curve were obtained. These results showed that the intensities of the light used in these experiments did not exceed a maximum intensity for the compound eye of the fruit fly. There are very close relationship between the light stimulations and its responses, but there is not yet proved that what kind of the receptor the fruit fly has.

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昆 蟲 之 受 器

二、東方果實蠅 (*Dacus dorsalis*) 複眼之網膜電圖

吳 京 一

取果實蠅，經 30 分鐘之暗適應後，以 15 種單色光，20 msec 之光照時間，照射其左側複眼之前面部。

另取極細之鎢絲電極，插入左複眼內。記錄其網膜電圖。結果發現複眼網膜電圖有三相性之活動電位，命名爲 γ, δ 及 ε 三種電壓，計測這些網膜電圖之電壓，畫出其光譜反應曲線 (spectral response curve)，得知果實蠅複眼之前面部有紫外光有最大之吸光 (λ max=348 nm) 外，對藍色光 (λ max=431 nm) 及青綠色光 (λ max=494 nm) 亦有最大吸光。